FOREWORD

The body of knowledge which comprises forensic science is a compilation of procedures adapted from other disciplines that encompass many of the physical and natural sciences. During the history of forensic science, a multitude of scientists have greatly contributed to the protocols, methods and procedures that have become a routine part of analysis. Every effort has been made in this manual to give proper recognition to the authors of specific procedures; however, in some instances, the original source of forensic procedures has been lost in antiquity. For others, the general procedures belong to the public domain and are recorded in many basic references concerning forensic science. In addition, many of the procedures described in this manual have been adapted from standard laboratory practices, and the citation of thousands of references which deserve credit for aiding in the development of these procedures is neither practical nor possible. To all those scientists who have contributed to the knowledge of forensic science contained herein, we do extend collective recognition and gratitude.

Procedures manuals which offer reliable information that is then combined with corresponding training manuals serve as the foundation for effective quality management of analyses. Extensive effort has been made to ensure that the routine procedures described herein will produce accurate and valid analytical results. However, not all possible analyses that may be encountered in casework can be appropriately covered in a procedures manual, nor can all possible variations to a described procedure be included. Therefore, this manual is written with the understanding that minor variations that do not significantly alter the described procedure may be used. An analyst may use a non-routine procedure not specifically stated in this manual, provided all the following conditions are met:

1. The procedure used is based upon documented and scientifically accepted practice.
2. A notation is made indicating the procedure followed is not specified in the procedures manual.
3. The analyst also indicates why the particular procedure was selected over a procedure contained in this manual. Rationale must be detailed sufficiently to withstand close scrutiny by independent examiners.
4. The analyst provides documentation showing that the non-routine procedure had been tested prior to application with evidence. Test criteria shall include test samples that approximate the characteristics of the evidence, the results obtained with the routine procedure, and the results obtained with the non-routine procedure. Documentation will also include related data concerning the non-routine procedure's sensitivity, precision and possible sources of error.
5. The non-routine procedure used will be recorded to a standard such that another scientist of similar skills and experience can understand fully the procedure used and the results obtained.

Additionally, there may be procedures which pertain to all sections. Such is the case with laboratory reagents. In order to standardize the testing and monitor the shelf life of reagents used by analytical sections, the Forensic Sciences Command has developed protocols which are universal for all sections. These protocols regarding reagent expiration and testing are found in the Command Quality Manual.

PREFACE

"Every contact leaves its trace." – Professor Edmond Locard

"Wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as silent witness against him. Not only his fingerprints or his footprints, but his hair, the fibers from his clothes, the glass he breaks, the toolmark he leaves, the paint he scratches, the blood or semen he deposits or collects — a great deal of ingenuity is required in the analytical approach — all of these and more bear mute witness against him. This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are, it is factual evidence, physical evidence cannot be wrong; it cannot perjure itself; it cannot be wholly absent, only its interpretation can err. Only human failure to find it, study and understand it, can diminish its value." – Harris vs. U.S., 331 U.S. 145, 1947.

These statements form the basis for the tasks performed in forensic microscopy. The function of a forensic micro/trace analyst is to do the following:

1. Observe or detect the clue.
2. Recognize the *potential value* of the clue.
3. Record the clue.
4. Collect and preserve the clue.
5. Examine the clue to determine from it as much information as possible.

The forensic micro/trace analyst plays a critical role in the determination of information from an item of physical evidence. Any breakdown in the five functions might prevent the truth from being discovered. The major portion of this manual will address the fifth function as mentioned above.

03/01/22

INTRODUCTION

rev. 10/06/2023

Statement of Purpose

The purpose of this manual is to provide the forensic scientist with useful procedures for the examination of physical evidence. The manual is not designed to be an all-inclusive collection of every possible procedure or variation of procedure which might be used in Micro/Trace. Instead, this manual is a presentation of methods which have been found to be workable by practicing forensic scientists.

Before any new procedure is used, a validation study must be conducted as is outlined in the Quality Manual.

Safety - Biohazardous Materials

The examiner will use appropriate Personal Protective Equipment (PPE) when working with biohazardous or potentially biohazardous materials. Each laboratory's Facility Operations Manual (FOM) have procedures dealing with biohazardous materials.

Micro/Trace Standard Laboratory Practices

1. For all trace materials analysis involving comparison of a known to an unknown, the unknown sample will be examined/reviewed to determine enough information is detected in the unknown to justify a comparison to known standards.

2. The examiner should change the examination paper between each evidence item.

3. There should be only one item opened at a time.

4. The examiner should change gloves and clean tools between examining the evidence from the victim and the evidence from the suspect.

5. If possible, the victim's evidence and suspect's evidence should be examined in separate rooms.

6. The examiner should follow all the biohazard procedures when working with biohazardous materials.

7. The examiner will use Clean Technique for examination of evidence that may be further analyzed in Biology/DNA (MT APP V)

Verifications

All meaningful fiber transfers and fabric impression comparisons detected in cases by the examiner issuing the report shall be verified by a second examiner conducting independent casework. Verifications shall be made before any contact is made with the agency regarding the transfer, except with the laboratory director's approval. All transfers shall be verified before a report is issued.

The same examiner shall make all the verifications in a case. Exception to this policy can be made by the laboratory director due to external circumstances (e.g. illness of the verifying examiner). The verification shall be made by the review of the pertinent items, data, and other documents which form the basis for the scientific conclusion.

General Search

General search encompasses all the steps taken from the time the evidence is first submitted to the laboratory until a specific examination is required.

Evidence Sign-in

Agencies submitting evidence for Micro/Trace are to provide comprehensive information about the items and examinations needed. If necessary, the agency should be contacted prior to examining the evidence to discuss or clarify the requests.

Interim Storage of Evidence

After submission, evidence is to be stored in appropriate vault locations. Possible perishable items may be stored in refrigerator or freezer vaults. Refer to the Evidence Submission Handout Appendices in the Illinois State Police Command Directives.

Preparation for the Examination

Before the actual examination, certain preparations should be made.

1. Review all the information provided to determine what questions the investigator needs to have answered. If a case is particularly complicated, it should be discussed with a colleague or supervisor to get a different viewpoint.

2. Plan the approach to the case. Certain items may have greater potential value for information than others. The analyst may want to examine these items first.

3. Prepare the work area. The bench must be clean and free of clutter. If large items such as clothing or bedding are to be examined, a large table or processing room should be available for this purpose. The work area should be covered with white paper to prevent the loss of small particles of evidence and to prevent the cross-transfer of materials from one item to another. The necessary tools and reagents for the examination should be conveniently placed. Adequate lighting should be provided to allow close visual inspection of evidence. If room lighting is insufficient, supplemental lighting may be used as necessary. Have note-taking materials at hand to record your observations. Have low power magnification available in the form of a hand lens, magnifying illuminator, or stereomicroscope.

4. Wear appropriate Personal Protective Equipment (PPE). Lab coats and gloves protect the examiner and the evidence from contamination.

PPE will be worn when examining biohazardous or infested evidence. In some instances, a nuisance odor mask may be helpful when dealing with foul smelling evidence.

The laboratory's Chemical Hygiene Plan should be followed when handling biohazardous materials.
Since the examination of evidence in the forensic science laboratory can often be a very tedious effort, the analyst should take stock of himself/herself and determine if he/she is up to the task. In the case of microscopic examinations, the analyst must be prepared to take breaks to rest his/her eyes since visual acuity drops markedly during extended periods of intense use. Fatigue can jeopardize the quality of the examinations.

Examinations

Once the analytical approach has been determined, the analyst should begin the examination in the following manner:

1. Deal with one item at a time, being sure the work area is cleaned between examinations. This usually will involve changing the paper entirely between each item.

2. Be certain that the previous item has been packaged and put away before opening another item on the work surface.

3. Remove packaging with care, remembering that materials of evidential value may be adhering to the item. Opening the evidence over the white surface paper will prevent the loss of these materials.

4. Mark the evidence for future identification with the Date, Initials, Case number, and Item number ('DICI').

Guidelines which cover a broad range of topics applicable to most evidence are described in a general sense within the following sections. Specific suggestions concerning different types of evidence are offered in their respective procedures.

The following topics in this Introduction do not pertain to evidence submitted for fire debris analysis.

Items Not Dried Before Submission

Evidence should be dried by an agency prior to submission to the laboratory. If, upon opening evidence, the examiner finds evidence to be wet or foul smelling, the container should be opened and allowed to air dry preferably in a vented hood area. Caution should be exercised to prevent the loss of other evidential material which might result from the movement of air currents inside the hood area. A telephone call to the submitting agency in order to explain the problem of objects submitted wet and to prevent a recurrence is appropriate.

Items Infested with Vermin

Fleas, lice, and insect larvae may be discovered while examining various objects. If such infestation occurs, the following steps should be taken:

1. Wear appropriate PPE.

2. Examine the item carefully on an isolated bench, if possible.

3. If the pests are still living, examine the object as quickly as possible, take samples of evidential material, and seal the object within a plastic bag with tape or a heat-sealing device and freeze it if possible.

Removal of Surface Debris (Does Not Apply to PGSR analysis)

Locard's Exchange Principle states that any time two objects come in contact, there is a cross-transfer or exchange of particles between them. The finding of these particles may have important evidential value. The surface debris containing these particles may consist of hairs, fibers, paint, wood fragments, glass, plant material, soil, and many other materials. Collection and preservation of this debris before any further examinations are conducted is important. A forensic scientist must realize that there is potential value in a great many types of particles which other sections of the laboratory could examine.

Removal of surface debris may take the form of any of the following:

1. Scraping: The item to be examined is suspended above the examination surface and gently scraped with a spatula. Scraping in a downward direction allows trace materials to fall onto the examination surface for collection.

2. Sticky tape: Clear plastic book tape can be applied sticky side down to the surface of the item to be examined, pressed down, and then pulled away. Trace materials will adhere to the adhesive on the tape. This is especially useful on large or dark-colored items on which evidence may be difficult to see. Trace materials may be removed from the tape with forceps or using hexane or xylene to dissolve the adhesive. A tape roller may be used as an alternative.
Taping should not be performed if paint traces may be probative.

3. Picking: Evidence may be removed manually using tweezers under various lighting conditions.

When all the surface debris has been removed and packaged for later examination, the analyst may then proceed to a general examination of the object.

General Examination

At this stage, the object can be spread out and examined carefully for any of the following:

1. Stains, soiling, smears, etc.

2. Damage such as rips, tears, cuts, missing portions, perforations, etc.

Observations should be recorded. Drawing a sketch of the object to describe the location of various stains, etc., may be helpful.

The general examination should continue until the object has been thoroughly searched for traces of evidential material.

Examination of Weapons (Does not apply to PGSR analysis)

Weapons are submitted frequently for forensic examinations. Often these requests require several sections to examine the same item. The scope of testing should be clearly outlined by the submitting agency and analysts should be aware of all analytical requests for the item. During subsequent examinations, care must be taken to preserve all possible evidentiary materials (biological, latent print, firearm, toolmarks, etc.).

1. Pertinent details to record about weapons include the following:
    a. Physical dimensions
    b. General condition
    c. Location of trace evidence observed
2. Examine under a stereomicroscope for additional trace materials of potential evidential value.
3. Carefully collect all trace evidence and securely package for further examination.

Examination of Clothing

Clothing is often submitted to the laboratory for examination. Items of clothing should be marked and then subjected to the procedures previously outlined involving the removal of surface debris. Next, the general examination can commence as follows:

1. Spread out the garment on the examining table, looking carefully at both sides.
2. Note or sketch any damage to the clothing which may have evidential value (torn or missing buttons, torn or cut areas on garments, damaged zippers, burned areas).
3. Note any soiled areas of evidential value on the garment (knees, buttocks, cuffs). Note whether the garment appears freshly washed or not.
4. Examine the pockets of the garment carefully. Turn pockets inside out and collect debris if appropriate. ***Caution is advised when placing the hand into a pocket. An unexpected sharp object could cause serious injury.***

5. Carefully examine the waistband, lining, cuff areas, and collar areas for material of evidential value.

6. Examine shoes very carefully. Shoes are less often discarded by criminals than other items of clothing. They also have many nooks and crannies which could retain materials of evidential value. Look carefully in the groove between the sole and upper shoe. Many times, evidence can be more easily seen if the shoe is viewed with oblique lighting.

Summary

In essence, the general search phase is the key to locating materials of evidential value, recording information about these materials, and collecting them for later examination. The imagination of the forensic scientist to recognize the potential value of these items is the key to a complete and fruitful analysis. Associating one person with another, or a person with a place, through the use of physical evidence is the result of a successful examination by the forensic scientist.

Physical Match

See Physical Match Procedures Manual

General Microscopic Trace Evidence Examinations

Since "trace evidence" encompasses a broad variety of materials, it is impossible to address every eventuality or possible situation in this section. However, some common principles can be applied to most categories of trace evidence examination. Trace evidence is not usually "positive identification"-type evidence. Trace evidence is, instead, associative evidence, which means that this evidence tends to associate people or a person with a place. The type of evidence and the location where it was found, taken in context of circumstances of the crime, determine the value of the trace evidence in the investigation.

The associative nature of trace evidence is directly related to Locard's Exchange Principle: "Every contact leaves its trace." Simply put, whenever two objects come in contact with one another, there is a transfer of material from the first to the second, and from the second to the first. The transfer may be sub-microscopic, and therefore goes undetected. However, depending on the nature of the materials comprising the two objects, the transferred material is often microscopic or larger and retained for long periods of time by the receiving object. Finding the transferred material shows that contact with some sort of object took place, and if the transferred material is identical to that which comprises a particular object, then the possibility is established that contact could have occurred with that object.

Not all the trace materials found in an investigation of a crime are useful evidence. The value of trace evidence is determined by the answers to some basic questions:

1. What information of fact can be established by the evidence? How does this information fit into the investigation?

2. Where is the evidence found?

3. How unique is the evidence?

When the analyst has a good understanding of what the investigator believes occurred during the crime, he or she can begin to see where the pieces of physical evidence fit into the investigation. The analyst needs to know if the various pieces of evidence were found in some place which is already associated with the victim or suspect. If fibers like those of the victim's clothes and fibers like those of the suspect's clothes are found in a location, such as an abandoned house, which is foreign to both of them, then there is good evidence that they may have both been in that place.

Many types of microscopic evidence are encountered in the crime laboratory. An analyst can develop the ability to recognize a vast assortment of materials he or she has personally seen, if the analyst has a large reference collection with which to work. The usefulness of a reference collection should not be underestimated. The samples in the collection need not be certified standards, or National Bureau of Standards reference materials. The collection need only consist of known bits and pieces of the world as they exist in real life. When an unknown substance is encountered, the collection can be searched for similar appearing particles. Careful observation of these may lead the analyst to an identification.

Labels and Documentation

Evidence items are to be labelled with the Date, Initials, Case number and Item numbers ('DICI').

Items that are separated from the parent item (such as a questioned hair to be sent to DNA) are to be sub-itemized.

Trace materials recovered in packets, pillboxes, tapings, slides, etc. are to be labelled, secured, and sealed. Notation about trace packages are to be documented in the case file. In general, trace materials are to be returned to the package of the parent evidence item. Packaging seals shall be marked with the examiners initials and the date sealed.

The case file shall sufficiently document the observations and examinations that were performed. The technical records included shall be such that, a peer reviewer can evaluate what was done and interpret the data.

For reference through-out this manual, any reference to LAM refers to the Laboratory Asset Manager module in the Laboratory Information Management System (LIMS).

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MICRO/TRACE
Last Review Date 08-01-2025

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fire Debris Analysis

Reviewed by:

_____
Forensic Scientist Adrienne Bickel, Chairperson
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Approved by:

_____
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Micro/Trace Command Coordinator
Forensic Sciences Command

MINIMUM STANDARDS & CONTROLS

I. DEFINITIONS

A. Extracted Ion Profile (EIP) – a group of ions that, together, represent a class of chemical compounds that are presented as a single chromatogram that is representative of that particular class.

B. Gas Chromatography/Mass Spectrometry (GC/MS) – an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated and identified.

C. Gaussian distribution – a chromatographic pattern where most peaks cluster in the middle of a range and the rest taper of symmetrically toward either end.

D. Identifiable Ion Extraction Pattern - It meets the ignitable liquid's minimum requirements for class identification for the group of ions listed.

E. Ignitable Liquid - a liquid which burns when held to an open flame.

F. *n*-alkane – "normal" alkane, meaning a straight chain of carbon atoms with hydrogens attached. Also known as paraffin, or an acyclic (non-ring) saturated hydrocarbon. (For this manual, the convention 'C_5' is pentane, 'C_6' is hexane, and so on.)

G. Stacked profile – the display of a reference collection profile and a sample profile in a vertical arrangement such that comparative peaks (ions) are readily located and viewed.

H. Total Ion Chromatogram (TIC) – represents the summed intensity across the entire range of masses being detected at every point in the analysis.

I. Chromatogram – data output from a gas chromatograph (GC) which is a plot of the amount of each chemical compound on the y-axis versus the time it emerges from the GC and detected on the x-axis.

II. MINIMUM STANDARDS OF ANALYSIS

A. Analytical Approach

1. Each item will be opened and inspected prior to analysis. On the basis of this examination, the analyst will decide which analysis technique will be used. While an identification can be made using any technique, a negative or inconclusive conclusion can be reported only if either neat liquid, solvent

extraction, or charcoal adsorption has been performed and analyzed by GC/MS. An exception would be alcohol or single component solvents.

a. If crime scene examination gloves are observed in the can, remove prior to analysis.

B. Instrumentation – the below guidelines are in addition to the Minimum Standards and Controls found in the Gas Chromatography/Mass Spectrometry method (MT-IXB)

1. Gas Chromatograph/Mass Spectrometer

a. An instrument performance check will be run each day that the instrument is used for casework. This run will use the same conditions as regular samples. The performance check is an identifiable EIP for Alkanes, Aromatics, Indanes and Naphthalenes from a solution containing 0.5 µL of gasoline and 0.5 µL of diesel fuel in 1 mL of CS_2. The *n*-propylbenzene peak and the pentadecane peak must be visible in the TIC.

b. A complete TIC that includes the LPD through HPD range must be recorded.

C. Documentation

1. All records from performance checks must be kept. Copies of the performance check (D.1.a.) will be documented in each case file.

2. A full scale, full page TIC and appropriate EIPs will be recorded for all samples analyzed.

3. If a sample is heated during analysis, the temperature of the extraction shall be recorded in the case file.

4. Identification or classification of samples must include comparison to a reference and must be documented by placing the reference in the case file.

a. If an in-house reference is available, a full-page TIC and appropriate EIPs must be included in the case file. If available, stacked TICs and EIPs may be used for comparisons instead of full page individual EIPs. The TIC and EIPs must be comparably scaled to feature individual areas of interest so as to be easily visualized by a peer reviewer.

b. If an external reference is used, a copy of the TIC (and all appropriate EIPs of that reference, if available) must be placed in the case file.

c. If required for identification and classification, mass spectra searched by computer software must be confirmed by visual comparison to the computer-generated spectra and must be documented by placing full page reference and questioned sample spectra in the case file. If available, stacked profiles of the spectral comparisons may be used instead of full-page individual spectrum. The spectra must be comparably scaled, so as to be easily visualized by a peer reviewer.

d. If a finding of "Inconclusive" is reported, the reasoning for the conclusion should be explained in the case file.

D. General

1. If a heating block is used to clean syringes, permit syringe needle to cool prior to drawing up a sample except when analyzing for oxygenates.

2. All submitted comparison samples will be analyzed.

3. If sufficient, headspace is not available in the submitted metal can for analysis, it may be necessary to transfer the evidence into a clean, unused metal can at the laboratory. The cleanliness of the transfer container should be verified prior to sample transfer. Cleanliness is determined by using the transfer container as the system blank using the same conditions as will be used for the questioned sample. One cleanliness is verified, allow the transfer container with sample to equilibrate for at least one hour before sampling.

4. The cleanliness of every new lot number of solvent must be verified prior to use in casework and the data from the check must be kept in LAM.

SPECIAL NOTES

I. RECOMMENDED PRACTICES IN FIRE DEBRIS ANALYSIS:

A. Use a pipet to add enough CS_2 to wet charcoal. Use a consistent volume for the system blank and its respective samples.

B. Use a timer for a consistent measurement of the time that CS_2 is in contact with the charcoal, usually five minutes minimum.

C. Change the septum often.

II. SAFETY:

A. This protocol involves hazardous materials, operations, and equipment. This protocol does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Proper caution should be exercised, and the use of personal protective equipment should be considered to avoid exposure to dangerous chemicals. Consult the appropriate Safety Data Sheet (SDS) for each chemical prior to use.

B. The vapor density of solvents used in fire debris analysis procedures are listed below. Remember, a vapor density greater than one will result in vapor concentration near the work surface.

Also, any solvent with a high flammability or health rating (greater than two) should be used in the fume hood with adequate ventilation.

Solvent	Vapor Density	Flammability Rating	Health Rating
Carbon Disulfide	2.6	3	2
Hexane	3.0	3	1
Pentane	2.5	4	1
Diethyl Ether	2.6	4	2

INSTRUMENT CONDITIONS

I. DISCUSSION:

The following are suggested techniques which may be adopted or modified for fire debris analysis. Any technique used must meet the Minimum Standards of Analysis.

II. GAS CHROMATOGRAPHY/MASS SPECTROMETRY TECHNIQUE:

A. GC Conditions:

Column: Fused silica capillary, dimethyl silicone 25 Meter, 0.33-micron film thickness, 0.20mm inner diameter
Carrier: Helium, 0.9mL/min
Injector: Split mode (33:1-50:1), 230°C-270°C

Pressure Program (Highly Volatile Compounds):

[For Instruments equipped with Electronic Pneumatic Control]

Oven Temp:	40°C Isothermal
Initial Pressure:	0.6 psi
Hold time:	0.35 min.
Ramp 1:	80 psi/min.
Final Pressure 1:	1.0 psi
Hold Time:	0.25 min.
Ramp 2:	80 psi/min.
Final Pressure 2:	14.8 psi
Final Hold Time:	6 min.

Temperature Program:

Initial Temp:	50°C
Initial Hold:	2 min.
Ramp:	10°C/min.
Final Temp:	250°C
Final Hold:	5 min.

B. Interface Temperature (Transfer Line): 250°-270°C

C. MS Conditions-
(For Temperature Program):

Solvent Delay:	2 min.
Scan Mode:	EI^+
Ionization Energy:	70 eV
Scan Range:	30-400 m/z
Source Temp:	200°C

(For Pressure Program):

No Solvent Delay

Scan Mode:	EI^+
Ionization Energy:	70 eV
Scan Range:	15-330 m/z
Source Temp:	200°C

INTERPRETATION OF IGNITABLE LIQUID TOTAL ION CHROMATOGRAMS

I. READING THE TIC:

For each sample extracted from fire debris, a TIC will be produced. The typical TIC displays a series of vertically tapering figures called "peaks," which differ in position and size. Collectively, these peaks characterize the sample's composition. The analyst reads the TIC and associated EIPs by visually comparing its peak features with those characterizing each of several distinguished "classes" of complex ("many peaks") liquids commonly encountered as ignitable liquid residues. When the comparison discloses a degree of similarity consistent with criteria as outlined in III, the analyst reports that the indicated class of ignitable liquid has been detected.

II. IGNITABLE LIQUID CLASSIFICATION SYSTEM:

A. Seven "classes" of complex ignitable liquid products may be identified by gas chromatography/mass spectrometry, extracted ion profiling, or a combination thereof when recovered from fire debris. It is a further attribute of the following system that products listed as examples of the same class may be indistinguishable from each other. These classes are outlined below. A miscellaneous category is included for those ignitable liquids that do not fit the criteria for inclusion into the seven major classes.

B. With the exception of the gasoline class, the major ignitable liquid classes may be divided into three subclasses based on boiling (*n*-alkane) range: light, medium and heavy.

1. *Light product range* (C_4-C_9); the majority of the pattern occurs in the range C_4-C_9, no major peaks associated with the ignitable liquid exist above C_{11}.

2. *Medium product range* (C_8-C_{13}); narrow range products, the majority of the pattern occurs in the range of C_8-C_{13}, no major peaks associated with the ignitable liquid below C_7 or above C_{14}.

3. *Heavy product range* (C_9-C_{20+}); typically, broad range products, the majority of the pattern occurs in the range C_9-C_{23}, with a continuous pattern spanning at least 5 consecutive *n*-alkanes. Also included in the subclass are narrow range (encompassing less than 5 *n*-alkanes) ignitable liquid products starting above C_{11}.

4. It may be necessary to characterize a product as "light to medium" or "medium to heavy" when the carbon number range does not fit neatly into one of the above categories.

CLASS	LIGHT ($C_4 - C_9$)	MEDIUM ($C_8 - C_{13}$)	HEAVY ($C_9 - C_{20+}$)
Gasoline-all brands, Including gasohol and E85	Fresh gasoline is typically in the range $C_4 - C_{12}$		
Petroleum Distillates	Petroleum ether Some cigarette lighter fluids Some camping fuels	Some charcoal starters* Some paint thinners Some dry cleaning solvents	Kerosene Diesel fuel Some jet fuels Some charcoal starters
Isoparaffinic Products	Aviation gas Some specialty solvents	Some charcoal starters Some paint thinners Some copier toners	Some commercial specialty solvents
Aromatic Products	Some paint and varnish removers Some automotive parts cleaners Xylenes, Toluene-based products	Some automotive parts cleaners Specialty cleaning solvents Some insecticide vehicles Fuel additives	Some insecticide vehicles Industrial cleaning solvents
Naphthenic-Paraffinic Products	Cyclohexane-based solvents/products	Some charcoal starters Some insecticide vehicles Some lamp oils	Some insecticide vehicles Some lamp oils Industrial solvents
Normal Alkane Products	Solvents Pentane Hexane Heptane	Some candle oils Some copier toners	Some candle oils Carbonless forms Some copier toners
Oxygenated Solvents	Alcohols Ketones Some lacquer thinners Fuel additives Surface preparation solvents	Some lacquer thinners Some industrial solvents Metal cleaners/gloss removers	
Others-Miscellaneous	Single component products Some blended products Some enamel reducers	Turpentine products Some blended products Some specialty products	Some blended products Some specialty products

Note: the products listed in the various classes of the above classification system are examples of known commercial uses of these ignitable liquids. These ignitable liquids are not intended to be all-inclusive. Reference literature materials may be used to provide more specific examples of each classification.

**Many of the examples can be prefaced by the word "some", as in "some charcoal starters."*

III. MINIMUM REQUIREMENTS FOR CLASS IDENTIFICATION:

Gasoline

Alkane: Pattern varies: *n*-alkanes above C_9 may be present, saturated branched alkanes must be present.

Aromatic: Petroleum pattern comparable to that of the reference ignitable liquid. Above C_7 the aromatic concentration is generally higher that the alkane concentration.

Dihydroindenes (Indanes): Petroleum pattern comparable to that of a known reference.

Condensed Ring Aromatic: Petroleum pattern comparable to that of known references are usually present. These compounds may be absent in some gasolines.

Cautions: The presence of high levels of alkenes and oxygenates may indicate significant pyrolysis of the substrate matrix and should make the recovery suspect. The presence of high levels of aromatics without the appropriate level of alkanes may indicate an aromatic product.

Petroleum Distillates

General: Predominant pattern associated with a homologous series of *n*-alkanes in a Gaussian distribution of peaks. Light distillates may not exhibit a recognizable pattern and may contain only one or two of the *n*-alkanes.

Alkane: Abundant. Predominant normal alkanes present with isoparaffinic compounds present.

Cycloalkane: Present, less abundant than alkanes. Pattern varies by boiling range and peak spread.

Aromatic: Usually present in medium and heavy distillates; less abundant than alkanes. Pattern varies by boiling range and peak spread. May be present in light distillates. *Note:* In some products the aromatic composition may be significantly reduced or completely absent.

Condensed Ring
Aromatic: May be present based on boiling range and peak spread.

Note: Based on the boiling point range of light petroleum distillates and the instrumental technique used, it is recognized that the entire Gaussian distribution of the alkane pattern will not be visible. Additionally, due to the nature of these products, branched-chain and cyclic alkanes may be higher in abundance in light petroleum distillates (possibly similar or greater abundance than the n-alkanes) than those seen in medium or heavy petroleum distillates.

Oxygenated Solvents

General: Major peaks present before C_8; major compound(s) may include alcohols, esters, ketones, toluene, or xylenes.

Alkane: May contain characteristic petroleum distillate pattern; pattern depends on formulation.

Cycloalkane: Pattern depends on formulation.

Aromatic: Pattern depends on formulation.

Condensed Ring
Aromatic: Not significant.

Cautions: The mere presence of oxygenated solvents such as alcohols or acetone does not necessarily indicate a foreign ignitable liquid is present in the sample. The questioned compound should be, minimally, one order of magnitude above the matrix peaks in the chromatogram before considering the finding of an oxygenated solvent significant.

Note: All oxygenated compounds must be identified by GC retention times and mass spectral identification.

Isoparaffinic Products

Alkane: Characteristic isoparaffinic product pattern present with no *n*-alkanes or only minor levels of *n*-alkanes. The boiling range and component pattern are dependent on the specific formulation.

Cycloalkane: Not significant. Note: Ions indicative of cycloparaffins are also present in smaller amounts in isoparaffinic compounds. "Cycloalkane" pattern representing isoparaffins may be present but significantly less abundant than the alkane pattern.

Aromatic: Not significant.

Condensed Ring
Aromatic: Not significant.

Normal Alkane Products

Alkane: Normal alkane product pattern present with no Isoparaffins or only minor levels of Isoparaffins. The boiling range and pattern are dependent on the specific formulation.

Cycloalkane: Not significant.

Aromatic: Not significant.

Condensed Ring
Aromatic: Not significant.

Note: All normal alkanes must be identified by GC retention time and mass spectral identification.

Aromatic Products

Alkane: Not significant.

Cycloalkane: Not significant.
Aromatic: Pattern depends on formulation.

Cautions: The relative intragroup ratios of the isomers of xylenes and C_3-alkylbenzenes do not vary significantly among petroleum products. Therefore, the relative ratios of these compounds should match, or nearly match, the ratios found in petroleum products, if they are to be reported. Further, the mere presence of toluene or xylenes does not necessarily indicate that a foreign ignitable liquid is present in the sample. There should be a large excess of the product (at least one order of magnitude above the matrix peaks in the chromatogram) before the analyst should consider the finding of toluene or xylenes.

Note: Some aromatic products may consist of single or just a few components. These components must be identified by GC retention times and mass spectral identification. For aromatic products that have many components, pattern recognition and ion profiling is acceptable for identification.

Naphthenic-Paraffinic Products

Alkane: Naphthenic-paraffinic product pattern present with no *n*-alkanes or only minor levels of *n*-alkanes. The boiling range and component pattern are dependent on the specific formulation.

Cycloalkane: Pattern depends upon formulation.

Aromatic: Not significant.

Condensed Ring
Aromatic: Not significant.

IV. A REFERENCE TABLE OF MAJOR IONS PRESENT IN MASS SPECTRA OF COMMON IGNITABLE LIQUIDS:

Compound Type	m/z
Alkane (C_nH_{2n+2})	57, 71, 85, 99
Alkylcyclohexanes	82, 83
Cycloalkane and Alkene	55, 69
Decalins	138, 96, 81

Aromatics/Alkylbenzenes	91, 105, 119; 92, 106, 120
C_4 Alkylbenzenes	119, 134
C_5 Alkylbenzenes	133, 148
Indanes	117, 118; 131, 132
Methylindanes	117, 132
Dimethylindanes	131, 146
Naphthalene (C_{10} H_8)	128
Methylnaphthalenes	142, 141
C_2 Alkylnaphthalenes	156, 141
C_3 Alkylnaphthalenes	170, 155
Alkylstyrenes (styrenes)	104, 117, 118, 132, 146
Alkylanthracenes	178, 192, 206
Alkylbiphenyls/Acenaphthenes	154, 168, 182, 196
Monoterpenes (Terpenes)	93, 121, 136
Ketones	43, 58, 72, 86
Alcohols	31, 45
Combinations	
Alicyclics and Olefinics	55, 69, 83, 97
Styrenes and Indanes	104, 118, 132, 146
Aromatics minus Terpenes	91, 105, 119, -93
Naphthalenes	128, 142, 156
Indanes to include Xylenes	103, 104, 117, 118
Olefinics minus Alkanes	55, 69, -57
Fatty Acid Methyl Esters	67, 74, 79

***The underlined ions are sometimes used for single ion extraction for the group.**

V. CHECKLIST FOR INTERPRETATION OF DATA:

A. Note the chromatographic conditions and verify correct operation of GC/MS by running an autotune, instrument performance check (Gas Chromatography/Mass Spectrometry method IXC), and an appropriate blank.

B. Obtain a TIC in which the major peaks are "on" scale (3/4 to full scale).

C. Note in what area of the TIC peaks are present.
   1. In what boiling range are peaks present, that is, light, medium, or heavy?
   2. What is the spread of the range of the peaks (narrow or wide)?

D. Once an area of interest is identified, look for characteristic features of a class of petroleum products. For example:

   1. Gasolines (C_4-C_{14}):

a. Ethyl toluenes and 1,2,4-trimethylbenzene grouping present and all peaks in correct relative rations.
b. Methyl naphthalenes are usually present.

2. Light Petroleum Distillate (C_4-C_{11}):
   a. Elute early.
   b. Often have a narrow boiling range.

3. Medium Petroleum Distillate (C_8-C_{13}):
   a. 2 to 3 normal alkanes usually present.
   b. A bell-shaped curve may exist.

4. Heavy Petroleum Distillate (C_9-C_{23}):
   a. Normal alkane series present in correct relative ratios.
   b. Pristane and phytane present in No. 2 fuel oil.
   c. Determine if FAME's are present.

5. Unidentified Petroleum Product (may fall into any carbon range):
   a. There are numerous commercial and industrial products which are ignitable but fall into more than one class or do not meet all the criteria necessary to be included in a particular class.
   b. Bacterial or environmental degradation, some substrate interference, or lack of some characteristic points of correlation with a reference ignitable liquid TIC/EIPs may be factors contributing to this finding.
   c. It may require the comparison of several reference ignitable liquids in order to attempt to match the sample TIC/EIPs.

   Note: Use these features to limit your possibilities. Rule out some classes of ignitable liquids and focus on features of remaining possibilities.

E. When comparing questioned TIC/EIPs to references, compare peaks in the reference to peaks in the questioned TIC/EIPs.

   Make sure both are run under comparable chromatographic conditions!!!

F. Remember, it may be necessary to concentrate the sample to look at minor peaks or use a different program to clarify an area of interest (higher resolution).

G. When interpreting the TIC/EIPs of an item, the interpretation will be made on the results of the analysis of that item and on the analysis of any appropriate comparison samples and references.

H. The peaks used for identification in the EIPs may be masked by pyrolysis products or overlapping peaks. This is information that should be considered by the analyst.

I. The TIC/EIPs of a reference must accompany all positive identifications which do not meet the stated class criteria.

J. The 55 m/e ion profile is useful to differentiate a petroleum distillate from a polymer.

K. The presence of the alkyl-diene, alkene, and alkane triplet is indicative of a polymer found in substrates.

L. Compounds required for class identification are to be determined by retention time and mass spectral identification.

1. A reported result of "Terpenes found. Terpenes are found in turpentine as well as naturally occurring in some types of wood." will be supported by all of the following:
   a. Alpha-Pinene present with significant abundance.
   b. Beta-Pinene, camphene, OR limonene present.
   c. Alpha-Pinene and the terpene from (b.) above will be identified by GC retention time and mass spectral identification.

REFERENCES

1. Keto, R.O. "GC/MS Data Interpretation for Petroleum Distillate Identification in Contaminated Arson Debris"; Journal of Forensic Sciences, May 1995, 40, 3, 412-423.

2. ASTM E1618-19 "Standard Guide for Ignitable Liquid Residues In Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry" ASTM International, West Conshohocken, PA, 2019, www.astm.org.

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4. Wineman, P.L.; Keto, R.O. "Target Compound Method for the Analysis of Accelerant Residues in Fire Debris"; Analytica Chimica Acta, March 1994, 288, 1-2, 97-110.

5. Neuman, R., et al. GC-MS Guide to Ignitable Liquids; New York: CRC Press, 1998.

6. ASTM E2997-16, "Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography – Mass Spectrometry"; ASTM International; West Conshohochen, PA., 2016, www.astm.org.

7. ASTM E2881-18, “Standard Test Method for Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography – Mass Spectroscopy”; ASTM International; West Conshohocken, PA., 2018, www.astm.org.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fire Debris Analysis

Method: Volatiles Recovery

Procedure: Solvent Extraction

Reviewed by:

_____

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Based on preferential ignitable liquid solubility in solvent.

A. Advantages:

1. Provides a representative full range sample of any ignitable liquid present.
2. High recovery rates.
3. Can detect 0.5 micro liters of ignitable liquid in a one-quart can.

B. Disadvantages:

1. Requires large quantities of pure solvents.
2. Picks up many contaminants which may make chromatogram interpretation difficult.
3. Picks up products which may "clog" GC/MS column.
4. May dissolve some plastics and other components in the debris.
5. If you are not careful, the extract will evaporate to dryness.

C. This can be used for liquid extractions.

SAFETY CONSIDERATIONS

A. Perform solvent extractions in the fume hood.

B. Do not permit a heat source to be located in the fume hood while performing the solvent extraction technique.

C. Keep the quantity of solvent used to a minimum.

D. See also MT-I, "Protocol: Fire Debris Analysis, SPECIAL NOTES, II. SAFETY"

PREPARATIONS

A. Several different solvents can be used. The ones we generally use are:

1. Hexane
2. Pentane

3. Diethyl ether
4. Carbon Disulfide

B. Whatman #1 Filter paper (or equivalent)

C. Whatman #1PS Filter paper (or equivalent)

INSTRUMENTATION

A. Gas Chromatograph/Mass Spectrometer

B. Instrument parameters: See MT-I, "Protocol: Fire Debris Analysis, INSTRUMENT CONDITIONS"

MINIMUM STANDARDS & CONTROLS

A. See MT-I, "Protocol: Fire Debris Analysis, MINIMUM STANDARDS AND CONTROLS"

B. Solvents recommended for use are hexane, pentane, diethyl ether and carbon disulfide (CS_2).

C. Blank: If the sample extract is to be evaporated, the blank will be evaporated a like amount.

D. The solvent extract will be saved by placing it in a vial and adding clean charcoal. The vial will be returned with the evidence. The inability to save the extract will be recorded in the case file.

PROCEDURE OR ANALYSIS

A. Evaporative:

1. Half fill a beaker of suitable size (larger than 600 mL is rarely necessary) with all or a portion of the debris sample furnished. Place beaker and sample in a fume hood.

2. Add reagent-grade solvent. The amount needed will vary with the amount and kind of debris. A normal range is 20-100 mL. Use only enough solvent to permit thorough "wetting" of the debris and will provide (10-25 mL) of solvent extract. If the debris is coarse and rigid (e.g., charred wood), add

the solvent slowly, washing the debris in the process; then agitate by gentle swirling (approximately 30 seconds) to induce further solvent-debris contact. Let soak for 20 minutes. With flexible debris (e.g., carpet), use a second (one-size smaller) beaker like a plunger to promote extraction.

3. Filter (Whatman #1) the extract (if necessary), collecting the filtrate in a suitably small breaker or evaporating dish. If the filtrate is contaminated with water, phase separator paper (Whatman #1PS) can be used to separate the water from the solvent.

4. Concentrate sample by evaporation, without heating, to a volume appropriate for providing a chromatogram. Blank solvents must be evaporated to the same volume as the sample.

5. Inject an appropriate amount (1 microliter or less) of the sample into the GC/MS.

B. Non-Evaporative:

1. One or two milliliters of solvent is used to extract the condensate regularly present on the interior lid and walls of containers.

2. Extract is filtered (if necessary).

3. Inject an appropriate amount (1 microliter or less) of the sample into the GC/MS.

C. Liquid Samples:

1. If the neat liquid is thought to be aqueous (possibly indicated by a negative ignition test or a miscibility test), a liquid-liquid extraction can be performed using a suitable solvent. If necessary, the extract or a portion of the extract can be evaporated down to a suitable volume and analyzed using GC/MS.

D. The solvent extract will be saved by placing it in a vial and adding charcoal. The vial will be returned with the evidence. The inability to save the extract will be documented in the case file.

REPORT WORDING

A. See Appendix I.

REFERENCES

1. ASTM E1386-15, "Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction"; ASTM International, West Conshohocken, PA, 2015, www.astm.org.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fire Debris Analysis

Method: Volatiles Recovery

Procedure: Headspace

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Based on the vapor pressure of the components.

Vapor pressure is a function of temperature. Raising the temperature raises the vapor pressure.

The higher the vapor pressure in a closed system, the greater the amount of a liquid component going into the vapor phase.

The purpose of a heated headspace is to vaporize the volatile components into the air space above the debris and sample.

A. Advantages:

1. Speed

2. Convenience

3. Good detection levels, 5 to 10 microliters gasoline in a quart can.

B. Disadvantages:

1. Severely affected by water presence. May cause loss of sample while heating.

2. Produces a disproportionate representation of low boilers (highly volatile) in the headspace. May result in swamping of headspace with more volatile components of ignitable liquid producing an incomplete pattern OR ignitable liquid mixtures may be masked by the presence of only the most volatile components.

SAFETY CONSIDERATIONS

A. Use “Standard Laboratory Practices”.
B. See MT-I, “Protocol: Fire Debris Analysis, SPECIAL NOTES, II. SAFETY”

PREPARATIONS

A. Not Required.

INSTRUMENTATION

A. Gas Chromatograph/Mass Spectrometer

B. Instrument Parameters: see MT-I, “Protocol: Fire Debris Analysis, INSTRUMENT CONDITIONS”

Consider an isothermal temperature program or a ramped temperature program which begins at a lower temperature (i.e. 35 – 40˚C).

MINIMUM STANDARDS & CONTROLS

A. Ignitable Liquids (not Oxygenates)

1. See MT-I, "Protocol: Fire Debris Analysis, MINIMUM STANDARDS AND CONTROLS"

B. Oxygenates

1. Daily Function Check: An instrument function check will be run each day the instrument is used for casework. Headspace: a vapor injection of any of the listed oxygenates should result in a chromatogram whose oxygenate peak exceeds 10 times the noise level. Liquid: a liquid injection of any of the oxygenates should result in a chromatogram whose oxygenate peak exceeds 10 times the noise level. This function test will be placed in each case file.

2. A blank will be run if the preceding instrument run was positive for an oxygenate.

PROCEDURE OR ANALYSIS

A. Headspace for Ignitable Liquids (not Oxygenates)

1. Punch or drill a small (1/32-1/16 inch) hole in the lid of the sample container.

2. Cover the hole with adhesive tape.

3. Place container in oven preheated to 80-85°C. Leave it there for at least 10-20 minutes, but no longer than 30 minutes <u>if</u> the sample was found to be "<u>wet</u>" during a preliminary examination. (Reason: To minimize ignitable liquid loss by "steam distillation" through the vent-hole in the container lid. Venting is required, in the case of wet samples, to prevent "lid blowing" as a result of steam pressure.) "Dry" samples should remain in the oven for at least 30 minutes and may "safely" remain there for several hours.

4. After the container is removed from the oven, inject a **0.1cc** maximum headspace sample into the GC/MS. Use a <u>warm</u> syringe.

   WARNING: Not heating syringe will cause severe loss of sensitivity of higher boiling components (i.e., fuel oils 1 and 2). The higher boilers tend to condense in a cold syringe.

5. The presence of the homologous alkane peaks in the MPD region should be checked for an HPD. If an HPD is suspected, the enhancement of the alkane region by adsorption/elution or solvent extraction is suggested.

B. Headspace (Heated or Room Temperature) for Oxygenate Analysis

1. This procedure is to be used for qualitative identification purposes. The instrument parameters used should be adjusted to permit the gas chromatographic separation and mass spectral identification of the common oxygenates.

2. Boiling points of common oxygenates. These are recommended for use.

   A. Methanol 64.9°C

   B. Ethanol 78.5°C

   C. Acetone 56.2°C

   D. Iso-propanol 82.4°C

   E. N-Propanol 97.1°C

PROCEDURE OR ANALYSIS

1. Inject a sufficient amount of any of the above listed oxygenates into the GC/MS (see this procedure's Minimum Standards and Controls, B.1.). Adjust instrument parameters to obtain the best resolution.

2. Document these parameters in the case file.

3. A headspace of the reference is used for cases in which debris samples are being analyzed. A liquid sample of the reference is used for cases in which a liquid is being analyzed.

4. The procedure selected must provide confirmatory information before an identification is reported.

5. Punch or drill a small (1/32-1/16 inch) hole in the lid of the sample container.

6. Cover the hole with adhesive tape.

7. If needed, heat the can in an oven for a minimum of 5 minutes or longer. The amount of time for heating is dependent upon the amount of material and the moisture level inside the can.

8. After the container is removed from the oven (if heating was required), inject a 0.1cc maximum headspace sample into the GC/MS.

REPORT WORDING

A. See Appendix I.

REFERENCES

1. ASTM E1388- 17, "Standard Practice for Sampling of Headspace Vapors from Fire Debris Samples"; ASTM International, West Conshohocken, PA, 2017, www.astm.org.

2. Reeve, V. et al. "Developments in Arson Analysis: A Comparison of Charcoal Absorption and Direct Headspace Injection Techniques Using Fused Silica Capillary Gas Chromatography"; *Journal of Forensic Sciences*, 1986, 31, 2, 479-488.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fire Debris Analysis

Method: Volatiles Recovery

Procedure: Adsorption Elution (Dynamic/Active)

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Adsorption-Elution is actually a total headspace sampling. It relies on volatility of the ignitable liquid. Volatilized ignitable liquid is removed by vacuum and trapped on an in-line charcoal tube. Ignitable liquid is removed from the tube and analyzed. This is also referred to as the "Purge and Trap Method."

The activated charcoal is coconut-based. The carbon makes up 95-98% of the activated granules. The remainder (3-5 weight percent) consists of inorganic oxides ("ash"). The 50-200 mesh charcoal is a more efficient adsorbent than the 6-14 mesh when adequate flow rate (0.1 - 1.0 liters/min.) is used.

A. Advantages:

1. Speed
2. Convenience
3. Provides a representative sample
4. Provides the analyst with a liquid sample which allows further analysis by other techniques (IR., etc.).
5. Can be automated.
6. Not severely affected by the presence of water.
7. Can detect less than 1 microliter of gasoline or other ignitable liquids in 1 quart can.

B. Disadvantages:

1. Pyrolysis products may become more obvious due to concentration.
2. Charcoal tube system may be subject to contamination by any hydrocarbons in the laboratory air. (This can be remedied by the analyst by appropriate means.)
3. Very concentrated samples can exhibit selective adsorption of the components.

SAFETY CONSIDERATIONS

A. Perform the CS_2 elution in an operating fume hood.

B. Warning: Carbon Disulfide is flammable. Use in Fume Hood.

C. See MT-I, "Protocol: Fire Debris Analysis, SPECIAL NOTES, II. SAFETY".

PREPARATIONS

A. Materials:

1. Disposable glass Pasteur pipets, 5-3/4 inches long (e.g., Kimble No. 72000).

2. Activated coconut charcoal, 50-200 mesh (e.g., Fisher No. 5-690). The charcoal will be heated to remove contaminants prior to use.

   a. Remove a small portion of charcoal from its original shipping container.
   b. Using a heat source of at least 300°C, heat the charcoal for a minimum of 2 hours.
   c. Cool the charcoal.
   d. Remove layer of ash which forms at the surface.
   e. Store charcoal in desiccator.

   Note: If a system blank using this treated charcoal shows a significant contamination, this process should be repeated.

3. Absorbent, "first-aid" type cotton.

4. Cotton swabs or applicator sticks.

B. Solvents

1. Carbon Disulfide (CS_2).

INSTRUMENTATION

A. Gas Chromatograph/Mass Spectrometer

B. Instrument Parameters: See MT-I, "Protocol: Fire Debris Analysis, INSTRUMENT CONDITIONS"

MINIMUM STANDARDS & CONTROLS

A. See MT-I, "Protocol: Fire Debris Analysis, MINIMUM STANDARDS AND CONTROLS"

B. Blank: Each day a charcoal adsorption is used, a whole system blank will be run. The charcoal tube will be eluted, and the solvent evaporated by the same method used in the case samples. This system blank will be analyzed using the same GC/MS conditions as the case sample.

C. A new charcoal inlet tube will be used for every item.

D. When charcoal adsorption is performed, the inlet tube should be checked for proper air flow. An example would be to use a Rotameter with a flow rate of 0.1 to 1 liter per minute or the flow rate determined by the laboratory.

E. If water is present in an item, do not remove prior to performing the charcoal adsorption.

F. Do not permit wet samples to exceed 100°C during charcoal adsorption.

G. The CS_2 extract and charcoal from each item will be saved by placing them together in a vial. The vial will be returned with the evidence. The inability to save the extract will be recorded in the case file. (Note: Clean charcoal can be added to the vial in place of the charcoal used to extract the sample.)

H. The cleanliness of each new batch of charcoal must be verified prior to use in casework and the data from this verification must be kept in LAM. To check the cleanliness of the new batch of charcoal, use the charcoal in a system blank, concentrate the extract and inject 1uL of the extract into the GC/MS. Ensure no interfering peaks are present. If any interfering peaks are seen, repeat the steps in "Preparations" above to clean the charcoal and redo the system blank.

PROCEDURE OR ANALYSIS

A. Preparation of Sample Tubes:

1. Using an appropriate tool, insert a piece of cotton into a Pasteur pipet until it begins to enter the tip, then tamp lightly with a cotton swab or applicator stick. A suitable quantity of cotton forms a plug approximately 1/4-3/8 inch long.

2. Using a short length of flexible tubing, connect the stem of a small funnel to the mouth of the pipet. Load the pipet with charcoal, introduced through the funnel, to a depth of approximately 25 mm. More charcoal (50 mm maximum) can be added for very concentrated samples.

3. After removing the funnel and connector from the pipet, introduce a second piece of cotton and tamp it lightly with a cotton swab or applicator stick into place against the top of the charcoal column.

4. Label one of the tubes with the case number and item number. This tube will be your outlet tube. In order to later differentiate between the tubes if needed, do not mark the inlet tube.

5. The tube is now ready for use. If not to be used immediately, or if a "stock" of tubes is being assembled, storage in a clean vapor-tight container is strongly advised.

B. Sampling Debris:

1. Place sample in paint can and seal.

2. Preheat oven at 90° ± 5° C. Caution: Avoid sample temperature above 100 C to minimize stripping of "light" components of the sample from the charcoal.

3. Use a nail or punch and puncture top of can with two holes. Enlarge the holes with a punch.

4. Place plastic electrical tape or equivalent over holes and puncture with nail or punch. Note: This tape may give a styrene peak on the chromatogram.

5. Place a charcoal tube in each of the taped holes. Make sure the outlet tube is labeled with the case number and item number.

6. Place can in oven. Connect the vacuum tube to the outlet charcoal tube. Since the vacuum tube extends into the oven, a thick wall vacuum tube must be used. Connect the inlet tube to the inlet charcoal tube.

7. Turn on vacuum pump and draw sample through charcoal for 20 minutes (may be less if high concentration is present). The time may need to be extended if high water content or a large amount of debris is present.

8. Check vacuum of inlet tube with a rotameter or equivalent. If the flow becomes minimal or seizes, replace the tubes.

9. If the item is to be checked for latent prints, sampling can be performed at room temperature. When this is done, minimally extend the run an additional 10 to 15 minutes.

C. Elution of Sample Tube:

1. Place one end of outlet charcoal tube in a small glass vial. Stand tube and vial in vertical position in fume hood.

2. Using a suitably small syringe or pipet, add to the upper end of the charcoal tube a sufficient amount of reagent-grade carbon disulfide to wet charcoal. Permit CS_2 to elute for at least 5 minutes.

3. Use a rubber bulb to force CS_2 from tube. Collect the CS_2 extract. (If this highly volatile extract cannot be chromatographed within minutes, it should be stored in a refrigerator or freezer.)

4. If necessary, concentrate the CS_2 extract to an appropriate volume by evaporation using a stream of dry, pure air or air dry in fume hood.

5. Inject an appropriate amount (i.e., 1 µL or less) of sample into the GC/MS.

6. The CS_2 extract and charcoal from each item will be saved by placing them together in a vial. The vial will be returned with the evidence. The inability to save the extract will be documented in the case file. (Note: Clean charcoal can be added to the vial in place of the charcoal used to extract the sample.)

REPORT WORDING

See Appendix I.

REFERENCES

1. ASTM E1413-19, "Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Dynamic Headspace Concentration onto an Adsorbent Tube", ASTM, International, West Conshohocken, PA, 2019, www.astm.org.

2. Sandercock, P.M.L. “Comparison of Passive Charcoal Adsorption with Dynamic Charcoal Adsorption Technique”; *Can. Soc. Forensic Sci. J*., 1994, 27, 3, 179-201.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fire Debris Analysis

Method: Volatiles Recovery

Procedure: Adsorption Elution (Static/Passive)

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Passive adsorption elution sampling is a total headspace sampling. It removes small amounts of ignitable liquid residues from samples of fire debris using activated charcoal to extract residue from the static headspace above the sample then elutes the adsorbent with a solvent.

A. Advantages:

1. Speed with multiple samples.

2. Convenience. Does not require the use of a vacuum pump and can be performed at room temperature.

3. Provides representative sample.

4. Not severely affected by the presence of water.

5. Capable of isolating amounts smaller than one microliter of gasoline or other ignitable liquids.

6. Chromatograms closely resemble neat sample chromatograms.

B. Disadvantages:

1. Pyrolysis products of substrates are also adsorbed.

2. May not detect naphthalenes as well as active adsorption elution.

SAFETY CONSIDERATIONS

A. See MT-I, "Protocol: Fire Debris Analysis, SPECIAL NOTES, II. SAFETY"

B. Warning: Carbon Disulfide is flammable. Use in Fume Hood.

PREPARATIONS

A. Materials:

1. Commercially available polymer strips of activated charcoal.

2. Oven minimum temperature range: 40° to 150°C.

3. String or wire (eg. paper clip).

4. Vials for strips

B. Solvents

1. Carbon Disulfide (CS_2)

INSTRUMENTATION

A. Gas Chromatograph/Mass Spectrometer
B. Instrument Parameters: See MT-I, "Protocol: Fire Debris Analysis, INSTRUMENT CONDITIONS"

MINIMUM STANDARDS & CONTROLS

A. See MT-I, "Protocol: Fire Debris Analysis, MINIMUM STANDARDS AND CONTROLS"

PROCEDURE OR ANALYSIS

A. Procedure for the use of charcoal strips

1. Use commercially available polymer strips of activated charcoal.

2. Strips may be cut in equal segments (greater than 10 mm) and still provide high adsorption efficiency.

3. Attach string or wire (e.g. paper clip) to strip and suspend in evidence container.

4. Allow the sample container with strip to set at room temperature overnight and then heat it in the oven (to a temperature of 70° to 90° C) for 1/2 hour to 2 hours or place in oven at 90° ± 5°C for 2 hours without the overnight requirement.

5. Let the sample container cool to room temperature.

6. Remove the strip from the evidence container and place in sample vial. Place a sufficient amount (wet charcoal) of carbon disulfide in the vial for 5 minutes to elute sample.

7. Allow the carbon disulfide to evaporate to a suitable volume.

8. Inject appropriate amount (e.g. 0.3 µL to 1.5 µL) into the GC/MS.

9. When analysis is completed place vial containing strip and eluent into original container. The vial will be returned with the evidence. The inability to save the eluent will be noted in the case file.

B. Notes and Cautions

1. Be sure hands are free of volatile organics when handling activated charcoal strips.

2. Don't heat-seal plastic bag containers with charcoal strips inside.

3. It's important to monitor the oven while the cans are in the oven. If a number of cans are put in the oven at the same time, you will notice the oven temperature will have dropped while loading the oven. Depending on the mass of the cans and contents it may take some time for the oven to return to the temperature desired.

4. If you have a strong sample (you can smell the ignitable liquid without opening the container), shorter room temperature adsorption times and shorter heating times will eliminate the chance of cross-contamination of charcoal strips in containers placed together in confined places like ovens and drawers where there is little air movement to sweep the odors away from the containers.

5. Wire used to suspend charcoal strip should be cleaned with appropriate solvent (e.g. CS_2) prior to use.

6. If the situation warrants a low temperature extraction, such as a DNA request, containers may be sampled at room temperature or heated in a 60-65° oven for 16 hours.

REPORT WORDING

A. See Appendix I.

REFERENCES

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Paint Analysis

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The challenge and responsibility of a Forensic Scientist performing a paint comparison is to find a significant difference, if it exists, of the paints being compared. Without question, paint chips will have differences. The goal of the Forensic Scientist is to demonstrate or find which of these differences, if any, are exclusionary. The absence of an exclusionary difference at the conclusion of an analysis constitutes or suggests similarity between the compared samples in all tested characteristics. The strength, or degree, of this similarity will depend on a number of factors, including the following: the type and number of matching physical characteristics; the type and relative abundance of components in the paint film; the presence or absence of studies quantifying the uniqueness of these components; and the discriminative power of the tests performed.

Limited sample size and sample preservation requirements dictate that the comparative tests must be selected and applied in a reasonable sequence to maximize the discriminating power of the test results.

Polymers are a common component of paint systems; therefore, the analytical scheme used for paint comparisons explained in this and subsequent procedures can be applied to polymer comparisons. Ensure all minimum standards and controls for paint are followed.

PAINT TERMINOLOGY

Additive (Modifier):	Any substance added in a small quantity to improve properties. Additives can include substances such as driers, corrosion inhibitors, catalysts, ultraviolet absorbers, plasticizers, etc.
Binder:	Non-volatile portion of a paint that forms the film and binds the pigment particles to one another and to the substrate. Most paint binders encountered in forensic cases are polymer based.
Migration:	A defect in which pigment from a lower coat of paint diffuses into an upper coat and discolors the latter.
Blistering (Bubbling):	The occurrence of air or solvent vapors as small bubbles in a paint film.
Blooming:	A powder-like deposit which sometimes forms on the surface of a paint film.
Coating:	A generic term for paint, lacquer, enamel, or other liquid or liquefiable material which is converted to a solid, protective decorative, or combination thereof, film after application.

Chalking:	The degradation of a paint film by gradual erosion of the binder. White powdery formation on surfaces of exterior paints.
Cracking:	Splitting of a paint film. Usually occurs as straight lines which penetrate the entire film thickness.
Checking:	Hairline cracks in the coating.
Cratering (Fisheyes):	Small round depressions in a paint film which may or may not expose the underlying surface.
Crazing:	The formation of surface cracks, often as a fine network, which do not penetrate to the underlying surface. Crazing is sometimes caused by the softening effect of solvents from successive paint coats.
Discriminate:	To distinguish between two samples based on meaningful differences; to differentiate.
Dispersion Coatings:	A type of paint in which the binder molecules are present as colloidal particles.
Drier:	A material that promotes or accelerates the drying, curing, or hardening of oxidizable coating vehicles. The principal driers are metal soaps of a monocarboxylic acid.
Drying Oils:	Naturally occurring triglycerides which form films principally by air oxidation. The same oils may be used as feedstocks for varnishes, alkyd resins, epoxy ester resins, oil modified urethane resins, and some plasticizers.
Enamel:	The term enamel does not intimate the chemical nature of the coating but implies a pigmented coating which dries to a hard gloss. Increasingly, the term has come to mean a cross-linked thermosetting resin.
Exclusionary Difference:	A difference in one or more characteristics between compared items that is sufficient to determine that the compared items did not originate from the same source, are not the same substance, or do not share the same composition or classification.
Extender:	A low cost white inorganic pigment used with other white pigments to modify the gloss, texture, viscosity, and other properties, and to reduce the cost of the finished product.
Face Rusting:	The appearance of rust on an apparently unblemished painted surface.

Floating (Mottle):	The tendency of pigment particles in a wet paint film to separate from one another and concentrate in particular areas.
Flooding:	Incomplete color pigment dispersion in the base paint. Produces a streaked, non-uniform color when applied.
Hammertone Finish:	A paint finish which makes the surface look as though it has been peened.
Hash Marks:	A defect of electrodeposition finishes resembling a series of parallel ripples across the part surface.
Lacquer:	Fast-drying coatings, clear or pigmented, that dry by evaporation of the solvent rather than by oxidation or polymerization.
Latex:	A suspension of a pigment in a water-based emulsion of any of several resins, for example, acrylic polymers, vinyl polymers, or styrene-butadiene polymers.
Meaningful difference:	A feature or property of a sample that does not fall within the variation exhibited by the comparison sample, considering the limitations of the sample or technique, and therefore indicates the two samples do not share a common origin. The use of this term does not imply the formal application of statistics.
Metallic Paint:	Paint which contains reflective metallic particles, usually in the form of tiny flakes.
Orange Peel:	An irregularity in the surface of a paint film resulting from the inability of the wet film to "level out" after being applied. Orange peel occurs as a characteristically uneven or grainy surface to the eye, but usually feels smooth to the touch.
Paint:	A material, which when applied as a liquid to a surface, forms a solid film for the purpose of decoration and/or protection. Generally, a paint contains a binder, solvent, and pigment. Commonly known as a pigmented coating.
Paint Standard:	Paint which is removed directly from a known source (for example: near the damaged area of a window frame or door area and a suspect's or victim's vehicle.) It is not transfer paint or a paint smear. It is paint that is thought to have been on the object prior to the incident.
Pigment:	A finely powdered solid which imparts a color and is essentially insoluble in the medium in which it is dispersed. Pigments may be inorganic, such as titanium

	dioxide, or organic, such as phthalocyanine. Besides color, a pigment can provide many of the essential properties of paint, such as opacity, hardness, durability, and corrosion resistance. White pigments are primarily intended to hide the underlying surface. A pigment is distinguished from a dye in that a dye is soluble in the vehicle while a pigment is not. The term pigment includes extenders.
Plasticizer:	A material incorporated into a polymer to increase its flexibility or workability.
Polymer:	A polymer is a substance that contains a molecular structure that mainly contains a large number of similar units. This includes synthetic organic materials such as resins and plastics.
Popping:	A paint defect characterized by raised bumps in the surface. Caused by solvent vapor forming within the paint after it has begun to "skin-over."
Primer:	A type of paint applied to a surface to increase its compatibility for the top coat or to improve the corrosion resistance of the substrate.
Reflective Paint:	Paint which contains reflective particles, usually in the form of tiny flakes. The flakes can be composed of a metal like aluminum.
Sagging:	The downward flow of a paint film as a result of its being applied as too heavy or too fluid a wet coat.
Sealer:	A coating used to increase the compatibility of a prime coat for a top coat.
Smear:	A transfer of paint resulting from contact between two objects; these transfers can consist of co-mingled particles from two or more sources, fragments, or contributions.
Solvent:	Organic liquids of various types having the function of dissolving the binder and thereby providing a consistency to the coating which is more suitable for application.
Stain:	A solution of a dye or a suspension of a pigment in a vehicle designed to impart a color to a wood surface rather than to form a protective coating.
Thermoplastic Polymer:	A resin which polymerizes without the necessity of heat. If the resin is heated below its decomposition temperature, it softens and hardens again upon cooling; hence, the term "thermoplastic."
Thermosetting Polymer:	A resin which can be made to form cross-linkages when baked.

Varnish: A homogeneous solution of drying oils and resins in organic solvents. The resins may be naturally occurring (for example, rosin or dammar), or synthetic (for example, products of phenol and formaldehyde).

Vehicle: The portion of a surface coating other than the pigment, the purpose of which is to enable the pigment to be distributed over the surface. The vehicle includes solvents, binders, and other additives. In general discussion, the term vehicle is frequently used to indicate the oil or resin which forms a continuous film and binds the pigment to the substrate.

Wrinkling: A paint defect in which the film surface skins over and then absorbs liquid within the film. The absorption results in swelling of the surface skin with the formation of a wrinkled texture.

MINIMUM STANDARDS & CONTROLS

1. Minimum Standards of Analysis with Adequate Samples:

   Microscopic examination of color, texture, layer sequence, pyrolysis gas chromatography/mass spectrometry, and elemental analysis (SEM-energy dispersive x-ray analysis). Other tests that can be used in conjunction with the above examinations are chemical reactivity tests and infrared spectroscopy.

   A physical match of paint and polymer samples provides the highest degree of association and should be assessed first. If these initial tests are inconclusive or not exclusionary, the examination proceeds with the selection of additional tests based on their potential for use in evaluating or discriminating among the samples of interest. Once an exclusionary difference is found between the known and unknown samples in any of the examinations, the analysis can end, and a report can be written.

2. A conclusion regarding a paint comparison can be rendered if one or more of the following exists:

   A. A physical match.
   B. Comparison of the physical characteristics, organic and inorganic components.
   C. A comparison of any physical or chemical property that results in an elimination.
   D. The observation or determination of a unique quality possessed by the comparison samples.

3. Documentation

A. All instrument copies generated from case items must contain the case number, item number, analyst's initials and date.
B. Pertinent instrument parameters used for an analysis must be documented in the procedures manual or a digital copy placed in the case file.
C. All system performance check data must be documented and contain the analyst's initials and date.
D. Worksheets will include the tests performed and the results obtained.
E. Identification or classification made by comparison to other than in-house reference collection must be noted in the case file.
F. Identification or classification made via computer search must be confirmed by visual comparison to computer's reference and noted in the case file.

4. Analytical Techniques

A. Pyrolysis Gas Chromatography/Mass Spectrometry (PGC/MS) See the Minimum Standards and Controls found in the Gas Chromatography/Mass Spectrometry method (MT-IXC) and the Minimum Standards and Controls found in the Paint Pyrolysis Gas Chromatography/Mass Spectrometry method (MT-IIB-4)

B. Fourier Transform Infrared Spectrometer (FTIR) See the Minimum Standard and Controls found in the Fourier Transform Infrared Spectroscopy method (MT-IXA)

C. Scanning Electron Microscope with Energy Dispersive X-Ray System (SEM/EDS) See the Minimum Standards and Controls found in the Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy method (MT-IXB)

PROCEDURE OR ANALYSIS

1. Paint, PDQ, or physical match analyses are not well known or understood by many user agencies. Therefore, prior to beginning any analysis, review any agency notes or information in LIMS. Determine if the request is probative and see if another analysis might be better than what was originally requested. If necessary, contact the agency for further information or to discuss the case.
   A. An example of when a conversation with the agency may be beneficial
      1.) A paint comparison was requested on a recovered vehicle part from the scene to paint standards collected off the suspect's vehicle. In this case, a call should be made to the agency to determine if they are still in possession of the suspect vehicle. If they are, it should be determined if a physical match analysis is possible instead of a paint comparison. If so, have the agency submit the additional evidence for a possible physical match analysis.

2. Paint Recovery
   A. See the "Intro" section at the beginning of the Micro/Trace Procedures Manual for

general collection guidelines.

B. Describe the evidence and note the condition (e.g., smear or damage presence and location, smear or particle color, stains, apparent biological material, if the item has been cut).
C. Conduct a thorough inspection of the item for any visible paint particles/deposits. Collect these in a small metal container, a paper fold, or or between 2 glass slides taped together (one containing a cavity well).
D. If a paint smear is observed, it should be closely examined using a stereomicroscope. The area containing the smear can be cut out if needed.
E. After collecting any noticeable paint particles/smears/deposits, the item should be scraped by holding the item over a clean sheet of paper using a clean spatula. Collect as much debris as possible in a small metal container. Examine contents under a stereoscope.
F. Tape should not be used to collect any paint evidence. The adhesive from the tape can interfere with paint analysis.
G. Plastic containers should not be used to store any potential paint evidence. Plastic evidence containers (bags, boxes, etc.) can build up static which can cause paint chips to adhere to the surface or fly off and be lost.

3. Vehicle Parts

A. Vehicle parts are common evidence submitted for paint or PDQ analysis
B. Parts submitted as a standard or known sample
   1.) Examine and record all paint layers along with the type of substrate present
   2.) Record where on the vehicle the part was taken, if known
C. Parts submitted as a questioned or unknown sample
   1.) Examine and record all paint layers along with the type of substrate present
   2.) If the substrate is different than the substrate in the known paint sample, stop the analysis and request additional standards from the submitting agency.
   3.) The vehicle part should be examined for any identifying markings that may indicate the make or model of the vehicle from which the part came. Any identifying markings should be notated in the notes and the report. If possible, a picture of the identifying marks should be included in the casefile.
D. If a part from a known vehicle is submitted with a questioned paint smear, the paint underneath the smear or from another area of the part can be used as a standard to compare to another item if necessary. This can be done only if the paint layers are intact, and the quality of the paint is sufficient.

SAFETY CONSIDERATIONS

1. This protocol involves the use of dangerous chemicals, temperatures, or radiation sources. This protocol does not purport to address all the possible safety hazards or precautions

associated with their application. It is the responsibility of the analyst prior to use to review and implement appropriate safety and health practices.
2. The SDS must be consulted unless the analyst is already aware of the hazards associated with the chemicals to be used.

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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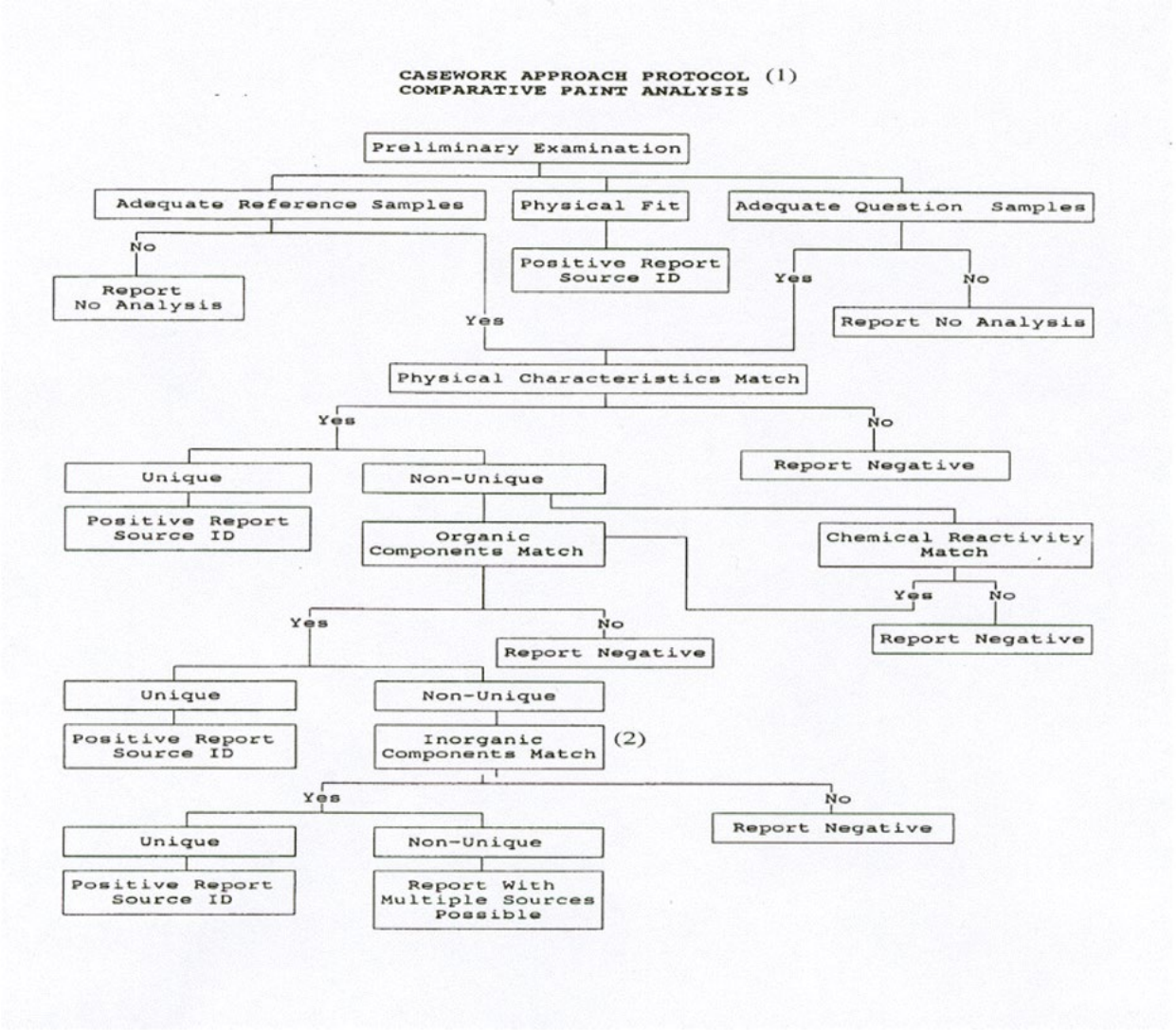
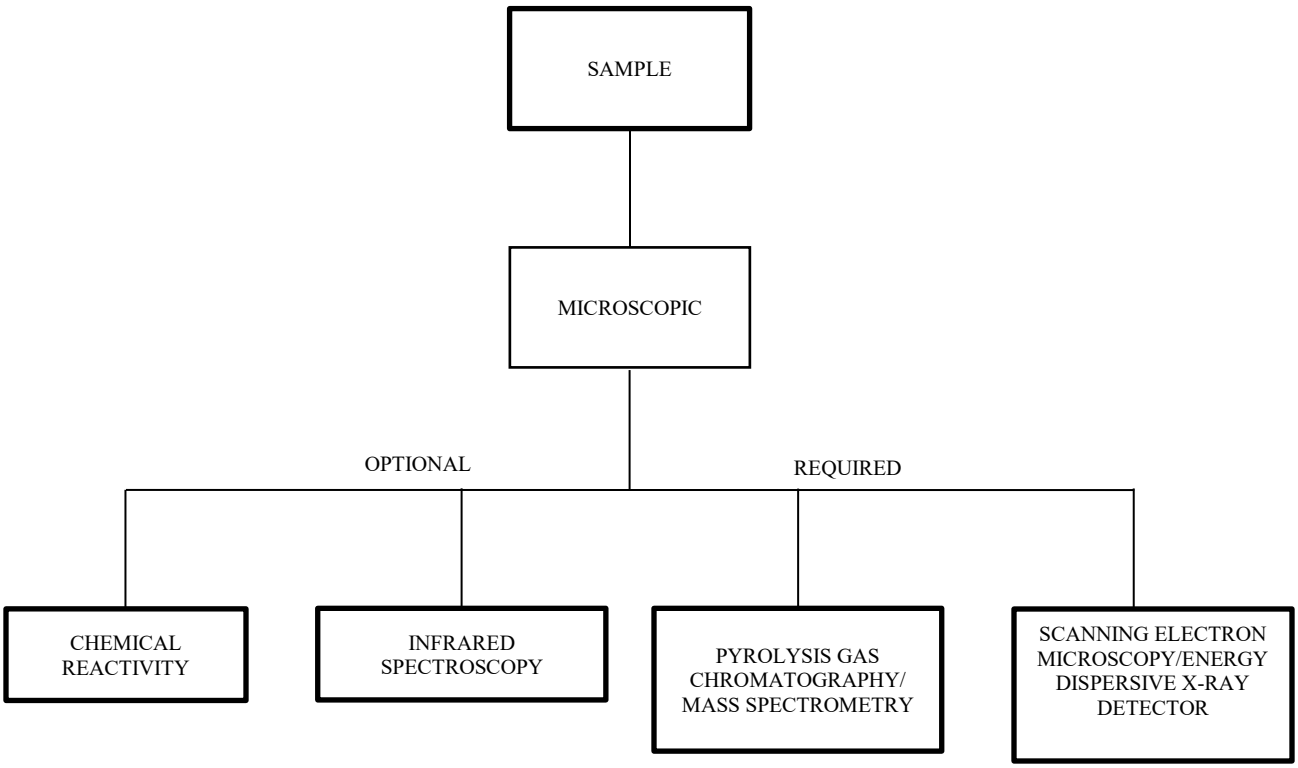


Note:

(1) This is a sequence of tests and doesn't imply the use of the same chip.
(2) The inorganic comparison may be performed before the organic.

ANALYTICAL FLOW CHART



ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Paint Analysis

Method: Examination Procedures

Procedure: Microscopic Examination

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

_____

Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The initial examination of paint with the microscope can provide important information about the physical characteristics of paint chips. The observed qualities would include, but are not limited to, the color, layer sequence, thickness, uniformity of the layers, striae, surface texture and features, contaminates and inclusions. This examination could also reveal common edges when two chips are compared.

SAFETY CONSIDERATIONS

See Protocol "Safety Considerations"

PREPARATIONS

A. Fine-tip tweezers
B. Fine-pointed needles or probe
C. Modeling Clay
D. Clean Paper (White, Black)
E. Scalpel
F. Spot Plate
G. Distilled Water
H. LR white resin, accelerator, embedding tray and block holders

INSTRUMENTATION

A. Stereomicroscope, preferably with dual fiber optics illumination.
B. Compound Microscope or Comparison Microscope
C. Microtome

MINIMUM STANDARDS & CONTROLS

See Protocol "Minimum Standards & Controls"

PROCEDURE OR ANALYSIS

A. Initially examine each item separately and record observations.
B. Place paint on a clean sheet of paper.
C. Place paper under the stereomicroscope and examine with magnification range of 7X to 60X.
D. Examine the paint chips to determine the number of layers, the color of the layers, the layer sequence, the texture of the paint, and the thickness of the layers. Also note the quality of the paint and any unusual features. A comparison of edges from standard and unknown may reveal a physical match.

E. The examination of the layer sequence and thickness may be enhanced by placing the chip in a spot plate, submerging the chip in water and examining it with a compound or comparison microscope under 40x to 100x magnification.
F. Record your observations.
G. Paint chips may be manipulated with tweezers or the fine point of a needle. Modeling clay may also be used to secure paint chips while examining their characteristics.
H. Microtome can be used to provide paint chip cross-sections for microscopic examination.
I. Paint chips can be separately embedded in LR white resin for microtoming.

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

PROTOCOL: Paint Analysis

METHOD: Analytical Techniques

PROCEDURE: Chemical Reactivity

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

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Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

A. Chemical reactivity tests are a destructive test and should only be used when adequate comparison samples are available. The primary use of this test is for exclusionary reactions between the questioned and known paint samples.

B. Three different chemical solvents can be used to examine paints. These solvents and the reactions expected with them are listed below.

CHEMICAL	PAINT	REACTION
Methyl Ethyl Ketone (MEK)	Acrylic Lacquer	Soluble
	Acrylic Enamel	Insoluble
	Nitrocellulose	Soluble
Chloroform	Acrylic Lacquer	Soluble
	Acrylic Enamel	Insoluble
	Nitrocellulose	Insoluble
Acetone	Acrylic Lacquer	Soluble
	Acrylic Enamel	Insoluble
	Nitrocellulose	Soluble

SAFETY CONSIDERATIONS

A. See Protocol "Safety Considerations"
B. Hazard Identification

Chemical	Health	Flammability	Reactivity
Chloroform	2	0	0
Acetone	1	3	0
Methyl Ethyl Ketone	1	3	0

PREPARATIONS

A. Methyl Ethyl Ketone (MEK): Reagent Grade
B. Chloroform: Reagent Grade

C. Acetone: Reagent Grade

D. Spot Plates
E. Stereomicroscope
F. Glass rod or spatula

INSTRUMENTATION

No Instrumentation Required.

MINIMUM STANDARDS & CONTROLS

A. See Protocol "Minimum Standards & Controls".
B. Worksheet will indicate all chemical tests performed and results.

PROCEDURE OR ANALYSIS

A. Wash surface of soiled chips.

1. If the paint chip is still soiled, put a small amount of detergent (e.g., alconox) and water into a beaker.

2. Dip a Kimwipe or cotton swab into the detergent solution and rub it across the painted surface.
3. Take another Kimwipe or cotton swab, add distilled water to it and rinse the painted surface.
4. Dry the paint surface with a third Kimwipe.

B. Place known and unknown paint chips in a spot plate next to each other to allow for their simultaneous observations.

C. Apply solvent directly onto each chip. Use one to two drops. When necessary, samples are submerged with a glass rod or spatula.

D. The effect of each solvent on the individual layers of one chip is compared to that on the corresponding layers of the other chip.

E. Observe and record the following qualities which apply.

1. Soluble
2. Partially soluble
3. Pigment leach
4. Swell

5. Curl
6. Discolor
7. Soften
8. Layer separation
9. Gas bubble formation
10. Other reactions
11. No reaction

F. The reactions given by each corresponding layer must be identical. Reactions should be observed immediately, (1) and (3) minutes after solvent is applied.

G. Proceed with other solvents as sample quantity permits.

H. Be sure to retain sufficient sample for instrumental analysis.

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Paint Analysis

Method: Analytical Techniques

Procedure: Fourier Transform Infrared Spectrometer (FTIR) Analysis

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Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

FTIR can provide some important information related to the chemical composition of the paint being examined. This information can be used in the classification of the paint film, identification of pigments, and/or a means of finding significant differences in the composition of the unknown as compared to the standard.

This technique is non-destructive and samples less than 25 microns can be analyzed with the FTIR Microscope.

SAFETY CONSIDERATIONS

A. Do not look directly into the laser.
B. If the FTIR Microscope is used, use personal protective equipment to cover eyes and skin while working with liquid nitrogen.

PREPARATIONS

A. Compression cell with Diamond windows and salt plates.
B. 2 mm KBr and/or NaCl Disks (plates).
C. Fine-tip tweezers.
D. Fine-pointed needles or probe.
E. Scalpel.
F. Liquid Nitrogen
G. Polystyrene Reference
H. Microtome
I. LR white resin, accelerator, embedding tray and block holders

INSTRUMENTATION

A. Stereomicroscope
B. Fourier Transform Infrared Spectrometer (FTIR). A microscope accessory is preferred.
C. FTIR Collection Parameters

1. Parameters must be adjusted to provide sufficient intensity and resolution for spectra comparison or classification.

2. The use of the microscope will require:
   a. Change in scan speed.
   b. Use of external mirror.
   c. Use of MCT Detector.
   d. Increase number of scans.

e. Proper adjustment of apertures with the same setting for sample and background collection.

MINIMUM STANDARDS & CONTROLS

See Method "Fourier Transform Infrared Spectroscopy" (MT-IXA)

PROCEDURE OR ANALYSIS

A. Samples must be prepared so the thickness is between 3 to 15 microns for transmittance operation.

B. Samples may be prepared by:

1. If a sample has multiple layers, sample and analyze each layer separately if possible.
2. Use a scalpel to remove individual paint films or slice a thick paint film.
3. Use a glass slide as a guide and cut a thin cross section from a paint chip.
4. Compress the paint film between two salt plates in a compression cell or diamond windows in the compression cell.
5. Use a microtome for cross section

C. Place sample on a salt plate. It may be retained in the compression cell during analysis.

D. Set instrument parameters.

E. Run polystyrene reference (see Method "Fourier Transform Infrared Spectroscopy" (MT-IXA) for acceptance criteria).

F. Place sample on microscope stage and adjust apertures to area of interest.

G. Run sample.

H. Move off sample and run background. The sequence of steps G & H is instrument dependent.

I. See Figures 1 and 2 for classification.

J. Documentation must meet the Minimum Standards and Controls requirements in Paint Analysis (MT-II) and Fourier Transform Infrared Spectroscopy" (MT-IXA).

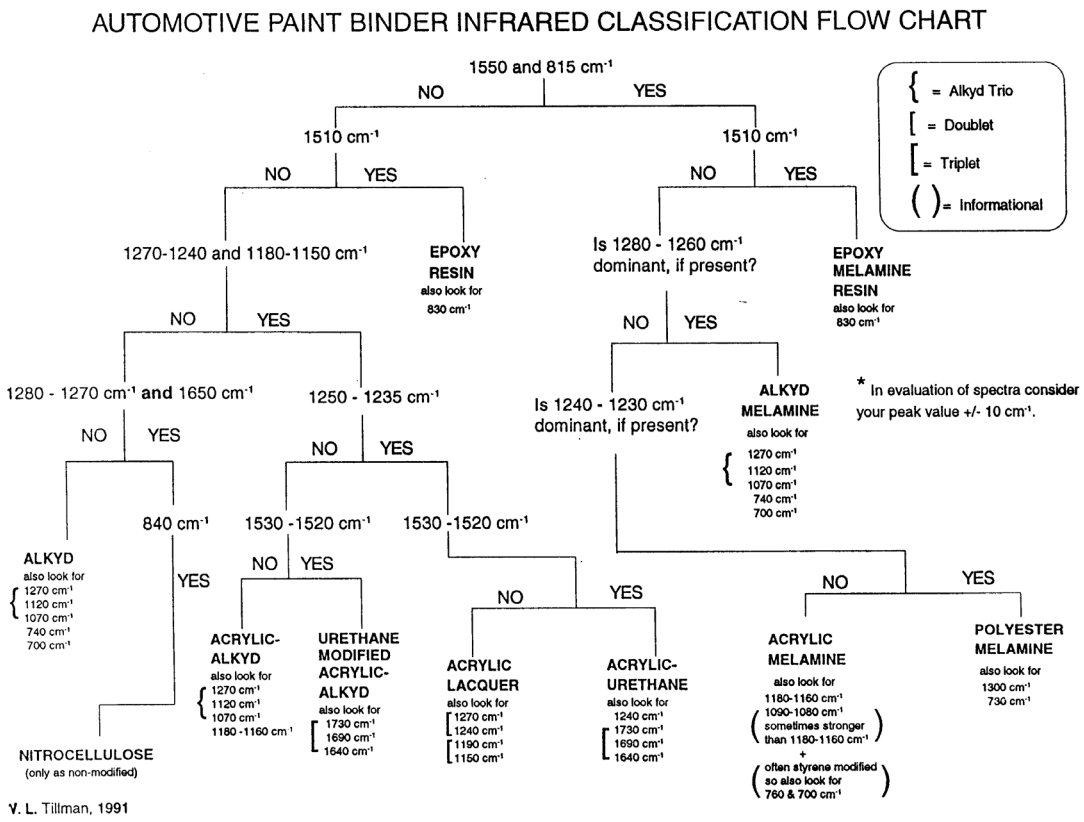
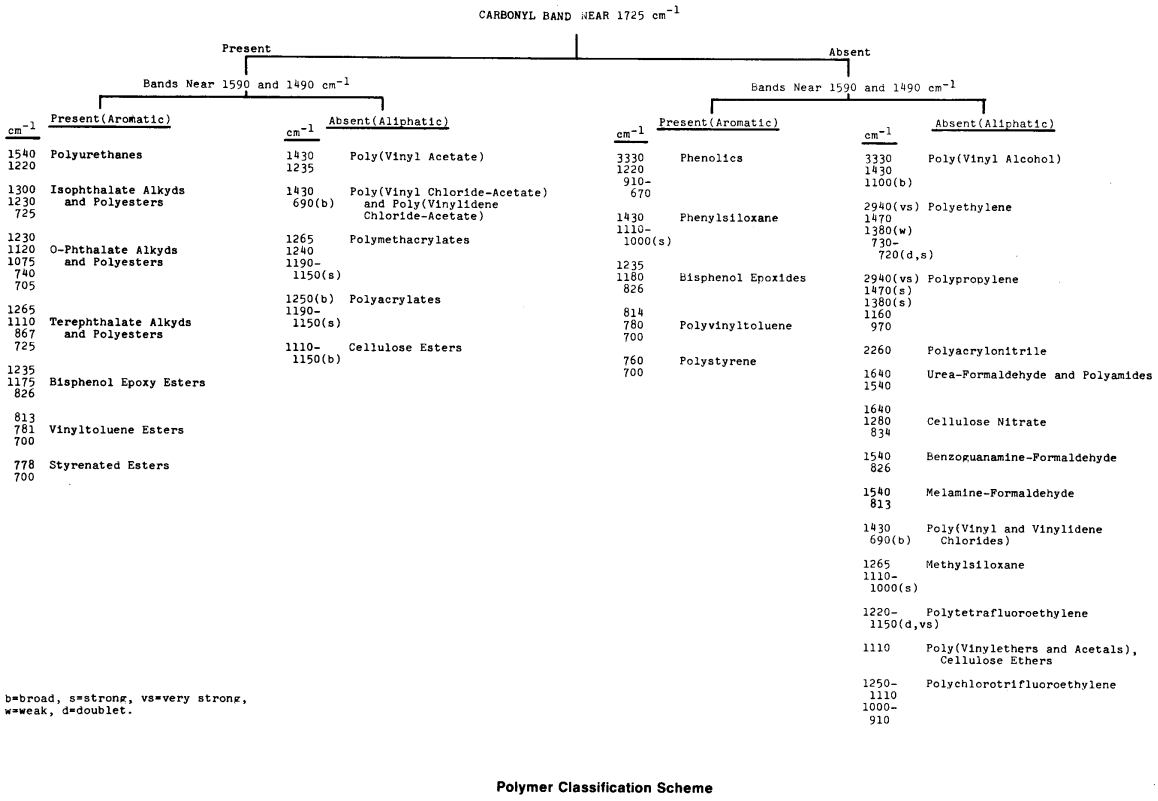


FIG. 1



Polymer Classification Scheme

FIG. 2

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Paint Analysis

Method: Analytical Techniques

Procedure: Scanning Electron Microscope/Energy Dispersive X-Ray

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Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The Scanning Electron Microscope/Energy Dispersive X-Ray Technique serves multiple purposes in paint and polymer analysis. It can provide layer structure information, insight into the texture of the layer (surface topography, distribution of inclusions), and element identification The SEM serves as a dual role of providing the energy to generate x-rays and isolating small regions for analysis. The Energy Dispersive X-Ray system collects and processes the x-rays for elemental identification.

PREPARATIONS

A. Sample stubs for SEM.
B. Carbon tape, discs, or colloidal suspensions for mounting sample.
C. Copper and aluminum for calibration.
D. Tweezers.
E. Scalpels.

INSTRUMENTATION

A. Scanning Electron Microscope.
B. Energy Dispersive X-Ray System.
C. Stereomicroscope.
D. Carbon Coater

MINIMUM STANDARDS & CONTROLS

A. See Appendix II.
B. See Method "Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy" (MT-IXB).
C. A semi-annual check of the instrument will be performed by analyzing a 316 Stainless Steel reference and ensuring specific elements in the reference are detected. If all required elements are not detected, the semi-annual check should be performed again. If elements are still undetectable, a service call should be placed. A laboratory record of this check will be maintained in LAM. See below for the composition of the 316 stainless steel reference sample and what elements are required. If paint analysis is performed infrequently on an SEM, this semi-annual check can be done prior to the case and does not need to be done semi-annually.

Element	Percent Composition	Required
Carbon (C)	0.044%	No
Silicon (Si)	0.509%	Yes
Phosphorus (P)	0.02%	No, overlaps with Mo

Molybdenum (Mo)	2.38%	Yes
Sulfur (S)	0.016%	No, overlaps with Mo
Vanadium (V)	0.047%	No
Chromium (Cr)	18.45%	Yes
Manganese (Mn)	1.64%	Shoulder on Cr peak
Iron (Fe)	64.345%	Yes
Nickel (Ni)	12.26%	Yes
Cobalt (Co)	0.12%	Shoulder on Fe peak
Copper (Cu)	0.169%	No

PROCEDURE OR ANALYSIS

A. To avoid interference from other layers individual paint layers should be sectioned with a scalpel and mounted onto an SEM stub instead of cross sections whenever possible.
B. Mount chips with carbon mounting medium.
C. If necessary, make a drawing of the surface of the stub in order to be able to locate the paint chips in the SEM.
D. To avoid charging, the paint samples may have to be carbon coated.
E. Perform a performance check on the energy dispersive x-ray system using the x-rays generated from the copper and aluminum on the sample mount stub.
F. Be sure to run the copper and aluminum with the same excitation voltage as the sample.
G. File your copper/aluminum data.
H. Proceed with the following:
    1. Isolate the area of interest on the sample.
    2. Set the appropriate voltage and spot size.
    3. Activate the x-ray collection system.
    4. Plot results.
    5. Properly mark digital copies.
    6. Remember, the electron penetration may be up to 10 µm at acceleration voltage of 20 keV. The x-ray generation area may be 5 µm beyond the scan region.
    7. Some EDS Systems may not detect chemical elements with an atomic number less than Sodium (Na). The sensitivity may be limited to elements with concentrations greater than 1%. The system being used should be checked for these limitations.

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

PROTOCOL: Paint Analysis

METHOD: Analytical Techniques

PROCEDURE: Pyrolysis Gas Chromatography/Mass Spectrometry Analysis

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Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

A. Pyrolysis Gas Chromatography/Mass Spectrometry (PGC/MS) is a destructive technique that is used to compare and identify the pyrolytic breakdown products of paint vehicles or resins.

B. The resultant pyrogram will be influenced by instrument conditions and placing of the sample in the pyrolysis boat or tube. Therefore, it is imperative that the procedure be carefully duplicated when comparing known and unknown samples.

C. Small differences in topcoat composition may not be detected when a multi-layer paint is analyzed. Therefore, if an analyst has a multi-layer automobile paint and enough sample, the top layer of the paint should be analyzed separately in addition to analyzing the various layers together.

D. The following is a list of some ions used for extracted ion profiles:

Compound Type	m/z
Styrene	103 + 117 +118 or 104 +117 + 118 + 132 + 146
Styrene + Phthalic Anhydride	76 +148
Methacrylates	41 + 69
Acrylates with Methacrylates	41 + 43 + 55 + 56 + 69 + 87
Acrylates with reduced Methacrylates	45 + 55 + 58 + 73 + 86
Phenol	66 + 94 + 121 + 136 or 65 + 66 + 79 + 94
Enhanced alcohols to the Methacrylates	41 + 42 + 43 + 56
Bisphenol A	213 + 228
Bisphenol A + Methacrylates	41 + 69 + 119 + 213 + 228
Isocyanates	81 + 85 + 98 + 123 or 85 + 99 +110 + 123
Aromatics	91 + 106

SAFETY CONSIDERATIONS

See Protocol "Safety Considerations"

Chemical	Health	Flammability	Reactivity
Methanol	1	3	0
Acetone	1	3	0
Hexane	1	3	0

PREPARATIONS

A. Fine-tip tweezers
B Fine-pointed needles or probe
C. Scalpel
D Stainless steel cup for oven pyrolyzer
E. Oven pyrolyzer
F. Methanol
G. Acetone
H. Hexane
I. Distilled water
J. Reference (Polyethylene, Polystyrene, or Paint)

INSTRUMENTATION

A. Stereomicroscope
B. Frontier oven pyrolyzer, or equivalent
C. Gas Chromatograph/Mass Spectrometer
D. The following are suggested instrument conditions which may be adopted or modified for paint analysis. Any method used must meet the minimum standard of analysis.

1. Chromatographic Parameters
   Inlet: 250°C
   Detector interface: 300°C
   Program Conditions: 50°C for 2 minutes
   10°C/min to 300°C
   300°C for 6 minutes

2. Carrier gas:
   Type: Helium
   Flow Rate: 1.5 mL/min
   Split Ratio: 100:1 with monomer samples or reduce sample size.
   50:1 with paint samples

3. Column:
   Phase: HP5 MS or equivalent
   ID: 0.25mm
   F.T.: 0.25µm
   Length: 30m

4. Detector Parameters:
   Solvent Delay: 1.0 min
   Mass Range: 35:400 m/z

5. Pyrolysis

   a. Oven pyrolyzer
      Interface Temperature: 275°C
      Oven Temperature: 550°C

6. Sample Size
   Monomers: 3-20µg
   Paint: 10-50µg

MINIMUM STANDARDS & CONTROLS

A. See Appendix II.
B. See Method "Gas Chromatography/Mass Spectroscopy" (MT-IXC).
C. A blank of the cleaned cup will be run immediately prior to each item. The cup can be cleaned with methanol, acetone, hexane, distilled water, or compressed air. Ensure the cup is dry prior to adding sample.
D. Control: a reference paint sample (usually acrylic enamel, styrene, or polyethylene) will be run each week case samples are run. The results will be checked for reproducibility of retention time, peak separations, and sensitivity. A copy of the pyrogram will be placed in the case file. The comparison to past control samples do not need to be retained.

PROCEDURE OR ANALYSIS FOR OVEN PYROLYZER

See Method Gas Chromatography/Mass Spectrometry (MT-IXC) for step-by-step directions.

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

PROTOCOL: Paint Analysis

METHOD: Analytical Techniques

PROCEDURE: FTIR Attenuated Total Reflection (ATR)

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

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Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The purpose of Internal Reflection Spectroscopy (IRS) is to provide a non-destructive procedure for the analysis and identification of organic and inorganic material. IRS produces Attenuated Total Reflection (ATR) spectra. This is accomplished by placing the sample in optical contact with the surface of an Internal Reflecting Element (IRE). These elements have a high refractive index and will resist mechanical damage and chemical attack. The most commonly used IRE is the diamond. The same considerations in determining and comparing spectra are used with the ATR accessory.

RELATED PROCEDURES

Paint Fourier Transform Infrared (FTIR) Spectroscopy (MT-IIB-2)
Instrumentation Fourier Transform Infrared Spectroscopy (FTIR) (MT-IXA)

SAFETY CONSIDERATIONS

A laser beam is used to align the mirrors during the operation of the instrument. Do not look directly into the beam as damage to the eyes can result.

PREPARATIONS

No preparation of the sample is necessary. The sample can be used directly on the IRE.

INSTRUMENTATION

A. FTIR Spectrometer
B. ATR Accessory

MINIMUM STANDARDS & CONTROLS

See Appendix II.
See Method "Fourier Transform Infrared Spectroscopy" (MT-IXA).

PROCEDURE OR ANALYSIS

A. A background spectrum must be collected initially.
B. A sample is placed on the IRE mount area and positioned to the optimal area. If necessary, use the monitor to aid in sample positioning. The clamp is secured (hand-tightened) to insure good contact between the sample and the IRE as indicated on the instrument.
C. Spectra of all questioned samples and known references shall be collected in the same manner and digital copies maintained in the case file.
D. The IRE should be cleaned between samples.

E. Blanks will be run before each sample.

The following are suggested FTIR method parameters:

Number of Scans = 32
Resolution = 8 cm^{-1}
Gain = 8
Apodization = Happ-Genzel
Starting wave number = 4000cm^{-1}
Ending wave number = 400cm^{-1}

Parameters may be adjusted to provide sufficient intensity and resolution for spectra comparison and/or identification. When comparing items, ensure the same parameters are used for each. The only exception to this is the number of scans performed. A smaller/thinner sample may require more scans to produce a useable spectrum.

REPORT WORDING

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Paint Analysis

Method: Make, Model, Year Determination

Procedure: Paint Data Query (PDQ)

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

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Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

Introduction

Automobile manufacturers tend to use specific combinations of pigments, extenders, and binders in each of the paint layers used. It is this specific combination in each layer that enables forensic scientists to suggest the make, model, and year of vehicles from which a paint smear/chip may have originated. Coded information from each available layer of an unknown is entered into a computer database, Paint Data Query (PDQ), and a list of automotive plants with vehicles similar by layer sequence and chemistry to the unknown is produced. By comparing the FTIR spectra of the layers of the unknown to those on the PDQ list, a refined list of the possible make, model, and year of the unknown can be produced.

Minimum Standards & Controls

A. See MT-APP-II "Minimum Standards and Controls".
B. Documentation
   1. Coding for PDQ is based on the FTIR spectra obtained by using the bench or microscope accessory only.
   2. Only Original Equipment Manufacturer (OEM) paint can be compared to spectra in the PDQ database.
   3. The exact codes entered into the PDQ database must be documented either on a worksheet or on a printout from PDQ.
   4. Any worksheets used to help eliminate/include automotive plants and years must be included in the case file.
   5. The hit list from PDQ must be printed and included in the case file.
   6. Sufficient spectra comparisons must be included in the case file to cover the plants and span of years determined to be possible sources of the unknown paint chip. Spectral comparisons of those in the hit list that are excluded as a possible source of the questioned paint need only be documented in the notes.
   7. If Automotive News is used, a digital copy of the portions used from Automotive News to determine the possible vehicle makes in the report should be included in the case file.
   8. The version of the PDQ software used should be documented somewhere in the case file.
   9. If the paint sample is too small for subsequent additional paint comparisons, permission to consume must be obtained and documented.

Safety Considerations

A. Do not look directly into the FTIR laser.
B. If the FTIR microscope is used, use personal protective equipment to cover eyes and skin while working with liquid nitrogen.

Preparations

A. Fine-tip tweezers
B. Fine pointed needles or probe
C. Scalpel
D. Liquid nitrogen
E. Polystyrene calibration standard
F. Compression cell with diamond windows or salt plates
G. KBr or NaCl plates
H. Roller tool

Instrumentation

A. Stereomicroscope
B. Fourier Transform Infrared Spectrometer (FTIR) with Know-It-All softwareA microscope accessory is preferred.
C. PDQ database from RCMP (Royal Canadian Mounted Police)

Procedure or Analysis

A. Refer to Micro/Trace Procedure MT-IIA-1 for the examination of paint samples.
B. By using the table below, microscopically try to determine if the paint sample is an original automotive finish (OEM). Check the number of layers, color, and approximate thickness.

What to look for:	**What it can mean:**
Thickness of the topcoat paint layers	Unusually thick layer(s) may suggest repaint layer(s).
Metallic flakes in basecoat	Unusually large metallic flakes in basecoat may suggest a repaint layer.
Thickness of the undercoat paint layer	Unusually thick layer(s) may suggest repaint layer(s).
Sanding marks	Paint layer(s) above a layer with sanding marks may be repaint layer(s) or factory applied repaint layers.
Body filler/Bondo	Paint layers above a layer of body filler are most likely repaint layer(s). To confirm a repaint layer, look for thickness, color and air bubbles in the primer layer.
Two different clearcoats/basecoats	If a paint system has 2 different clearcoats/basecoats, this may suggest repaint layer(s).

C. If original finish is suspected, prepare the samples so that each layer of the paint chip can be analyzed separately and be thin enough to allow for transmission IR.

D. Refer to Micro/Trace Procedure MT-IIB-2 for FTIR analysis of paint layers. Obtain an IR spectrum of each original finish paint layer.

E. By looking at the IR spectra, code the components of each layer (excluding the base coat) using the RCMP coding reference chart (see figures 1-2). For topcoats only code binders, do not code pigments and extenders. For undercoats, code both the binders and pigments/extenders. If it is unclear if a component is present, or is present in trace amounts, do not code it but make note for later reference if needed. See figures 1-2 to help determine the components of the paint layer system.

F. By using the table below, again try to determine if OEM finish.

What to look for:	**What it can mean:**
Melamine	Topcoat paints over metal substrates usually contain melamine with some exceptions. The lack of melamine in a topcoat may suggest a repaint layer, but it could also suggest a layer above a non-metal substrate (plastic, fiberglass, etc.), a late 90's to present paint layer or other possibilities. Some topcoat layers from the late 90's to present contain trace amounts of melamine and polyurethane. Melamine has to be heated to cure unless it is mixed with polyurethane. If melamine is present without polyurethane, it is a good indication that the paint is OEM.
Polyurethane	Polyurethane in a topcoat paint may suggest a repaint, a paint layer above a non-metal substrate, a late 90's to present paint layer or other possibilities. Some topcoat layers from the late 90's to present contain trace amounts of melamine and polyurethane. Also, some manufacturers are using polyurethane in the topcoat with melamine so the paint does not require heat to cure.
Talc/Clay	Unusually high amounts of talc or clay in an undercoat may suggest a repaint layer. Look for hints such as sanding marks or repainted topcoats to confirm that it is a repaint layer.
Calcium Carbonate	Undercoats that are primarily composed of calcium carbonate may be a repaint layer(s) such as body filler (Bondo). Look for hints such as unusual thickness, color and air bubbles in this primer layer.
E-coat	Most metal frames of a vehicle have an e-coat layer. Metallic substrates which lack the e-coat layer may suggest repaint layer(s). There are a few exceptions, for example, there is a plant in Mexico that does not cover the body with an e-coat. The lack of an e-coat may also suggest that the paint system is very old (before the mid 80's).

G. If the paint sample appears to be a refinish do not proceed with the make, model, year analysis because only original finish layers are to be searched in the PDQ database. For the OEM paint layers, enter the codes for each layer's components into the PDQ database and the location of that layer in the paint system. The layer location describes the type of paint (O = original, R = manufacturer's repaint), whether it is an Undercoat (U) or Topcoat (T) and where it is in relation to the interface of the original topcoats and undercoats. An example for a 4-layer OEM paint system might be OT2 for the clear coat, OT1 for the basecoat (do not enter this layer into PDQ), OU1 for the primer surfacer layer and OU2 for the e-coat layer. If not sure exactly which layer is present OT* can be used for all topcoats and OU* can be used for all undercoats (OU* should be used for all ECoats to account for the possibility of an anti-chip layer).

1. If it is certain that a component is not present in the sample, exclude it in the software by holding the SHIFT key and selecting the component. That component will turn red. This will eliminate any paint systems that have that component.
2. For best results, enter the clearcoat chemistry with Layer Sequence OT*. This allows the clearcoat to be in any OT position.

H. Generate a hit list of plants from potential manufacturers. This list can be exported to Excel. In Excel, a "Comments" column can be added to describe the reasoning for eliminations.

I. A preliminary examination can be done in the PDQi software by doing a quick comparison of the layers and eliminating any with big differences to the unknown paint sample.

J. Using the hit list, begin comparing database reference spectra to the unknown spectrum for each layer in the Know-it-All software. Due to differences in colors/pigments, the basecoat layer is not compared to reference spectra.

1. Use the spectrum of the most unique or discriminating layer, usually the clear coat or primer surfacer layer, along with the Property Fields such as Layer Type, Plant, Year range, Substrate, etc., from the PDQ Hit List. For layer type use CC for clear coat, BC for basecoat, PS for primer surfacer, and EC for ECoat. The specific layer database can be used for each layer instead of inputting the layer type, if desired.

J. Continue to look at all layers of the paint system. If there are spectral differences in the manufacturing plant, it can be eliminated from the PDQ hit list. If there are comparable hits, transfer this plant and year range to a PDQ Spectral Search Sheet for further comparison.

K. Do a combination search using the unknown spectra and the name of the layer, plant, and year to determine whether this particular layer is comparable to the unknown. If there is one spectrum that is similar, place a check mark in the box for that plant and year. If no spectra in the database are similar to the unknown, place an x. If it is unclear whether a spectrum is similar, a ? can be placed into the box.

L. Continue until the entire year range from the hit list, plus a few extra on each side for good measure, have been compared to the unknown. Be sure to have at least 3 X's in a row to signal a change and difference in paint formulation. If no paint

sample is in PDQ for a particular year at a particular plant, place a – or 0 in the box. A – or 0 mark must be treated as a check mark when determining the year range for a plant.

M. Layers with overlapping check marks, ?, -, or 0's will indicate a window or year range. This year range can be used to determine possible manufacturing plant/year range of vehicles with a similar paint system as an unknown and therefore should be considered as a possible source.

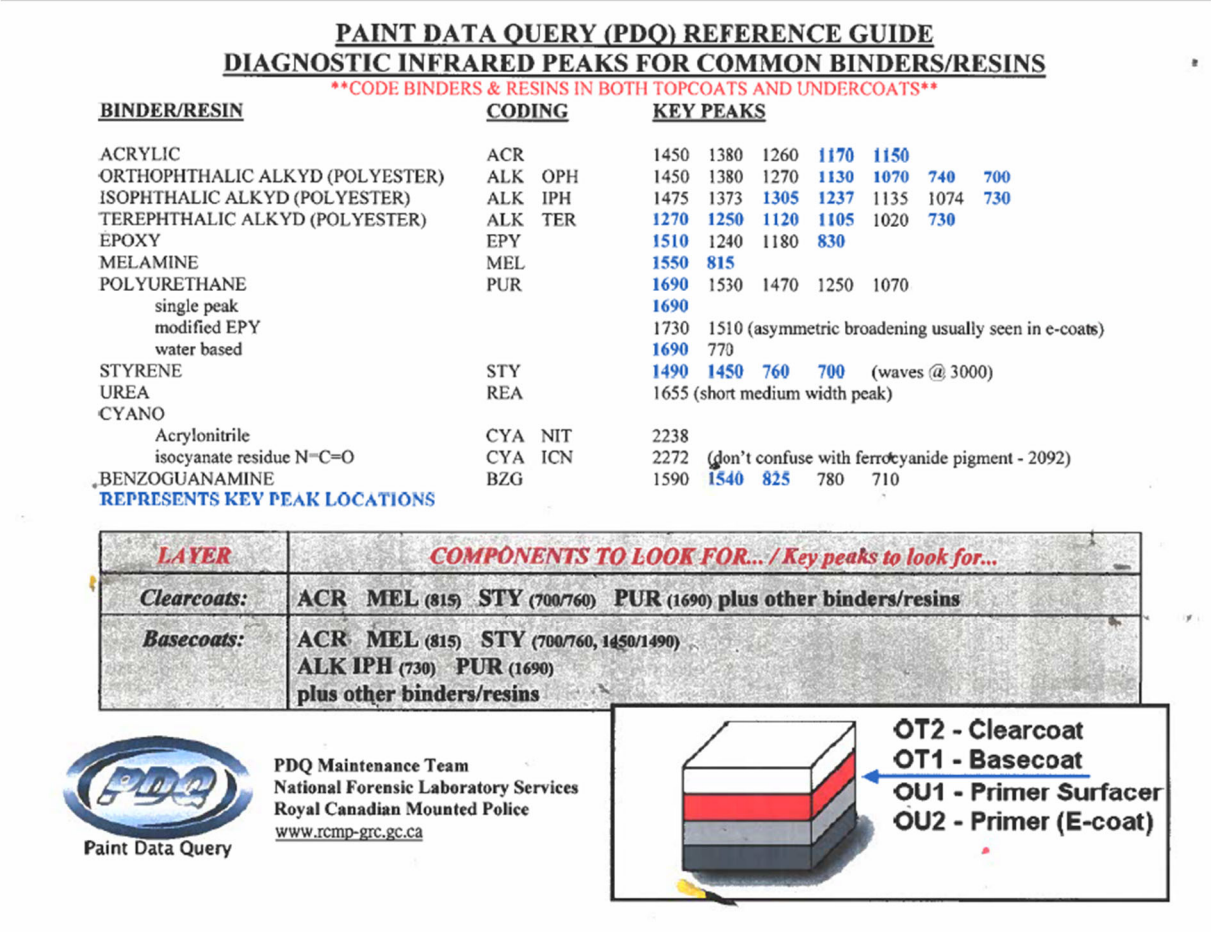
N. Add one year on each side of the window to allow for the phasing in and out of paints.

O. If the basecoat of the unknown is present and large enough to get an accurate description of its color, it may be beneficial to search refinish books to narrow the range of years even further. This step is performed to verify that the color in question was actually used by the auto manufacturer(s) in the year(s) suggested by the results of the database queries.

P. Automotive News is a listing of the models manufactured at assembly plants in North American for years 1990-present. Look up the plant and years of the comparable hits from the spectral search to determine what models were produced at the manufacturing plant during that time period. If an automotive plant outside North American was found to have comparable chemistry to the unknown, internet searches will need to be performed to determine the models produced at these plants during these years.

Q. With this information, a final list of possible make/model/year range is determined and provided to the requesting agency.

R. If a suspect vehicle is subsequently identified, analysis of the paint from that vehicle should be completed and compared to the questioned paint in accordance with the Paint Analysis (MT-II) protocols.



PAINT DATA QUERY (PDQ) REFERENCE GUIDE
DIAGNOSTIC INFRARED PEAKS FOR COMMON BINDERS/RESINS
CODE BINDERS & RESINS IN BOTH TOPCOATS AND UNDERCOATS

BINDER/RESIN	CODING	KEY PEAKS
ACRYLIC	ACR	1450 1380 1260 **1170** **1150**
ORTHOPHTHALIC ALKYD (POLYESTER)	ALK OPH	1450 1380 1270 **1130** **1070** **740** **700**
ISOPHTHALIC ALKYD (POLYESTER)	ALK IPH	1475 1373 **1305** **1237** 1135 1074 **730**
TEREPHTHALIC ALKYD (POLYESTER)	ALK TER	**1270** **1250** **1120** **1105** 1020 **730**
EPOXY	EPY	**1510** 1240 1180 **830**
MELAMINE	MEL	**1550** **815**
POLYURETHANE	PUR	**1690** 1530 1470 1250 1070
single peak		**1690**
modified EPY		1730 1510 (asymmetric broadening usually seen in e-coats)
water based		**1690** 770
STYRENE	STY	**1490** **1450** **760** **700** (waves @ 3000)
UREA	REA	1655 (short medium width peak)
CYANO		
Acrylonitrile	CYA NIT	2238
isocyanate residue N=C=O	CYA ICN	2272 (don't confuse with ferrocyanide pigment - 2092)
BENZOGUANAMINE	BZG	1590 **1540** **825** 780 710

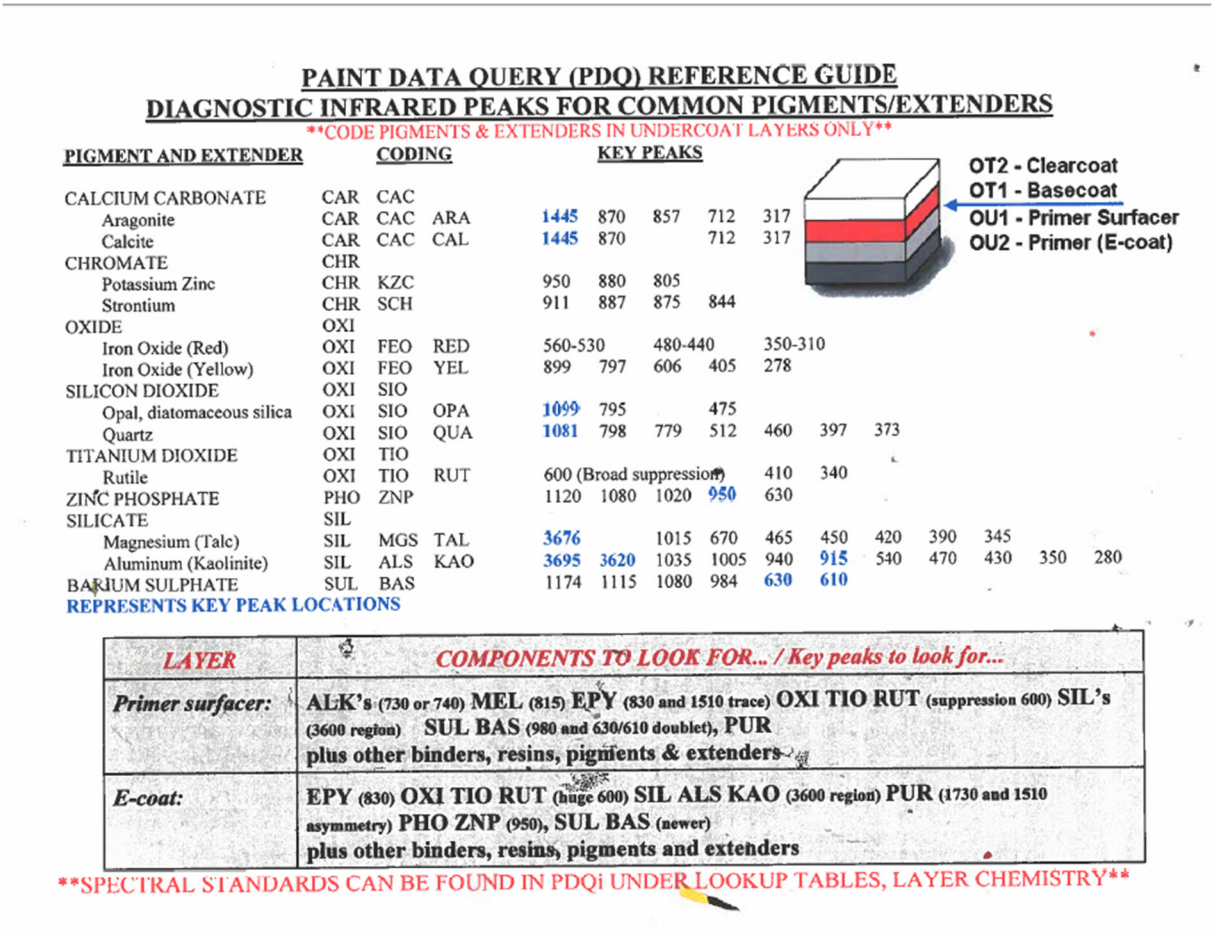
REPRESENTS KEY PEAK LOCATIONS

LAYER	COMPONENTS TO LOOK FOR... / Key peaks to look for...
Clearcoats:	**ACR MEL (815) STY (700/760) PUR (1690) plus other binders/resins**
Basecoats:	**ACR MEL (815) STY (700/760, 1450/1490) ALK IPH (730) PUR (1690) plus other binders/resins**

Paint Data Query

PDQ Maintenance Team
National Forensic Laboratory Services
Royal Canadian Mounted Police
www.rcmp-grc.gc.ca

Figure 1



PAINT DATA QUERY (PDQ) REFERENCE GUIDE
DIAGNOSTIC INFRARED PEAKS FOR COMMON PIGMENTS/EXTENDERS
CODE PIGMENTS & EXTENDERS IN UNDERCOAT LAYERS ONLY

PIGMENT AND EXTENDER	CODING	KEY PEAKS
CALCIUM CARBONATE	CAR CAC	
Aragonite	CAR CAC ARA	1445 870 857 712 317
Calcite	CAR CAC CAL	1445 870 712 317
CHROMATE	CHR	
Potassium Zinc	CHR KZC	950 880 805
Strontium	CHR SCH	911 887 875 844
OXIDE	OXI	
Iron Oxide (Red)	OXI FEO RED	560-530 480-440 350-310
Iron Oxide (Yellow)	OXI FEO YEL	899 797 606 405 278
SILICON DIOXIDE	OXI SIO	
Opal, diatomaceous silica	OXI SIO OPA	1099 795 475
Quartz	OXI SIO QUA	1081 798 779 512 460 397 373
TITANIUM DIOXIDE	OXI TIO	
Rutile	OXI TIO RUT	600 (Broad suppression) 410 340
ZINC PHOSPHATE	PHO ZNP	1120 1080 1020 950 630
SILICATE	SIL	
Magnesium (Talc)	SIL MGS TAL	3676 1015 670 465 450 420 390 345
Aluminum (Kaolinite)	SIL ALS KAO	3695 3620 1035 1005 940 915 540 470 430 350 280
BARIUM SULPHATE	SUL BAS	1174 1115 1080 984 630 610

REPRESENTS KEY PEAK LOCATIONS

OT2 - Clearcoat
OT1 - Basecoat
OU1 - Primer Surfacer
OU2 - Primer (E-coat)

LAYER	COMPONENTS TO LOOK FOR... / Key peaks to look for...
Primer surfacer:	ALK's (730 or 740) MEL (815) EPY (830 and 1510 trace) OXI TIO RUT (suppression 600) SIL's (3600 region) SUL BAS (980 and 630/610 doublet), PUR plus other binders, resins, pigments & extenders
E-coat:	EPY (830) OXI TIO RUT (huge 600) SIL ALS KAO (3600 region) PUR (1730 and 1510 asymmetry) PHO ZNP (950), SUL BAS (newer) plus other binders, resins, pigments and extenders

SPECTRAL STANDARDS CAN BE FOUND IN PDQi UNDER LOOKUP TABLES, LAYER CHEMISTRY

Figure 2

Report Wording

See Paint Analysis Report Wording MT-IID

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

PROTOCOL: Paint Analysis

METHOD: Report Wording

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Section Advisory Committee

Approved by:

Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

Paint Report Wording Guidelines

	CRITERIA	RECOMMENDED REPORT WORDING
A.	Positive	
	Physical Match: The realignment of two or more fragments that demonstrates these fragments were at one time joined to form a single object.	Report wording: see Physical Match Appendix 1 Report Wording (PM APP-I)
B.	Similar Comparisons	
	Physical Characteristics, Organic and inorganic analysis similar	Findings: Description of paint chips found (color, layer sequence, reflective particles, type of paint, quantity, etc.) Conclusions: The paint in Item ____ is similar in color, layer sequence, and chemical composition to the paint in Item ___. Therefore, the paint in Item ___ could have originated from the same source as the paint in Item__.
	Only partial analysis performed. Paints are similar.	Findings: Description of paint chips found (color, layer sequence, reflective particles, type of paint, quantity, etc.) Conclusion: "Preliminary examination showed similarities in the paints from Items____; however, due to insufficient quality/quantity of the paint in Item___ no further analysis could be completed. OR Conclusion: "Similarities were noted between the paints in Items____; however, due to the insufficient quality/quantity of the paint in Item___, no further tests were performed."
	Slight variation in physical or chemical properties.	Findings: Description of paint chips found (color, layer sequence, reflective particles, type of paint, quantity, etc.) Conclusion: While there are similarities in the __in Items ____, because of the differences in the primer layers it is inconclusive as to whether Item/Items ___originated from the same vehicle as Item/Items____. Submission of further paint standards taken directly from the damaged areas of the suspect/victim vehicle may lead to a more conclusive finding.
	No paint found or insufficient paint found.	No paint was found/observed/recovered. OR Nothing of apparent evidential value was found.

C. Negative

Differences found in paint samples	Findings: Description of paint chips found (color, layer sequence, reflective particles, type of paint, quantity, etc.) Conclusion: "The paint in Item __ is dissimilar in (color, layer sequence, chemical composition) to the paint in Item __. Therefore, the paint in Item __ did not originated from the same source as the paint in Item __."

D. No standard submitted

Only microscopic examination or no suitable comparison in FTIR reference library	Findings: Description of paint chips found (color, layer sequence, reflective particles, type of paint, quantity, etc.). The paint appears to be automotive/non-automotive.
Microscopic examination and FTIR analysis with suitable comparison in FTIR reference library	Findings: Description of paint chips found (color, layer sequence, reflective particles, type of paint, quantity, etc.). The questioned paint appears to be a ___(add color and possible chemical composition) automotive/non-automotive paint
Additional optional Remark	If determined to be probative, further analysis may be performed upon specific request and submission of required standards.

Below is an example of report wording

ITEM	DESCRIPTION	FINDINGS
1	Paint standard from suspect's Ford truck	Paint chips with a layer sequence of white/metallic blue/grey/black were observed. Used for comparison to Item #2.
2	Questioned paint from victim's Dodge van	Paint chips with a layer sequence of white/metallic blue/grey/black were observed.
3	Paint standard from front door	Paint chips with a layer sequence of white/green were observed. Used for comparison to Items 4, 5, and 6.
4	Crowbar	White/blue paint found.
5	Screwdriver	White paint found.
6	Screwdriver	White paint found.
7	Screwdriver	No paint was found.

CONCLUSIONS

The paint in Item 2 is similar in color, layer sequence, and chemical composition to the paint in Item 1. Therefore, this paint in Item 2 could have originated from the same source as the paint in Item 1.

The paint in Item 4 is dissimilar in color and layer sequence to the paint in Item 3. Therefore, the paint in Item 4 could not have originated from the same source as the paint in Item 4.

Preliminary examination showed similarities in the white paints in Items 3 and 5; however, due to the poor quality of the paint from Item 5, no further tests were performed.

The paints in Items 3 and 6 are dissimilar in organic composition and could not have originated from the same source.

Paint Data Query (PDQ) Report Wording Guidelines

All findings are similar to Paint Report Wording Guidelines

	CRITERIA	RECOMMENDED REPORT WORDING (CONCLUSIONS)
A.	Search of unknown paint layer system results in PDQ hits	The color, layer sequence, and chemical type data of the _____paint in Item __were searched against the Paint Data Query (PDQ) database. The data from the recovered paint is most similar to paint from vehicles manufactured at the (list possible plant) _____ during the years of (list possible years). Vehicles manufactured at these plants during these times are listed below. (After possible makes and years if base coat is present) The vehicle part from which the paint originated would have a silver appearance. AND Please note that your search for a suspect vehicle should include, but not be limited to, these vehicles. Not every layering system for every vehicle is included in the database; therefore, it is possible that the questioned paint chips are from a vehicle not listed in this report.
B.	The unknown paint does not appear to be OEM paint	The _____paint in Item ___ does not appear to be an Original Equipment Manufacturer (OEM) paint based on the layer sequence and paint type; therefore, a Paint Data Query (PDQ) database search to determine possible make and model sources could not be performed. However, the vehicle part(s) from which the paint originated would have a _____ appearance. OR The color, layer sequence, and chemical type data of the ____ paint chip in Item ___ were searched against the Paint Data Query (PDQ) database. No meaningful make nor model information was developed as a possible source for the recovered chips. Please note that the paint type data of the ____ paint chip indicates that it may be consistent with refinish paint and, therefore, may not be represented in the PDQ database. However, the vehicle part(s) from which the paint originated would have a _____ appearance.
C.	The unknown paint is of insufficient quantity/quality for PDQ search.	The ____paint in Item ____was of insufficient quantity/quality for a meaningful Paint Data Query (PDQ) database search; therefore, no vehicle make and models could be determined as possible sources of the ___ paint. However, the vehicle part(s) from which this paint originated would have a ____ appearance.
D.	The PDQ search resulted in a very large hit list that could not be narrowed down.	The color, layer sequence, and chemical type data of the ____ paint chip in Item ___ were searched against the

		Paint Data Query (PDQ) database. The search resulted in a wide range of vehicle make and models: therefore, no meaningful make nor model information was developed as a possible source for the recovered chips. However, the vehicle part(s) from which this paint originated would have a ____ appearance.
E.	After the PDQ search results and the refinish books were searched for the color.	The uppermost color layer of the question chips was ____; therefore, the source vehicle is likely ____. Refinish chip collections were searched for similar colors. ____is the name of the color available on _____vehicles that most resembled the questioned paint color.
F.	The unknown paint chip/transfer only contains the basecoat.	____ single layer paint chips/transfers were observed. These paint chips/transfers are unsuitable for Paint Data Query (PDQ) database search for a possible source vehicle. However, the vehicle part(s) from which these chips/transfers originated would have a _____ appearance.
G.	The PDQ search resulted in zero hits.	The color, layer sequence, and chemical type data of the ____ paint chip in Item ___ were searched against the Paint Data Query (PDQ) database. No original finish layers in the database were found to be similar to the question paint chip layers; therefore, no year/make/model information of possible source vehicles could be determined. However, the vehicle part (s) from which these chips/transfers originated would have a _____ appearance.

Below is an example of report wording

ITEM	DESCRIPTION	FINDINGS
1	Victim's coat	Several paint chips were recovered with the following layer sequence: clear coat/reflective silver/light grey/grey.
2	Paint transfer from the hood of the suspect's vehicle	Reflective silver transfer paint was observed.
3	Victim's pants	Several paint chips were recovered with the following layer sequence: clear coat/white/filler material/grey.

Conclusions

The clear coat/reflective silver/light grey/grey paint chips recovered in Items 1 appear to be Original Equipment Manufacturer (OEM) paint. The color, layer sequence, and chemical type data of these chips were searched against the Paint Data Query (PDQ) database. The data from the paint recovered is most similar to paint from a vehicle manufactured at the General Motors Lansing Delta, Michigan plant during the years 2007 - present day or the General Motors Oshawa, Ontario plants during the years 2005 - 2013. Vehicles manufactured at these plants during these times are listed below.
Buick Allure 2005
Buick Enclave 2007 - present
Buick LaCrosse 2005 - 2009
Buick Regal 2011 - 2013
Cadillac XTS 2012 - 2013
Chevrolet Camaro 2009 - 2013
Chevrolet Equinox 2010 - 2013
GMC Acadia 2007 - 2017, 2024 - present
GMC Acadia Denali 2010.
The vehicle part from which the paint originated would have a silver appearance. Refinish chip collections were searched for similar colors. Dark Micastone and Silver Birch Effect were the names of the two colors available on 1991-2000 General Motors vehicles that most resembled the question paint color.

Please note that your search for a suspect vehicle should include, but not be limited to, these vehicles. Not every layering system for every vehicle is included in the database; therefore, it is possible that the questioned paint chips are from a vehicle not listed in this report.

The single layer reflective silver paint transfers in Item 2 are unsuitable for Paint Data Query (PDQ) database search for a possible source vehicle. However, the vehicle part(s) from which these transfers originated would have a silver appearance.

The paint in Item 3 does not appear to be an Original Equipment Manufacturer (OEM) paint based on the layer sequence and paint type; therefore, a Paint Data Query (PDQ) database search to determine possible vehicle make and model sources could not be performed. However, the vehicle part(s) from which the paint originated would have a white appearance.

If a suspect vehicle is developed, please resubmit the above evidence along with a known paint standard from the suspect vehicle for paint comparison.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Primer Gunshot Residue Analysis (PGSR)

Method: Primer Particle Gunshot Residue Analysis (PPGSR)

Procedure: Primer Gunshot Residue (PGSR) Analysis Using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS)

Reviewed by:

_____

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

This method of primer gunshot residue (PGSR) analysis is performed by scanning electron microscopy/energy dispersive x-ray spectrometry (SEM/EDS). This analysis uses automated software to control the SEM and EDS systems during the analysis of a PGSR sample to identify candidate particles that may be associated with primer gunshot residue. At the completion of the automated analysis, the analyst will manually control the SEM/EDS system to perform confirmatory analyses and classifications of candidate PGSR particles on the sample. The analyst will also review candidate particle morphology. This method of morphological and elemental particle examination forms the basis for conclusions relative to firearm discharge.

This analysis is a non-destructive technique.

TERMINOLOGY

A. Consistent particle – particle composed primarily of one or two of the following elements: antimony, barium, or lead.
B. Tri-component PGSR particle – particle composed primarily of antimony, barium, and lead.
C. Load factor – (particles/mm2) the total number of primer gunshot residue particles confirmed divided by the area analyzed.
D. Morphology – refers to the size, shape, structure, and texture of a particle.
E. Adhesive stub – a sample device used to collect materials for SEM/EDS analysis.
F. Background sample – a sample that contains < 3 confirmed tri-component particles and a load factor of < 3.75.
G. Adhesive blank – a sample that contains a variety of particle types, but the total number of particles must be no more than 50 and there may be no confirmed tri-component particles.
H. Contaminated sample – a room or kit control sample that has a load factor of 3.75 or greater and/or 3 confirmed tri-component particles.
I. Tri-component and consistent sample – a sample that contains a minimum of 3 confirmed tri-component particles and some consistent particles.
J. Consistent sample – a sample that contains < 3 confirmed tri-component particles and a load factor of > 3.75.
K. Stage point – a sample point saved to denote the edge of a sample.
L. Performance check – a copper and aluminum tape sample used to check the energy lines of the x-ray detector.
M. Instrument blank – an unused adhesive tape sample analyzed with questioned samples to monitor the cleanliness of the sample chamber's environment.
N. PGSR control – a PGSR sample that contains known PGSR particles which is analyzed with question samples. Its purpose is to test the accuracy of the instrument's capability to detect PGSR particles.

O. Threshold level (line scan) – levels that are set to optimize the brightness and contrast on a group of particles of interest such as PGSR particles.

SAFETY CONSIDERATIONS

A. Blood on adhesive stubs should be processed as a biohazard.
B. See Method Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (MT-IXB) for additional safety considerations.

PREPARATIONS

A. Stereomicroscope – may be used to examine the sample condition
B. Carbon Coater
C. Sample gripper

INSTRUMENTATION

A. Scanning electron microscope with an energy dispersive x-ray analyzer
B. Backscatter detector
C. Instrumental operation software and particle analysis software

MINIMUM STANDARDS & CONTROLS

A. A record of the instrumental conditions is to be kept with each case file.
B. An instrument blank and a primer gunshot residue control sample are to be analyzed with each automated analysis. The data are to be retained in the case file.
C. A laboratory log of each primer gunshot residue control sample analyzed is to be maintained. The total number of PGSR particles identified in each analysis will be documented and statistically monitored. If the total number of PGSR particles identified fall outside of 3 standard deviations of the average, the data of any subsequent analysis will not be accepted and must be reanalyzed.
D. The x-ray detector will be checked using a copper/ aluminum performance check once a day at the start of an automated analysis or particle relocation. The performance check will be maintained in a laboratory log and with the case file. See Method Scanning Electron microscopy/Energy Dispersive X-Ray Spectroscopy (MT-IXB) for defined acceptability ranges.
E. An EDS scan of the primary PGSR elements of lead, antimony and barium will be checked at least semi-annually and will be within 50 electron volts (eV) of accepted values. If the value is outside the 50 eV A copper/aluminum performance check should be performed. See Section D under Minimum Standards and Controls. The semi-annual check should be

performed again. If centroid values are still outside limits, a service call should be placed. A laboratory record of this check will be maintained.

PROCEDURE OR ANALYSIS

A. Sample Preparation
   1. Sample stubs can be carbon coated if needed to reduce charging.
   2. Sample Identification
      a. Mark sample stubs as needed for identification and/or orientation in the SEM sample stage.
      b. Insert sample onto sample stage and note location.
      c. Evacuate chamber.
   3. Prepare the instrument for operation by entering sample data information and ensure that the correct instrumental SEM conditions are set.

B. Automated Analysis
   1. ASPEX Explorer SEM only: select backscatter detector.
   2. Adjust operational threshold level (line scan).
   3. Perform instrumental setup of each sample loaded into the sample stage. For each sample, optimize the focus and define the area to be analyzed. Typically, there are at least three stage points per sample stub to focus and save into the PGSR setup. Included with these samples should be the instrument blank and PGSR control.
   4. Readjust the backscatter detector operational threshold as needed.
   5. Start the system to perform automated analysis.
   6. ASPEX Explorer SEM only: the automated analysis can be paused as needed to download and assess any completed analytical data. Particle relocation can be performed during pause and the automated analysis can be restarted, if needed.
   7. When automated analysis is completed, individual sample data is to be retained in the case file.

C. Particle Relocation and Identification
   1. Assess candidate primer gunshot residue particles in each sample and determine whether they are PGSR type or not. Utilize this information to formulate a conclusion about the sample.
   2. Relocate, analyze, confirm, and document any PGSR tri-component particles. A minimum of 3 tri-component particles in each sample must be confirmed where conclusion is a possible discharge.
   3. If particles in the instrument classification of 'Pb-Ba-Sb' cannot be confirmed, they should be designated as non-PGSR or Other and the deficiencies of that classification should be noted.
   4. Confirmed particles must not be in the same electronic field.

5. The major elements (Pb, Ba, Sb) in a PGSR particle must have two emission lines identified.
6. All data classifications for the sample shall be examined during the data review process.
7. Samples must be further analyzed by rotating the stub 180 degrees when 2 tri-component particles are confirmed. However, if any sample in a submitted kit has a minimum of 3 confirmed tri-component particles, any remaining samples in the kit with 2 confirmed particles do not need to be rotated and reanalyzed.
8. All samples without a minimum of 3 confirmed tri-component particles must meet the following criteria depending on the instrument used
    a. Aspex Explorer: 85% of the fields analyzed
    b. Phenom: 85% of the area analyzed
        i. If less than 50% of the area is analyzed after the first analysis, change the parameters of the instrument to include all particles and rerun.
        ii. If 51-84% of the area is analyzed after the first analysis, rotate the sample 180 degrees and reanalyze.
        iii. To determine percentage of area analyzed use following equations:
        (*Total fields on stub) x 0.09554mm^2 = total area of stub
        (*Area Analyzed/Total area of stub) x 100% = percentage of area analyzed
        Total fields on stub and Area Analyzed are found on GSR Analysis Summary Report

REPORT WORDING

A. See Appendix I.

REFERENCES

1. Walton, G. M.; Nesbitt, R. S.; Calloway, A. R.; Loper, G. L.; Jones, P. F. "Final Report on Particle Analysis for Gunshot Residue Detection"; Law Enforcement Development Group, Aerospace Corporation Contract # J-LEAA-025-73 Law Enforcement Assistance Administration U. S. Department of Justice.

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Primer Gunshot Residue Analysis (PGSR)

Procedure: Collection and Preservation of Gunshot Residue Samples

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Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Various types of evidence can be encountered in gunshot residue analysis. The purpose of this procedure is to give the analyst guidance on the appropriate flow and the proper techniques of sample collection for a typical gunshot residue case.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

PREPARATIONS

A. Sample stubs for SEM.
B. Carbon tape or discs.

MINIMUM STANDARDS AND CONTROLS

INSTRUMENTATION

No instrumentation required.

PROCEDURE OR ANALYSIS

A. Utilize the flow chart on the following page to gather case information, sampling location and evidence submission information.
    1. Primer gunshot residue (PGSR) kits collected from the hands of individuals will almost always be analyzed before any kits collected from clothing, vehicles, or other items. If no hand kits are submitted, PGSR kits from other items may be analyzed if deemed probative.
    2. PGSR kits from clothing items may be analyzed if the hand kit indicates particles consistent with background samples or adhesive blank and an owner is identified for the item or the results generated would be probative.

B. Clothing items submitted for Forensic Biology/DNA and PGSR analysis should be examined by Forensic Biology/DNA first. Communication can be documented if PGSR collection occurs first. (EVH26 III.C.1.)

C. Care should be taken to prevent contamination by cleaning and placing clean butcher paper on the work area where samples are to be collected and by wearing appropriate personal protective equipment.
    1. Clean technique must be employed when examining any evidence that will also be examined for Forensic Biology/DNA. (MT-APP-V)
    2. A control sample is to be opened and exposed to the sampling area environment during collection of PGSR samples.

3. Collection of samples from separately recovered items should be done at different times and area cleanup performed between collections.

D. Illinois State Police Laboratories will routinely collect PGSR samples without agency direction from items involved in shooting events such as long sleeve garments, gloves, or any other item that may be present in the area of the hand or wrist.
   1. The cuff area of long sleeve garments will be sampled. For reversible garments, samples should be collected from the inside and outside cuff areas.
   2. The back of the thumb, index finger and web area of a glove will be sampled. If right or left hand is not indicated, then both sides of the designated collection area should be sampled.
   3. PGSR samples collected from items other than those detailed above should be based on locations provided by the agency.
      a. Collection should be limited to areas in the vicinity of discharged firearm or in contact with a PGSR related item.
      b. The whole item should not be sampled.

E. Samples should be sub itemized in one of the following ways. These are merely examples as there may be more than three samples per item. For a coat that is listed as Item 5:
   1. The three sample vials from the two cuffs and the room control may be sub-designated as 5-1, 5-2 and 5-3 or
   2. The PGSR kit may be sub itemized Item 5A and the three sample vials from the two cuffs and the room control would be 5A-1, 5A-2 and 5A-3.

F. Sampling device consists of a carbon-based adhesive on a ½ inch aluminum pin mount stub in a protective vial.

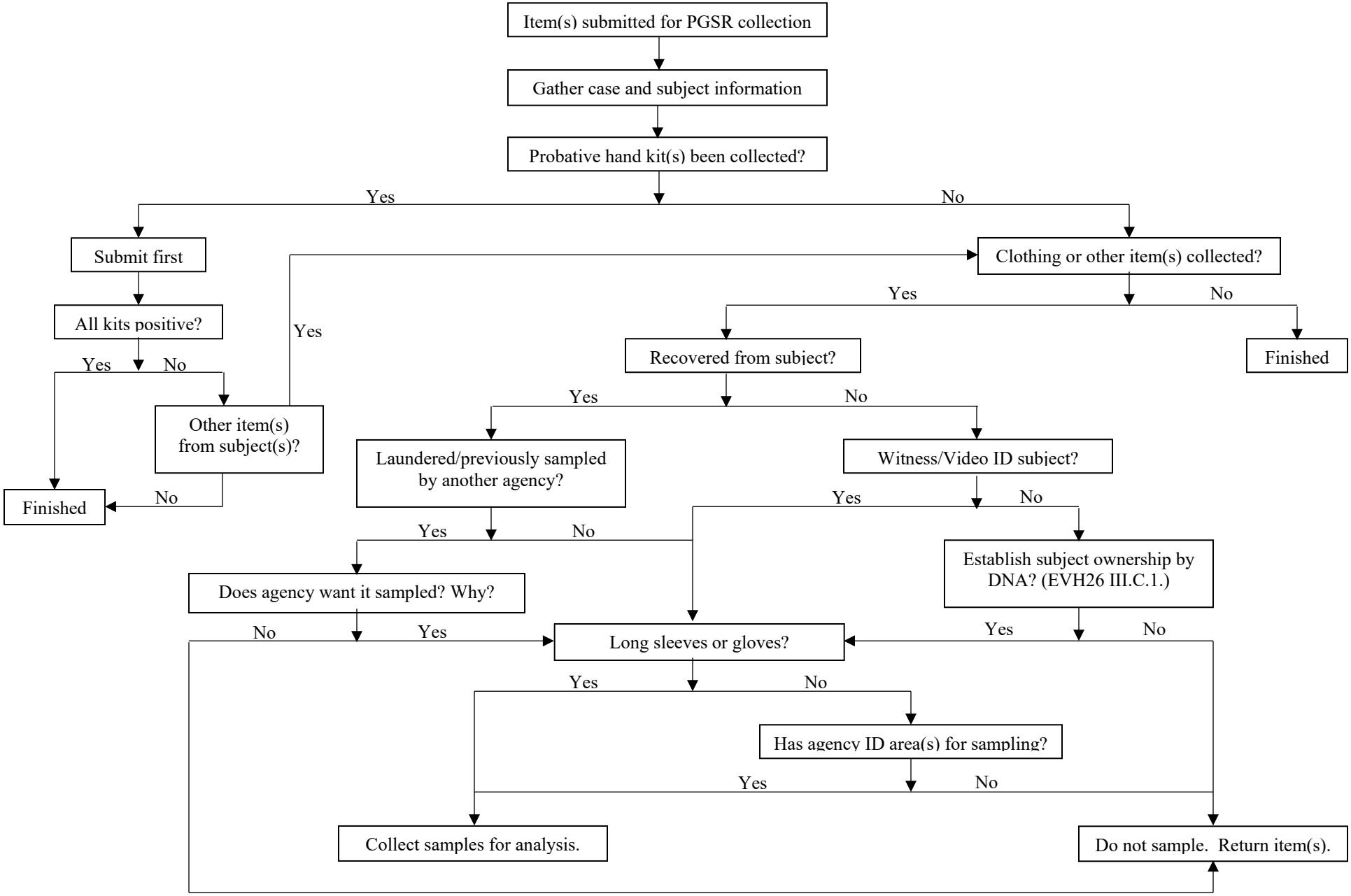
G. Particle collection is achieved by dabbing the sample adhesive to the item throughout the defined location until adhesive tackiness is diminished or the area has been sufficiently sampled.

H. All samples shall be carbon coated prior to SEM analysis.

I. Documentation is achieved by filling out the appropriate LIMS notes describing the item, where the samples were collected, date collected, and subitem numbers.

REPORT WORDING

See Appendix I – Report Wording


Item(s) submitted for PGSR collection
Gather case and subject information
Probative hand kit(s) been collected?
Yes
No
Submit first
All kits positive?
Yes
No
Other item(s) from subject(s)?
Yes
No
Finished
Clothing or other item(s) collected?
Yes
No
Finished
Recovered from subject?
Yes
No
Laundered/previously sampled by another agency?
Yes
No
Witness/Video ID subject?
Yes
No
Does agency want it sampled? Why?
No
Yes
Establish subject ownership by DNA? (EVH26 III.C.1.)
Yes
No
Long sleeves or gloves?
Yes
No
Has agency ID area(s) for sampling?
Yes
No
Collect samples for analysis.
Do not sample. Return item(s).

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: General Unknowns

Method: General Unknowns

Procedure: General Unknowns Identification

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

This protocol is a compilation of specialized analyses which do not appear in any of the proceeding protocols in this manual. This protocol will serve as a resource and guide for general unknown types of analyses which may be requested of the trace chemist.

Examinations of unknown substances are a dynamic series of actions. The process of determining the identity of an unknown substance is called qualitative analysis. This protocol utilizes a qualitative analytical scheme using simple chemical and physical tests followed by confirmatory instrumental analyses to identify unknown substances.

Qualitative analytical schemes are generally summarized by a flow diagram or flow chart. A flow chart is designed with a sequence of procedural operations directed toward identifying the unknown liquid, solid, or gas. The flow charts found in this protocol include the appropriate level of detail to give the trace chemist the analytical tools and guidance required to carry out these chemical analyses and identify an unknown sample.

SAFETY CONSIDERATIONS

A. This protocol involves hazardous materials, operations, and equipment. This protocol does not purport to address all of the safety concerns associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Proper caution should be exercised and the use of personal protective equipment should be considered to avoid exposure to any dangerous substances. Consult the appropriate Safety Data Sheets (SDS) for each chemical prior to use.

Because of the potential hazards related to general unknown evidence, it is highly recommended that the analyst contact the submitting agency and obtain a detailed case history and verify specific analytical requests regarding any potential toxic or hazardous material before beginning any analysis.

Evidence containing totally unknown powders, as well as, all liquids and gases should be initially opened and examined in a ventilated fume hood.

Exercise caution when opening and collecting compressed gases from their containers. The use of pressure regulator valves, appropriate collection devices, and personal protective equipment is highly recommended.

Collect and preserve an unknown gas in an appropriate collection device such as a Tedlar® collection bag or air-tight syringe. The bulk of the collected sample should be stored in a ventilated fume hood during the analysis. Safely release the sampled gas into a ventilated fume hood once the analysis is completed. If consumption is

an issue, refer to Command Directives – EVH32.

B. It is noted that the ISP Forensic Command Micro/Trace Section does not accept extremely toxic (e.g. cyanide, anthrax, and sarin gas) or hazardous materials (e.g. perchloric acid and hydrofluoric acid), poisons, bacteriological, radiological, or nuclear materials as evidence for analysis. Prior to submission, the laboratories may reject these materials and may direct the submitting agency to contact other agencies (Department of Public Health, FBI, ATF, and Department of Homeland Security) for subsequent submission and identification.

PREPARATIONS AND INSTRUMENTATION

The information from the submitting agency and the nature, quality, and quantity of the evidence will dictate what tools, equipment, and instrumentation is required**.** Any and all instrumentation available in the Micro/Trace section may be used. The following suggested instrument parameters are put forward for consideration and may be adjusted by the analyst to obtain the best data for identification, comparison, or characterization of a particular sample.

A. Gas Chromatograph/Mass Spectrometer:

1. GC Conditions:
   - a. Column: Dimethyl Silicone, 0.2 mm (ID) X 25 m, 0.33 µm film
   - b. Carrier: Helium, 0.9 ml/min
   - c. Injector: Split mode (20:1), 250˚C
   - d. Temperature Program:
     - i. Initial Temp.: 40˚C/Hold for 2 minutes
     - ii. Temp. Ramp: 10˚C/min.
     - iii. Final Temp.: 250˚C/Hold for 10 min.

2. GC (Pyrolysis) Conditions:
   - a. Column: 5% Phenyl Methyl Siloxane, 0.25 mm (ID) X 25 m, 0.25 µm film
   - b. Carrier: Helium, 1.5 ml/min
   - c. Injector: Split mode (50:1), 250˚C
   - d. Pyrolyzer:
     - i. Furnace Temp.: 550˚C
     - ii. Interface Temp.: 320˚C
   - d. Temperature Program:
     - i. Initial Temp.: 50˚C/Hold for 2 minutes
     - ii. Temp. Ramp: 10˚C/min.
     - iii. Final Temp.: 300˚C/Hold for 6 min.

3. MS Conditions:

a. Solvent Delay: 0 min.
b. Scan Mode: EI^+
c. Ionization Energy: 70 eV
d. Scan Range: 15-500m/z
e. Source Temp.: 280˚C

B. Fourier Transform Infrared Spectrometer:
1. Transmittance mode
2. Frequency range: 500-4000 cm^{-1}
3. Number of sample scans: 32
4. Resolution: 4

C. Scanning Electron Microscope / Energy Dispersive X-ray:
1. Accelerating voltage: 25KV

D. X-ray Diffractometer:
1. Range: 3.0˚ – 80˚ 2Θ
2. Step: 0.02˚
3. Speed: 2.0˚ / minute
4. Power: 40KV, 15mA
5. Kβ filter
6. Copper tube

E. Stereomicroscope

F. Polarized Light Microscope (PLM)

MINIMUM STANDARDS AND CONTROLS

A. See Minimum Standards and Controls, APP II
B. See Method Fourier Transform Infrared Spectroscopy (MT-IXA)
C. See Method Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (MT-IXB)
D. See Method Gas Chromatography/Mass Spectroscopy (MT-IXC)
E. See Method X-Ray Diffraction (MT-IXD)

PROCEDURE OR ANALYSIS

A. Examinations performed on totally unknown substances are referred to as non-targeted examinations. Examinations performed to confirm an adulteration of one substance by another or to compare, characterize, or identify two or more substances are referred to as targeted examinations. These types of analyses may involve visual observations, wet chemistry techniques, chemical reactivity tests,

physical match, microscopy, microcrystalline tests, physical measurements, physical and chemical separation techniques, and instrumental analysis.
The flow charts in this protocol may be used as a guide to direct the course of action for the analyst to follow. Multiple techniques of increasing selectivity and sensitivity are included in the flowchart and can be used to obtain bulk property or chemical class characterizations and / or exact structural chemical identification of an unknown sample. For targeted examinations, the initial techniques employed in the sequence of the flow chart are left to the discretion of the analyst based on the level of characterization of the analyte(s) required.
The analyst may research for information on the sample. Sources include but are not limited to: on line search of the manufacturer's technical product information, household product database, Safety Data Sheets (SDS) (if available), Particle Atlas, Chemical Dictionary, and The Merck Index.

B. Record a detailed physical description of the evidence. Report the type of packaging and/or container, any markings or images, information on labels, tags, etc., and comprehensive information regarding the contents. Photographic records should be considered but are not required.

C. Bulk Property Characterization
   1. Magnified Optics
      a. A stereomicroscope is useful for initial inspection and assessment of the general class of material(s) present. Record any physical properties (color, transparency/opacity, size, shape, surface texture, etc.) observed. An unknown solid sample containing homogeneous particles may be indicative of a relatively pure sample, while a sample displaying a variety of particulate types is more likely to be composed of a mixture of different chemical species. Gentle pushing with a needle or probe on a particle of the material can reveal hardness, tackiness, or elasticity. If the material is apparent metal, magnetic properties can be shown by utilizing a small magnet and observing the behavior of the material. Comparison to material found in the Microscopy Reference Collection is recommended. A PLM may be used to further classify the unknown material.
      b. Examination of an unknown liquid under magnification may determine the presence of particulates, separate phases or viscidness of the liquid.
   2. Flame Test
      a. The unknown liquid sample may be taken up in a glass pipette or saturated onto a cotton-tipped wooden applicator. Exposure of an unknown liquid to an open flame may characterize the liquid as organic or flammable. Liquid hydrocarbons will form a flammable vapor/air mixture and produce a nearly invisible blue flame, a clean

yellow flame, or a sooty yellow flame depending on the chemical compound class or the formulation of the liquid.

b. A small sample of the unknown solid may be held in a small spatula or made to adhere to a moistened nichrome wire. Exposure of an unknown solid to an open flame may characterize the sample as inert, inorganic, organic, or an energetic mixture. The unknown solid may remain nonreactive, glow, melt, burn, or rapidly combust. Most inorganic compounds are not flammable but will liquefy if their melting points are reached. Exceptions are finely powdered metals such as aluminum, titanium, and magnesium which will burn. Typically, organic compounds are flammable by their nature (they contain carbon and hydrogen). Energetic mixtures contain a fuel and an oxidizer and will rapidly combust. Some metal and metalloid ions also give out colored light when heated in a flame. The color of an element in a flame occurs when electrons in an atom are excited to a higher energy level and then make a transition from that level back to their normal energy state. In that downward transition, energy is released as a photon of light at a specific wavelength which the analyst perceives as a visible color.

   i. A flame test uses a piece of nichrome wire loop attached to an insulated handle.
   ii. Dip the wire in concentrated hydrochloric acid (HCl) and then hold in a Bunsen burner flame.
   iii. If the wire is clean, the color of the flame will not change. If the flame changes color, repeat step ii., above, until the flame shows no color change.
   iv. Grind the unknown solid to a fine powder (if necessary).
   v. Dip the wire loop into concentrated HCl and then into the solid you are testing. If metal ions are present, a small amount of metal chloride is formed on the wire.
   vi. Aqueous metal and metalloid ion solutions may also be tested for color, if the ionic concentration is high enough. Dip the wire loop into the solution you are testing.
   vi. Hold the wire in the Bunsen burner flame. Observe and record any color change. See flame test colors (Table 1).
   vii. Run a control by repeating steps i. through vi., above, with a known reference material.
   ix. Limitations of this test:
      - The test cannot detect low concentrations of most ions.
      - The brightness of the signal varies from one sample to another.

- Impurities or contaminants affect the test results. Sodium, in particular, is present in most compounds and will color the flame with an intense yellow.
- The test cannot differentiate between all elements. Several metals produce the same flame color. Some compounds do not change the color of the flame at all.

c. Gases can be classified into three groups: oxidizers, inert gases and flammable gases. Oxidizers, such as oxygen and chlorine, are not flammable on their own but will act as an oxidant and aid combustion. Inert gases are not combustible and are sometimes used in fire suppression systems. Carbon dioxide and helium are examples of inert gases. Flammable gases can be explosive when mixed with air in the right proportions. If the mixture is to lean or to rich the mixture will not ignite. Hydrogen, butane, methane and ethylene are examples of flammable gases. **Due to the uncertainty in the level of concentration and the potential risk of fire or explosion it is recommended never to ignite an unknown gas**.

3. pH Test
   a. pH will characterize the unknown liquid, the aqueous solution of an unknown solid, or an unknown gas bubbled through deionized water as acidic, basic, or neutral (See Table 3).
4. Miscibility/Solubility
   a. A number of solvents can be used to attempt to show miscibility of an unknown liquid or solubility of an unknown solid. Entering into solution with a polar or non-polar solvent provides an indication of the polarity of an unknown liquid or the ionic character of an unknown solid. See solubility tests flowchart (Figure 4) and solubility of ionic compounds in water (Table 2).
5. Phase Separator Paper
   a. Silicone treated filter paper (e.g. 1PS - Whatman®) allows for the characterization of an unknown liquid as organic or aqueous. Aqueous solution droplets will bead on the filter paper while organic liquid droplets will disperse across the paper.

D. Characterization and Chemical Identification
1. If additional compound class characterization or exact chemical structural identification is required the analyst may perform supplementary techniques of increasing sensitivity and selectivity.
2. Screening techniques can include, but are not limited to:
   a. Chemical spot tests and Microscopic crystalline tests
      i. In a typical spot test, a drop of chemical reagent is added to

a drop of an unknown liquid or a small particle of solid. If the target compound is present, a chemical reaction characterized by one or more observables is produced. Visualization may include bubbling, color change, or the formation of specific microcrystalline shapes. This visual change can reveal the compound class of which the unknown sample is composed. Both blanks (negative control – a sample of similar solution or matrix, such as deionized water that is known not to contain the target compound) and appropriate control samples (positive control – sample of a known substance) must be run concurrently with the unknown sample. A negative test for a target compound class may preclude the need for further sample processing. A positive test is presumptive and identification, if required, will require the appropriate confirmatory techniques. See reagents, chemical reactivity tests, and microscopic crystal tests.

b. Density

i. Density is a physical property of matter. A physical property can be measured without changing the chemical identity of the substance. Density is defined as the mass per unit volume of a substance (D = grams / cm^3). Since pure substances have unique density values, measuring the density of the substance can help identify that substance or may preclude the need for further sample processing.

a) The density of a liquid may be measured directly using a density meter (e.g. Anton Paar DMA 4500).

b) The density of a non-water soluble solid may be measured using the technique of water displacement (Archimedes Principle).

i) Fill a graduated cylinder with water to a known volume. This is the initial volume.

ii) Determine the mass of the unknown solid.

iii) Add the unknown solid to the graduated cylinder and note the new volume of the water. This is the final volume.

iv) Final volume minus initial volume equals the volume of water displaced. Volume of water displaced equals the volume of the unknown solid.

v) Density of the substance equals the mass of the substance per volume of water displaced.

c) Relevant lists of densities may be found in the

periodic table of the elements, Handbook of Chemistry and Physics, or on-line references.

c. Fourier Transform Infrared Spectroscopy (FTIR)

3. Confirmatory techniques can include, but are not limited to:
   a. Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM / EDS)
   b. Gas Chromatography/Mass Spectrometry (GC/MS)
   c. Fourier Transform Infrared Spectroscopy (FTIR)
   d. X-ray Diffractometry (XRD)

E. Unknown Liquid Analysis

1. Using the flowchart (Figure 1), the unknown liquid may be examined optically for multiple layers and particulate matter, flammability, extreme pH ranges for corrosiveness, miscibility, and phase separator paper reactivity. The information from these bulk properties characterizations may preclude the need for further sample processing.
2. See General Unknown Protocol, Specialized Analysis, Acids / Bases / Bleaches Identification, MT-IVA-1, Intoxicating Compound Identification, MT-IVA-2, and Lachrymator Identification, MT-IVA-3, if any of these analyses are required for the unknown liquid.
3. If additional characterization is necessary, FTIR can be performed to reveal the functional groups present in the sample. The information from the presence or absence of any specific compound class characterizations of inorganic or organic functional groups or the identification of the unknown liquid by successful library search and/or comparison to a reference material may preclude the need for further sample processing.
4. The detection of multiple organic species (complex FTIR spectra) may require the analysis of the sample by GC/MS to identify the exact molecular structure of one or more of the chemicals in the matrix or to create chromatographic patterns and extracted ion profiles from highly complex materials, such as refined petroleum products or distillates. See Fire Debris Analysis Protocol, MT-I, for the identification of ignitable liquids.

F. Unknown Solid Analysis

1. Using the flowchart (Figure 2), the unknown solid may be examined optically for homogeneity, combustibility, solubility, and, if soluble in water, pH. The information from these bulk properties characterizations may preclude further sample processing.
2. See General Unknown Protocol, Specialized Analysis, Acids / Bases / Bleaches Identification, MT-IVA-1, or Lachrymator Identification, MT-IVA-3, if either of these analyses is required for the unknown solid.
3. Chemical spot tests and microcrystalline tests coupled with FTIR can reveal the general chemical compound class of which the unknown solid is

composed. A negative result for a target compound class may preclude further sample processing, while a positive result, because of its presumptive nature, must be confirmed by an additional instrumental method such as XRD.

4. If additional characterization is necessary, FTIR can be performed to reveal the functional groups present in the sample. The information from the presence or absence of any specific compound class characterizations of functional groups or the identification of the unknown solid by successful library search and/or comparison to a reference material may preclude the need for further sample processing.
5. Unknown solids displaying simple FTIR spectra, indicated by one or two main absorption bands, must be confirmed by SEM/EDS and XRD.
6. Unknown solids producing complex FTIR spectra may be dissolved in a suitable solvent and analyzed by GC/MS or, if insoluble, may be analyzed directly by Pyrolysis GC/MS.

G. Unknown Gas Analysis

1. Using the flowchart (Figure 3), the unknown gas will, at a minimum, be analyzed by FTIR. An FTIR equipped with a gas cell accessory will rapidly determine the bulk composition of an unknown gas. The detection and successful library matching of the major chemical functional groups may identify the unknown gas and may preclude further testing. Functional groups of other components in the bulk gas may be detected by this method if the concentration of those components is in the ppm range or higher.
2. See General Unknown Protocol, Specialized Analysis, Intoxicating Compound Identification, MT-IVA-2, if a FTIR gas cell analysis is required for the unknown gas/vapor.
3. GC/MS can provide high-confidence information about the exact chemical structure of the unknown gas. A small volume of gas may be directly injected into the GC/MS using a gas tight syringe. For GC/MS to be confirmatory, the use of a specialized chromatographic column (e.g. PLOT, porous layer open tubular) should be employed to produce reference comparable peak(s) with retention time and mass spectral data. However, the mass spectrum of an un-retained peak on a general purpose column, such as a HP1-MS, may provide supporting chemical information about the unknown gas.
4. If enough volume of unknown gas is available, a portion can be bubbled through deionized water and the pH of the water measured. Basic substances such as ammonia gas will increase the pH of the water, while acidic substances such as hydrogen chloride gas will lower the pH of the water. The pH may provide supporting chemical information about the unknown gas.
   a. Working in a ventilated fume hood, samples of the unknown gas are

drawn into a large air-tight syringe or may be sampled directly from the container if a regulator is used. If a regulator is used, close the pressure adjusting knob so that the delivery pressure gauge reads zero.

b. Attach an appropriate length of small diameter tubing to the syringe or regulator.

c. Prepare a small beaker with approximately 20 mL of deionized water.

d. Insert the open end of the tubing into the water and release the unknown gas into the water (by slowly depressing the plunger on the syringe or opening the pressure adjusting knob on the regulator) so that small bubbles of gas percolate through the deionized water at approximately one bubble per/second.

e. Continue to release the unknown gas for approximately one minute.

f. Check the pH of the deionized water/gas product. Concurrently perform blanks and pH controls.

REPORT WORDING

A. See Report Wording, APP I

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: General Unknowns

Method: Specialized Analysis

Procedure: Common Acids, Bases, and Bleaches Identification

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

This procedure provides the analytical approach to the identification of various common acids, bases, and bleaches. The identification of an acid, base, or bleach is based upon the results from a series of tests. It is the responsibility of the analyst to select the appropriate tests that will exclude all other possible acids, bases, or bleaches before an identification is reported.

SAFETY CONSIDERATIONS

A. This protocol involves the use of dangerous chemicals, temperatures, or radiation sources. This protocol does not purport to address all the possible safety hazards or precautions associated with their application. It is the responsibility of the analyst prior to use to review and implement appropriate safety and health practices.

B. The SDS must be consulted unless the analyst is already aware of the hazards associated with the chemicals to be used.

C. Common acids (e.g. HCl, HNO_3, and H_2SO_4), common bases (e.g. NaOH and KOH), and common bleaches (e.g. NaOCl and CaOCl) may be encountered as evidence. They are found in a variety of easily obtained household and commercial products (e.g. battery acid, cement cleaner, oven and drain cleaners, household bleach, and pool disinfectant). These chemical compounds are very corrosive. Corrosive is defined as the ability to destroy something progressively by chemical action. Eye and skin protection must be used while handling these chemicals.

D. Strong acids may be very reactive with chlorates, acetone, flammable liquids, and water. Strong bases react with metals, such as aluminum, to produce hydrogen gas. When heated to decomposition, sodium hypochlorite emits hydrochloric acid fumes and chlorine gas. Spontaneous combustion occurs when calcium hypochlorite reacts with brake fluid and possibly other organic materials. The interaction of these compounds must be avoided.

MINIMUM STANDARDS & CONTROLS

A. See Minimum Standards and Control, APP II.

PREPARATIONS

A. Distilled or deionized water.

B. pH paper or pH meter.

C. Diphenylamine (DPA) Reagent

D. Barium chloride ($BaCl_2$) Reagent

E. Silver nitrate ($Ag\ NO_3$) Reagent

F. Calcium chloride ($CaCl_2$) Reagent

G. Copper chloride ($CuCl_2$) Reagent

H. Iron II chloride ($FeCl_2$) Reagent

I. Iron III chloride ($FeCl_3$) Reagent

J. Nitron Reagent

K. Aniline sulfate Reagent

L. Zinc chloride ($ZnCl_2$) Reagent

M. Fluorescein Reagent

N. Potassium iodide (KI)

O. Potassium bromide (KBr)

P. Starch

Q. Acetone

R. Ammonium hydroxide

S. Ammonia solution

T. Nitric acid

INSTRUMENTATION

A. Fourier Transform Infrared Spectrometer (FTIR).

B. Gas Chromatograph/Mass Spectrometer (GC/MS).

C. X-Ray Diffraction (XRD).

D. Scanning Electron Microscope/Energy Dispersive X-Ray (SEM/EDS).

E. Unless specified in this procedure, suggested parameters for the above instrumentation may be found under the General Unknown Protocol, MT-IV.

F. Polarized Light Microscope (PLM).

PROCEDURE OR ANALYSIS

A. Record a physical description of the container and information on labels, etc. Check Safety Data Sheets (SDS) (if available), household product database, or manufacturer's technical product information for details regarding the evidence. If the evidence is contained on a substrate, record a description of the suspect area with regard to any stain or discoloration (i.e. size and pattern, apparent damage to the substrate, residue, color changes, detectable odor, etc.).

B. pH

1. Place 2 mL of deionized water in a shell vial. Slowly add a few drops of the unknown liquid. If the unknown is a solid, add a small portion of the solid to 2 mL of deionized water. Mix and check pH. An appropriate pH control and deionized water blank must also be run. Suggested acid control is HCl. Suggested base control is NaOH. Suggested bleach control is NaOCl.

2. If the pH is ≤ 2, a strong acid is indicated. If the pH is ≥ 10, a strong base is indicated. If the pH is $\geq$ 10 and the color indicator pads become "bleached" over time, a hypohalogenite is indicated. See Table 3 for pH.

C. Chemical Reactivity tests

1. Specific reagents react with the sample to reveal the general compound class of which the sample is composed. The results of the pH testing and the indication of a target ion from the chemical reactivity tests will direct the course of the analysis. Blanks and controls must be performed concurrently with the chemical reactivity tests. A positive test is presumptive and will require confirmation by instrumental analysis. A negative test may preclude further examination. However, the comprehensive chemical reactivity tests for hypohalogenites are sufficient to demonstrate the presence of a specific hypohalogenite. No known materials will give a false positive for the series of chemical reactivity tests for hypohalogenites. No two hypohalogenites can coexist together in solution.

Target Ion	Reagent	Color or Precipitate	Suggested Control
Cl^-	Silver nitrate	White precipitate	HCl
Br^-		Cream precipitate	KBr
I^-		Yellow precipitate	KI
NO_3^-	Diphenylamine	Blue color	KNO_3
SO_4^{-2}	Barium chloride	White precipitate	H_2SO_4
OH^-	Calcium chloride	White precipitate	NaOH
	Copper chloride	Blue precipitate	
	Iron II chloride	Green precipitate	
	Iron III chloride	Brown precipitate	
ClO^-	Zinc chloride Fluorecein/KBr	Salmon	NaClO
BrO^-	Zinc chloride Fluorecein	Red	NaBrO
IO^-	Zinc chloride Starch	Blue	NaIO

2. Utilize the silver nitrate and barium chloride flow charts in Figures 5 and 6, as well as the analytical sections for the individual target compounds below for additional detail regarding these chemical reactivity tests.

3. Continue and identify using the appropriate acid, base, or bleach test(s).

D. Strong Acids: Strong acids completely dissociate (ionize) in water, forming H^+ and an anion.

There are six strong acids:

- HCl - hydrochloric acid
- H_2SO_4 - sulfuric acid
- HBr - hydrobromic acid
- HI - hydroiodic acid
- HNO_3 - nitric acid
- $HClO_4$ - perchloric acid*

HCl and H_2SO_4 are encountered most frequently in casework.

*$HClO_4$, perchloric acid, is a strong oxidizing agent. It reacts violently or explosively with many organic and inorganic compounds. It should not be accepted by any laboratory for analysis.

1. Tests for Hydrochloric Acid:

    a. Vapor in a gas cell using FTIR may be run as a confirmatory test.

        i. Gas Cell Procedure

            a) Attach the gas cell to a rotary vacuum pump with flexible tubing attached to one stopcock and open the stopcock.

            b) Evacuate the gas cell for approximately 2 minutes and close the stopcock.

            c) Collect and save a background on the FTIR.

            d) Run the evacuated gas cell as a blank on the FTIR.

            e) In a ventilated fume hood, collect approximately 3 milliliters of the vapor over the suspect liquid in a syringe.

            f) Attach the syringe directly to the gas cell inlet with flexible tubing and open the stopcock. Release the vapor from the syringe into the gas cell. The volume of vapor required for analysis will be dependent upon the concentration of the vapor over the liquid.

            g) Run the vapor in the gas cell on the FTIR.

            h) Complete a library search or compare spectra obtained to reference material run under the same parameters.

        ii. Suggested FTIR parameters:

            a) Number of scans: 32

            b) Range: 2550 – 3125 cm^{-1}

            c) Resolution: 0.5 cm^{-1}

    b. Microcrystalline test and Instrumental analysis

        i. Place approximately 1 mL of sample into a test tube.

        ii. Carefully add two drops of silver nitrate reagent (see Figure 5, silver nitrate flowchart).

iii. A heavy white precipitate forms.

iv. Add 3 drops of concentrated nitric acid. If the white precipitate remains, the presence of a chloride ion is indicated.

v. Centrifuge. Collect, rinse with deionized water, and dry the precipitate. Color of the precipitate may darken when precipitate is exposed to light.

vi. Run the precipitate on SEM/EDS and XRD for confirmatory identification.

vii. The precipitate formed should be AgCl, if HCl is present.

viii. Place the precipitate in a marked vial and return with the evidence.

2. Test for Sulfuric Acid:

a. Microcrystalline test and Instrumental analysis

i. Place approximately 1 mL of sample into a test tube.

ii. Carefully add two drops of barium chloride reagent (see Figure 6, barium chloride flowchart).

iii. A heavy white precipitate forms.

iv. Centrifuge. Collect, rinse with deionized water, and dry the precipitate.

v. Run the precipitate on SEM/EDS and XRD for confirmatory identification.

vi. The precipitate formed should be $BaSO_4$, if H_2SO_4 is present.

vii. Place the precipitate in a marked vial and return with the evidence.

3. Test for Hydrobromic Acid:

a. Microcrystalline test and Instrumental analysis

i. Place approximately 1 mL of sample into a test tube.

ii. Carefully add two drops of silver nitrate reagent (see Figure 5, silver nitrate flowchart).

iii. A heavy cream-colored precipitate forms.

iv. Add 3 drops of concentrated nitric acid. If the precipitate remains, the presence of bromide ion is indicated.

v. Centrifuge. Collect, rinse with deionized water, and dry the precipitate. Color of the precipitate may darken when precipitate is exposed to light.

vi. Run the precipitate on SEM/EDS and XRD for confirmatory identification.

vii. The precipitate formed should be AgBr, if HBr is present.

viii. Place the precipitate in a marked vial and return with the evidence.

4. Test for Hydroiodic Acid:

   a. Microcrystalline test and Instrumental analysis

      i. Place approximately 1 mL of sample into a test tube.

      ii. Carefully add two drops of silver nitrate reagent (see Figure 5, silver nitrate flowchart).

      iii. A heavy yellow colored precipitate forms.

      iv. Add 3 drops of concentrated nitric acid. If the precipitate remains, the presence of iodide ion is indicated.

      v. Centrifuge. Collect, rinse with deionized water, and dry the precipitate. Color of the precipitate may darken when precipitate is exposed to light.

      vi. Run precipitate on SEM/EDS and XRD for confirmatory identification.

      vii. The precipitate formed should be AgI, if HI is present.

      viii. Place the precipitate in a marked vial and return with the evidence.

5. Test for Nitric Acid:

   a. Preliminary color test for Nitric Acid:

      i. Place 2 drops of DPA reagent into a spot plate well.

ii. Add one drop of the sample to the DPA.

iii. Immediate development of a deep blue color indicates the presence of nitrate ion or nitrate containing compounds.

b. Preliminary microcrystalline test for Nitric Acid:

i. Place a drop of sample on a microscope slide.

ii. Place a drop of nitron reagent on the slide in close proximity to the sample drop.

iii. Draw the nitron reagent drop into the sample drop using a needle or wire.

iv. A white precipitate forms.

v. Using a Polarized Light Microscope (PLM), long thin needles and imperfect radiates ("bow ties") appear showing high-order retardation colors if nitrates are present.

c. Confirmatory test for Nitric Acid, as Nitron nitrate:

i. Place approximately 2 mL nitron reagent in a large test tube.

ii. Add approximately 2 mL of the sample to the tube.

iii. A white precipitate forms.

iv. Centrifuge. Collect, rinse with deionized water, and **air** dry precipitate.

v. Run precipitate on FTIR/ATR for confirmatory identification.

vi. The precipitate formed should be $C_{20}H_{16}N_4 \cdot HNO_3$, if HNO_3 is present.

vii. Place the precipitate in a marked vial and return with the evidence.

6. Tests for Other Acids:

a. Test for Phosphoric Acid:

i. Microcrystalline test and Instrumental analysis

a) Place approximately 6 mL of acetone into a large test tube.

b) Add approximately 3 drops of the sample into the test tube and mix.

c) Add approximately 3 drops of ammonium hydroxide and mix.

d) A white precipitate should forms.

e) Centrifuge, remove the liquid, and rinse the precipitate with acetone.

f) Remove acetone and dry precipitate.

g) Run precipitate on XRD for confirmatory identification.

h) The precipitates formed should be $NH_4H_2PO_4$ and $(NH_4)_2\ HPO_4$, if H_3PO_4 is present.

i) Place the precipitate in a marked vial and return with the evidence.

b. Tests for Acetic Acid:

i. Test for Acetic Acid using GC/MS.

a) Inject 0.2 microliter of the sample into the GC/MS. Use the column and instrument parameters for oxygenate analysis (see TC IIA-2).

b) Alternatively, inject 0.1cc of vapor into the GC/MS. Use the column and instrument parameters for oxygenate analysis (see TC IIA-2).

c) Inject an acetic acid reference for retention time and spectral comparisons for confirmatory identification.

ii. Test for Acetic Acid vapor in a gas cell using the FTIR.

a) See section D.1.a.i. in Procedure or Analysis above for gas cell procedure.

b) Suggested FTIR parameters:

i) Number of scans: 32

ii) Range: 500 – 4000 cm^{-1}

iii) Resolution: 4 cm^{-1}

E. Strong Acid on Clothing or other Substrates

1. If the clothing or substrate is wet or damp, attempt to collect the liquid directly with the use of a Pasteur pipet and bulb. If liquid is extracted, continue with steps 4 and 5. If recovery of liquid is not possible, take a pH measurement directly in the suspect area and then proceed to steps 2, 3, and 5.

2. If appropriate, remove a swatch, approximately 5 cm x 5 cm, of the suspect area along with an equal sized "protected" area swatch for use as a control. A protected area contains no strong acid, has no stains or discoloration, and is likely to be found on the back side of a garment or opposite from the direction of contact of the corrosive liquid. If removal of a swatch is not possible, swab the suspect and control areas with separate swabs wet with deionized water and continue with step 4. Record the physical location of these sampled areas on your worksheet.

3. Extract the suspect swatch and control swatches with approximately 10 mL of deionized water. If additional deionized water is required it should be applied equally and sparingly to both the suspect and control swatches.

4. Check pH. If the suspect extract needs to be concentrated, gently warm on a hotplate. The control extract needs to be concentrated to the same extent as the sample extract.

5. Filter the suspect and control aqueous extracts and analyze using steps in sections B.2., C., and D. in Procedure or Analysis above.

6. Place any liquid recovered from the substrate in a marked vial and return with the evidence.

F. Strong Bases: Strong bases completely dissociate (ionize) in water forming OH^- (hydroxide ion) and a cation.

The hydroxides of the Group I and Group II metals are considered to be strong bases:

- NaOH - Sodium hydroxide
- KOH - Potassium hydroxide
- LiOH - Lithium hydroxide
- RbOH - Rubidium hydroxide
- CsOH - Cesium hydroxide
- Ca(OH)2 - Calcium hydroxide (not very soluble in water)
- Sr(OH)2 - Strontium hydroxide (not very soluble in water)
- Ba(OH)2 - Barium hydroxide (not very soluble in water)

NOTE: NaOH and KOH are encountered most frequently in casework.

1. Tests for Hydroxide ion:

    a. Microcrystalline tests and Instrumental analysis

        i. Hydroxide will precipitate metal ions.

        ii. A series of test tubes is prepared each containing approximately 2 mL of each of the following reagents:

            a) 10% Calcium chloride

            b) 10% Copper chloride

            c) 10% Iron II chloride

            d) 10% Iron III chloride

        iii. Add approximately 2 mL of sample into each test tube.

        iv. A precipitate should form.

            a) The table below shows the metal ion and precipitate formed.

Metal Ion	Precipitate
Calcium Ca^{2+}	white $Ca(OH)_2$
Copper Cu^{2+}	blue $Cu(OH)_2$
Iron Fe^{2+}	green $Fe(OH)_2$
Iron Fe^{3+}	Brown $Fe(OH)_3$

            Note: Precipitate colors should be visualized immediately. The green precipitate of $Fe(OH)_2$ turns brown over time due to the oxidation of the Fe^{2+} ions in air.

        iv. Centrifuge. Collect, rinse with deionized water, and dry the **white** precipitate.

        v. Run the precipitate on SEM/EDS and XRD for confirmatory identification.

        vi. The precipitate formed should be $Ca(OH)_2$, if hydroxide is present.

vii. Place the precipitate in a marked vial and return with the evidence.

b. Direct instrumental analysis of a liquid sample.

i. Evaporate approximately 10 mL of sample in a beaker in an oven at 90˚C +/- 5˚C.

ii. Run the residue on SEM/EDS and XRD for confirmatory identification.

iii. The results will identify the cation, the hydroxide, the hydroxide hydrate, and the carbonate in varying concentrations (e.g. Na^+, NaOH, $NaOH{\cdot}H_2O$, Na_2CO_3), if a strong base is present.

iv. Place the residue in a marked vial and return with the evidence.

Note: Hydroxides constantly absorb carbon dioxide (CO_2) from the air, are converted to carbonates, and loose strength as a base. The more complete the decomposition of the hydroxide, the more carbonate is recovered in the residue.

G. Strong Base on Clothing or other Substrates

1. If the clothing or substrate is wet or damp, attempt to collect the liquid directly with the use of a Pasteur pipet and bulb. If liquid is extracted, continue with steps 6. and 7. below. If recovery of liquid is not possible, take a pH measurement directly in the suspect area and then proceed to steps 4., 5., and 7. below.

2. Conversely, if the clothing or substrate is dry and residue is observed, scrape to remove and collect any residue from the clothing or substrate.

3. Analyze the residue using steps in sections B.2., C., and F. in Procedure or Analysis above.

4. If no residue is observed or recovery is not possible, and if appropriate, remove a swatch, approximately 5 cm x 5 cm, of the suspect area along with an equal sized “protected” area swatch for use as a control. A protected area contains no strong base, has no crystalline residue or discoloration, and is likely to be found on the back side of a garment or opposite from the direction of contact of the corrosive liquid. If removal of a swatch is not possible, swab the suspect and control areas with separate swabs wet with deionized water and continue with step 6. Record the physical location of these sampled areas on your worksheet.

5. Extract the suspect and control swatches with approximately 10 mL of deionized water. If additional deionized water is required it should be applied equally and sparingly to both the suspect and control swatches.

6. Check pH. If the suspect extract needs to be concentrated, gently warm on a hotplate. The control extract needs to be concentrated to the same extent as the sample extract.

7. Filter the suspect and control extracts and analyze using steps in sections B.2., C., and F. in Procedure or Analysis above.

8. Place any liquid or residue recovered from the substrate in a marked vial and return with the evidence.

H. Hypohalogenite Bleaching Agents

1. A bleaching agent is a material that lightens or whitens through chemical reaction often via oxidation.

2. The most common bleaching agents fall into two categories: hypohalogenite (e.g. sodium hypochlorite, calcium hypochlorite) and peroxygen (e.g. sodium percarbonate, peroxyacetic acid, hydrogen peroxide).

   Note: Non-hypohalogenite (peroxygen) bleaching agents may be analyzed using the unknown liquid or solid flowcharts in this protocol.

   Sodium hypochlorite (household bleach) and calcium hypochlorite (bleaching powder or swimming pool algaecide) are most commonly encountered in casework, although, hypobromites and hypoiodites may be encountered.

3. Hypohalogenite, Liquid or Solid (in aqueous solution)

   a. pH

      i. See section B, Procedure or Analysis above. If pH $\leq$ 7, it is not a hypohalogenite. If a pH indicator strip is used, look for the "bleaching" of the color indicator pads over time to indicate presence of hypohalogenite.

   b. Chemical Reactivity tests

      i. See section C. of Procedure or Analysis above for reactivity table.

ii. Blanks, if applicable, and controls must be run concurrently.

iii. Place 1 drop of zinc chloride reagent into the well of a white spot plate.

iv. Add a few grains of starch.

v. Add 1 drop of the unknown liquid. A dark blue color indicates the presence of OI^-. If this occurs continue to step vii. If no color appears continue with step vi.

vi. Add a few small crystals of potassium iodide. A dark blue color indicates the presence of ClO^- or BrO^-. Continue with step vii. to differentiate between ClO^- and BrO^-. If no color appears, stop here, no hypohalogenite is present in the unknown liquid.

vii. To an unused white spot plate well, place 1 drop of zinc chloride reagent.

viii. Add 1 drop of fluorescein reagent.

ix. Add 1 drop of the unknown liquid. A red color indicates the presence of BrO^-. If this occurs, stop here. BrO^- is indicated and differentiated from ClO^- because two hypohalogenites cannot exist together in solution. If no color appears, continue to step x.

x. Add a few grains of potassium bromide. A salmon color indicates the presence of ClO^-.

c. Instrumental analysis

i. Dried liquid hypohalogenites will yield the halogen and the halogenate salts, as well as the identity of the cation. (e.g. Na^+, NaCl, $NaClO_3$)

ii. If the unknown is a liquid, filter and evaporate to dryness in a ventilate fume hood. Continue with step iv. or step v.

iii. If the unknown is a solid, dry in a desiccator (if necessary). Proceed to step v.

iv. Chemical Reactivity tests on <u>rehydrated</u> sample from step ii above. (optional)

a) Place 1 drop of aniline sulfate reagent into a well of a white spot plate.

b) Add 1 drop of the concentrated aqueous solution of the unknown from step ii. above.

c) Add two drops of concentrated sulfuric acid. A blue ring at the aqueous/acid interface indicates the presence of ClO_3^-.

d) To an unused white spot plate well, place 1 drop of silver nitrate reagent.

e) Add 1 drop of the concentrated aqueous solution of the unknown from step ii. above. A white precipitate indicates Cl^-. Add 2 drops of concentrated nitric acid. If the white precipitate remains, Cl^- is indicated. See silver nitrate flowchart, Figure 5, for possible additional precipitates/ions.

f) Run the precipitate on SEM/EDS and XRD for confirmatory analysis.

g) The precipitate formed should be AgCl, if chloride ion is present.

h) Place the precipitate in a marked vial and return with the evidence.

v. Run the residue from the evaporated liquid from step ii. or original solid sample from step iii. above on SEM/EDS and XRD.

vi. The result will identify the cation present along with the halogen and the halogenate salts as decomposition products (e.g. NaOCl only exists in aqueous solution. If allowed to dry, NaCl and $NaClO_3$ will be found. $Ca(OCl)_2$ can exist as a solid. $Ca(OCl)_2$, $CaCl_2$, and $Ca(ClO_3)_2$ will be found).

vii. Place the evaporated residue from the liquid sample in a marked vial and return with the evidence.

4. Hypohalogenites on Clothing or other Substrate

a. If the clothing or substrate is wet or damp, attempt to collect the liquid directly with the use of a pasteur pipet and bulb. If liquid is extracted, continue with steps d and e below. If recovery of liquid is not possible, take

a pH measurement directly in the suspect area and then proceed to steps b, c, and e below. If the clothing or substrate is dry proceed with the step b.

b. If appropriate, remove a swatch, approximately 5 cm x 5 cm, of the suspect area along with an equal sized "protected" area swatch for use as a control. A protected area contains no hypohalogenite, has no stains or discoloration, and is likely to be found on the back side of a garment or opposite from the direction of contact of the corrosive liquid. If removal of a swatch is not possible, swab the suspect and control areas with separate swabs wet with deionized water and continue with step d. Record the physical location of these sampled areas on your worksheet.

c. Extract the suspect swatch and control swatch with approximately 10 mL of deionized water. If additional deionized water is required it should be applied equally and sparingly to both the suspect and control swatches.

d. Check pH. If the suspect extract needs to be concentrated, gently warm on a hotplate. The control extract needs to be concentrated to the same extent as the sample extract.

e. Filter the recovered liquid or suspect and control extracts and analyze using steps in section H.3. steps b. and c. in Procedure or Analysis above. If the clothing or substrate was dry, evaporate the suspect and control extracts to dryness and analyze using steps in section H.3. step c. in Procedure or Analysis above.

REPORT WORDING

A. See Report Wording, APP I

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: General Unknowns

Method: Specialized Analysis

Procedure: Intoxicating Compounds Identification

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

A. An act prohibiting the sale or use of certain compounds for the purpose of inducing in the user an intoxicated condition. Illinois Compiled Statues - 1995.

1. Chapter 720, Section 690/1 (use) (1) "No person shall breathe, inhale or drink any compound, liquid or chemical containing toluol, hexane, trichlorethylene, acetone, toluene, ethyl acetate, methyl ethyl ketone, trichoroethane, isopropanol, methyl isobutyl ketone, methyl cellosolve acetate, cyclohexanone, or any other substance for the purpose of inducing a condition of intoxication, stupefaction, depression, giddiness, paralysis or irrational behavior, or in any manner changing, distorting or disturbing the auditory, visual or mental processes. For the purposes of this Act, any such condition so induced shall be deemed to be an intoxicated condition."

2. Chapter 720, Section 690/2 (sale) (2) "No person shall knowingly sell or offer for sale, deliver or give to any person under 17 years of age, unless upon written order of such person's parent or guardian, any compound liquid or chemical containing toluol, hexane, trichloroethylene, acetone, toluene, ethyl acetate, methyl ethyl ketone, trichloroethane, isopropanol, methyl isobutyl ketone, methyl cellosolve acetate, cyclohexanone, or any other substance which will induce an intoxicated condition, as defined herein, when the seller, offer or or deliverer knows or has reason to know that such compound is intended for use to induce such condition."

3. Chapter 720, Section 570/312 (Dispensing Controlled Substances) (9) "No person shall distribute or dispense butyl nitrite for inhalation or other introduction into the human body for euphoric or physical effect."

B. This procedure may be used for any liquid or vapor (gas) suspected of being an intoxicating compound.

C. Liquids such as toluene, acetone, methyl ethyl ketone, and alkyl nitrites; petroleum products such as lacquer thinners and paint strippers; and compressed gases, such as chlorofluorocarbons, isobutane, nitrous oxide, found in aerosol dispensers or cylinders may be submitted as evidence related to inhalant abuse.

1. Commercial products that contain alkyl nitrites may have peculiar labels such as: "Rush", "Locker Room", "Jungle Juice", "Quick Silver", "Hardware", "Bolt", or "Bullet". These products may be marketed and sold as "head cleaner" or "room odorizers". Referred to in a slang term as "poppers", these products are usually packaged in 10 mL and 30 mL screw cap sealed brown bottles that routinely may also contain a small white

spherical object. This object is intentionally added by the manufacturer and is a desiccant that absorbs water and helps stabilize the product.

2. Prescription inhalers or crushable ampules may contain amyl nitrite.

3. Alkyl nitrites may be prepared at the laboratory following the procedure found in 91-TRACE-6. The nitrite is prepared by mixing a parent alkyl alcohol with sulfuric acid and a solution of sodium nitrite. Nitrites are not stable compounds. Over time and upon exposure to air, moisture, and light, the alkyl nitrite will degrade to the parent alkyl alcohol. The parent alkyl alcohol will be found with the target alkyl nitrite in varying percentages in the sample depending on the state of degradation. Cyclic nitrites have also been encountered in casework, but are not common (See 05-TRACE-3).

4. Alkyl nitrite information:

NAME	CAS NUMBER	BOILING POINT (˚C)
Ethyl Nitrite	109-95-5	17
Isopropyl Nitrite	541-42-4	39
N-propyl Nitrite	543-67-9	44
Tert-butyl Nitrite	540-80-7	63
Iso-butyl Nitrite	542-56-3	67
Sec-butyl Nitrite	924-43-6	68
N-butyl Nitrite	544-16-1	75
Iso-amyl Nitrite (Amyl Nitrite)	110-46-3	99
N-amyl Nitrite (Pentyl Nitrite)	463-04-7	104

MINIMUM STANDARDS & CONTROLS

A. See Minimum Standards and Controls, APP II.

SAFETY CONSIDERATIONS

A. This protocol involves hazardous materials, operations and equipment. This protocol does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Proper caution should be exercised and the use of personal protective equipment should be considered to avoid exposure to dangerous chemicals. Consult the appropriate Safety Data Sheets (SDS) for each chemical prior to use.

B. Intoxicating compounds are volatile substances that must be handled in a ventilated fume hood. FTIR with ATR accessory is **NOT** recommended due to the volatile nature, physiological effects, and health hazards associated with intoxicating compounds. Liquids analyzed by FTIR must be analyzed in a sealed system such as a liquid cell or gas cell.

C. Compressed tanks and cylinders contain gas under high pressure. The gas, when released, as well as the tank or cylinder, may become very cold. The use of personal protective equipment (PPE) is highly recommended.

D. See Safety Considerations – General Unknown Protocol, MT-IV.

PREPARATIONS

A. Microliter syringe
B. Disposable air-tight syringe (0.5cc – 10cc)
C. Short length of small diameter flexible tubing sized to fit air-tight syringe
D. Solid-Phase Micro-extraction syringe
E. Vapor cell (e.g. 10 cm path length)
F. Liquid cell
G. Rotary vane vacuum pump, or equivalent
H. Thick-walled vacuum tubing
I. Latex balloon or other commercial sampling bags (TEDLAR®, Teflon)

INSTRUMENTATION

A. Gas Chromatograph/Mass Spectrometer - suggested parameters

1. GC Conditions:

a. Column: 25 meter Dimethyl Silicone, 0.2 mm (i.d.) X 0.33 µm

b. Carrier: Helium, 0.9 ml/min.

c. Injector: 250°C Split mode (25:1)

d. Temperature Program:

i. Initial Temp: 40°C/Hold for 2 min.

ii. Temp. Ramp: 8°C/min.

iii. Final Temp: 180°C/Hold for 0.5 min.

2. MS Conditions:

a. Solvent Delay: 0 min.

b Scan Mode: EI^+

c Ionization Energy: 70 eV

d. Scan Range: 15-400 m/z

e. Source Temp: 230°C

B. Fourier Transform Infrared Spectrometer (FTIR) – suggested parameters

1. Resolution: 4 cm^{-1}

2. Scan Range: 400 - 4000 cm^{-1}

3. Number of Scans to Average: 32

Note: FTIR is not recommended for the identification of simple or complex mixtures.

PROCEDURE OR ANALYSIS

A. Record a physical description of the container and information on labels, etc. Check Safety Data Sheets (SDS) (if available), household product database, or manufacturer's technical product information for details regarding the evidence.

B. Single Compound Identification

1. Liquid

a. GC/MS

i. Inject approximately 0.02µL of the liquid (or use the wet needle technique) into a GC/MS to identify compound.

ii. Complete a library search and compare spectrum and retention times to reference material run under the same parameters.

b. FTIR/Liquid Cell Procedure

i. Collect and save a background on the FTIR.

ii. Run the empty liquid cell as a blank on the FTIR.

iii. In a ventilated fume hood, the liquid sample is introduced into the cell by vacuum. Two syringes are employed, one at the top syringe (luer) port filled with sufficient liquid to fill the cell and an empty one at the bottom syringe port. The quantity of sample depends on the capacity (volume) of the specific cell. The sample is introduced by pulling the plunger of the empty syringe until the sample shows at its bottom. A few seconds pause to allow for pressure equilibration is recommended. Then, the cell should be placed in the vertical position, syringes removed, and the luer lock ports plugged with stoppers.

iv. Run the liquid on the FTIR.

v. Complete a library search or compare spectrum obtained to reference material run under the same parameters.

c. FTIR/Gas Cell Procedure

i. Collect and save a background on the FTIR.

ii. Run the gas cell as a blank on the FTIR.

iii. Place 1-3 drops of the liquid directly in the gas cell.

iv. Pause approximately 1 minute to allow the vapor to equilibrate in the gas cell and then run the vapor in the gas cell on the FTIR.

v. Complete a library search or compare spectrum obtained to reference material run under the same parameters.

Note: Use of both FTIR techniques above may allow you to identify more than one component in a mixture (i.e., one component may predominate in liquid phase and another in the vapor phase).

2. Vapor (gas)

a. GC/MS procedure

i. Collect the sample as in step v. Sampling, below.

ii. Inject approximately 0.1 cc of vapor into a GC/MS to identify the compound.

iii. Complete a library search and compare spectrum and retention times to reference material run under the same parameters.

b. FTIR/Gas Cell Procedure

i. Attach the gas cell stopcock to a vacuum pump with flexible tubing and open the stopcock.

ii. Evacuate the gas cell for approximately 2 minutes and close the stopcock.

iii. Collect and save a background on the FTIR.

iv. Run the evacuated gas cell as a blank on the FTIR.

v. Sampling

a) Vapor over the liquid - In a ventilated fume hood, collect approximately 3 mL of the headspace vapor sample in a gas-tight syringe.

b) Compressed gas tanks with valves and/or regulators - In a ventilated fume hood, collect the sample in an appropriate container (Tedlar® bag or gas-tight syringe). Approximately 3 mL of the gas sample in the Tedlar® bag will need to be transferred to a gas-tight syringe for analysis.

c) Small compressed gas cylinders (8, 12, and 16 gram size): These small sealed steel cylinders usually contain carbon dioxide or nitrous oxide gas. The nitrous oxide cylinders may be colored and are used commercially as whipped cream chargers (Whip-It!™, Whip-eez™, Bestwhip™). The carbon dioxide cylinders are typically unpainted and are used

commercially for air guns, paintball guns and to carbonate single-serving beverages. To access the gas inside a "cracker" or specialized dispenser must be used. In a ventilated fume hood, place the cylinder in the dispenser, and tighten the screw cap. As the cap is tightened, a small hollow pin in the dispenser cap will puncture the top of the cylinder and a rubber o-ring will seal off the opening. A trigger on the dispenser will release the gas. The outlet of the dispenser can release the gas directly through the stopcock into the gas cell.

Caution: The dispenser and gas cylinder may become very cold and/or freeze during this operation, especially if they are not kept in an upright position.

Note: The gas in these small disposable cylinders will be consumed in analysis since there is no way to save the sampled gas or reseal the cylinder once it is punctured. If consumption of the evidence is an issue, see Command Directive EVH 32.

vi. Attach the syringe directly to the gas cell inlet and open the stopcock. Release the vapor/gas from the syringe into the gas cell. The volume of vapor/gas required for analysis will be dependent upon the concentration of the vapor/gas and the volume of the specific gas cell.

vii. Run the vapor/gas in the gas cell on the FTIR.

viii. Complete a library search or compare spectrum obtained to reference material run under the same parameters.

C. Simple Mixture Identification (2-5 Compounds)

1. Inject into a GC/MS either a liquid sample, as in step B.1.a.i. above, or vapor sample, as in step B.2.a.i. above, using either an isothermal or temperature program that will sufficiently resolve all of the components of the mixture.

2. Complete a library search.

3. Each individual compound identified in a simple mixture must have both chromatographic retention time and mass spectral comparison to reference material.

D. Complex Mixture Identification

1. Inject into a GC/MS either a liquid as in step B.1.a.i. above, or vapor sample, as in step B.2.a.i. above, using a temperature program that will sufficiently resolve all of the components of the mixture.

2. Complete a library search.

3. The class of a complex mixtures (e.g., gasoline, paint thinner, etc.) can be identified from their chromatographic patterns and extracted ion profiles using the same criteria as per MT-I (Fire Debris Protocol) or each individual compound identified in a complex mixture must have both chromatographic retention time and mass spectral comparison to reference material.

E. Intoxicating Compound on Substrate

1. Substrates containing an intoxicating compound **must** be submitted by the agency in an air-tight sealed container such as a clean paint can with a friction fit lid. If the evidence is received in a paper or plastic bag, the intoxicating compound(s) may be wholly or partially lost through evaporation or contaminated due to the permeability of the containers. Such evidence is not suitable for analysis due to improper packaging and will be returned to the submitting agency without analysis.

2. In a ventilated fume hood, open the sealed can to quickly view the evidence.

   i. Verify the evidence is safe to examine.

   ii. Record a physical description of the substrate.

   iii. Take note if the substrate is wet.

3. If the substrate is wet, attempt to collect any liquid directly by using a Pasteur pipet and bulb. Place any recovered liquid in a marked vial to be analyzed and then returned with the evidence. Even if no liquid is recovered, trace amounts of volatile material may still be present on the substrate and analyzed.

4. If liquid was recovered, analyze for intoxicating compounds using either steps B.1.a. or B.1.b., C or D in Procedures or Analysis above based on single compound, simple mixture, or complex mixture identification.

5. If liquid was not recovered, analyze for intoxicating compounds using the analytical procedure(s) referenced in MT-IA-2 (Heated Headspace), MT-IA-3 (Adsorption/Elution – Dynamic/Active), or MT-IA-4 Adsorption/Elution – Static/Passive) based on single compound, simple mixture, or complex mixture identification. All of the procedures will utilize GC/MS for identification using either an isothermal or a temperature program that will sufficiently resolve all of the components of the mixture. See Instrumentation, A. above for suggested GC/MS parameters.

6. Place any extract from the procedures in step 5. above in a marked vial and return with the evidence.

REPORT WORDING

A. See Report Wording, APP I.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: General Unknowns

Method: Specialized Analysis

Procedure: Lachrymators: Extraction and Identification

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

A. Lachrymators are the active ingredient present in tear gas and self-defense sprays. These compounds produce an inflammation of the skin and eyes and respiratory irritation. Dissolved in a solvent and contained within an aerosol dispenser, some lachrymators are commonly used by the military and law enforcement agencies to control crowds while some lachrymators are available to the public commercially as personal protection and animal repellant sprays.

B. Three different lachrymators are commonly used in aerosol chemical sprays.

1. Military - CS (o-Chlorobenzalmalononitrile), a white crystalline solid, e.g. ABC-M7A2, ABC-M7A3, "SABRE®" brand.

    a. Some commercial brands may be a mixture of CS and capsacin.

2. Law enforcement - CN (2-Chloroacetophenone), a white crystalline solid, e.g. "Mace®" brand.

    a. Some commercial brands may be a mixture of CN and capsacin.

3. Personal use pepper spray - OC (Oleoresin Capsicum, i.e. Capsaicin) (8-Methyl-N-vanillyl-trans-6-nonenamide), an amber to dark red-orange viscous liquid, e.g. "Halt!®" brand animal repellant

    a. The major capsaicinoids of the capsicum species are capsaicin, dihydrocapsaicin, and nordihydrocapsaicin.

    b. Nonivamide, also called pelargonic acid vanillylamide or PAVA (*N*-[(4-Hydroxy-3-methoxyphenyl)methyl]nonanamide) is a capsaicinoid found in chili peppers but is commonly manufactured synthetically. It may be present in some brands of personal protection sprays ("Captor" – UK), found in over the counter topical ointments for arthritis or muscle pain ("Finalgon®" – Germany) or as a food additive. Nonivamide may be differentiated from natural capsaicin in both infrared and mass spectral data.

CS and CN products contain 1-3% active ingredient in an alcohol or inert oil based carrier. OC Pepper sprays contain 0.2 -10% capsaicinoids in inert solvent based carriers such as trichloroethylene, dipropylene glycol, or light petroleum distillate.

SAFETY CONSIDERATIONS

A. This protocol involves hazardous materials, operations, and equipment. This protocol does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Proper caution should be exercised and the use of personal protective equipment (PPE) should be considered to avoid exposure to dangerous chemicals. Consult the appropriate SDS for each chemical prior to use.

B. Tear gas contains lachrymatory compounds which irritate or inflame the eyes, mucous membranes, and respiratory system. These chemicals or aerosol dispensers containing these chemicals must be kept in a ventilated fume hood. To minimize exposure, analytical preparation and post analysis clean-up must be performed in a ventilated fume hood.

C. NFPA hazard ratings for chemicals used in this procedure:

Chemical	Health	Flammability	Reactivity
Methanol	1	3	0
Acetonitrile	2	3	0
Chloroform	2	0	0
Sodium Hydroxide	3	0	1
Hydrochloric Acid	3	0	1
Hexane	1	3	0

PREPARATIONS

A. Methanol
B. Acetonitrile
C. Hexane
D. 2N Sodium hydroxide
E. 3N HCl
F. Chloroform
G. Hot plate
H. 500 mL beaker
I. Separatory funnel

INSTRUMENTATION

A. Gas Chromatograph/Mass Spectrometer (GC/MS)

1. When an oil based carrier is present, CS, CN, or capsaicin can be identified using the following suggested parameters:

a. Column: 25 meter HP-1MS (methyl silicone) capillary column

b. Injector: 250°C

c. Transfer line: 280°C

d. Carrier: Helium, 0.9 mL/min.

e. Oven: Initial: 150°C (2 min.)
Ramp: 10°C/min
Final: 250°C (10 min.)

f. Ion Range: 30 to 400 amu

2. Suggested alternate parameters:

a. Column: 20 meter HP-5 (5% phenyl methylpolysiloxane) capillary column

b. Injector: 250°C

c. Transfer line: 280°C

d. Carrier: Helium, 0.9 mL/min.

e. Oven: Initial: 60°C (1 min.)
Ramp: 15°C/min
Final: 300°C (2min)

f. Ion Range: 30 to 400 amu

3. Major ions present in the mass spectra of common lachrymators:

a. CS: 126, 137, 153, 188

b. CN: 51, 77, 105

c. Capsaicin: 137, 152, 305

d. Nonivamide: 137, 151, 195, 293

B. Fourier Transform Infrared Spectrometer (FTIR)

1. CS or CN in alcohol based carriers can be analyzed by FTIR. Evaporate the liquid to dryness and analyze the CS or CN crystals by FTIR.

2. Any work outside of a sealed system may result in the analyst's exposure to the lachrymator's potential acute health effects. Analysis by ATR is not recommended.

MINIMUM STANDARDS & CONTROLS

A. See Minimum Standards & Controls, APP II.

PROCEDURE OR ANALYSIS

A. Record a physical description of the container and information on labels, etc. Check Safety Data Sheets (SDS) (if available) or manufacturer's technical product information for details regarding the chemical lachrymator.

B. Collection and Extraction of Liquids from Aerosol (Dispenser) Canisters

1. Some aerosol canister actuators are equipped with a safety or locking mechanism. This mechanism must be released in order to dispense the contents. Products may vary and it is advised to contact the manufacturer if there is any question regarding the safe operation of the device.

    a. Valves and nozzles on lachrymator spray canisters are designed as stream delivery systems. There is considerable pressure released with the liquid contents. The effective range of these sprays is between 4 and 6 feet, although some sprays are reported to have a maximum range of 25 to 30 feet.

2. Collection (In a ventilated fume hood and wearing PPE)

    a. Hold a 500 mL beaker at an angle of 45 degrees with the opening of the beaker facing the back of the hood.

    b. Hold the canister in an upright position and dispense approximately 5 to 10 mL of the contents from the canister down the inside wall of the beaker.

    c. Keep the discharged aerosol canister and the beaker with the liquid contents in the ventilated fume hood until the analysis is completed.

3. CS & CN Extractions

a. Care should be taken to avoid losing the target compound when warming on a hotplate to evaporate the carrier solvent or concentrate the extract. CN has a melting point of 56.5˚C (133.7˚F), CS has a melting point of 93˚C (199.4˚F), and OC has a melting range of 62 to 65 °C (144 to 149 °F).

b. Oil based carrier - Extract with methanol. Approximately 5 mL of the liquid sample is extracted with approximately 5 mL of methanol.

   i. Concentrate the methanol to approximately 1 mL by evaporating with a stream of air and gentle warming on a hot plate.

   ii. Analyze for CS or CN by GC/MS (See Instrumentation, section A.1.).

   iii. The concentrate is placed in a marked vial and returned with the evidence.

c. Solvent based carrier - Approximately 5 mL of the liquid sample is evaporated to dryness with a stream of air and gentle warming on a hot plate.

   i. The solid residue is taken up in approximately 1 mL of methanol.

   ii. Analyze for CS or CN by GC/MS (See Instrumentation, section A.1.).

   iii. The residue is placed in a marked vial and returned with the evidence.

4. Capsaicin Extraction

   a. Extract approximately 2 mL of the sample with approximately 2 mL of acetonitrile and concentrate the acetonitrile to approximately 0.5 mL with a stream of air and gentle warming on a hot plate.

      i. Analyze for capsaicin by GC/MS (See Instrumentation, section A.1).

      ii. The concentrate is placed in a marked vial and returned with the evidence.

   b. Alternate Procedure

      i. Use this procedure if the analysis of the acetonitrile extraction of the sample results in a complex matrix that masks the capsaicinoids in the data. The alternate extraction procedure below may provide a “cleaner” sample by eliminating some of the matrix before subsequent analysis by GC/MS for the target compounds.

ii. Approximately 5 mL of the liquid is extracted with approximately 10 mL of a 2N sodium hydroxide solution in a separatory funnel.

iii. Mix by repeatedly inverting the funnel. Vent the funnel by opening the stopcock after each inversion. Use this technique for the subsequent mixing and extractions below.

iv. Retain the aqueous layer and extract two times with hexane. Remove and discard the hexane layer after each extraction.

v. The aqueous extract is then acidified with approximately 5 mL of 3N HCl.

vi. Extract the acidified solution with approximately 3 mL of chloroform. The chloroform layer is retained and evaporated to dryness.

vii. The residue is dissolved in approximately 0.5 mL of methanol.

viii. Analyze for capsaicin by GC/MS (See Instrumentation, section A.1.).

ix. The extract is placed in a marked vial and returned with the evidence.

C. Extraction Procedure for Clothing or other Substrates

1. Many lachrymator canisters also contain dyes, either visible or UV reactive, to mark skin or clothing in order to enhance identification by law enforcement.

   a. Some stains are visibly red to orange while some may fluoresce under a hand-held UV light or in a UV light box. These stains should be the primary areas to extract suspect lachrymators.

2. Record a description of the clothing or substrate suspect area(s) with regard to any stain or discoloration: size and pattern, location, powdery or oily residue, color, fluorescence, etc.

3. If appropriate, remove a swatch, approximately 5 cm x 5 cm, of the suspect area along with an equal sized swatch from a "protected" area for use as a control. A protected area has no stains or discoloration and is likely to be found on the back side of a garment, in an area opposite to the direction of contact or in an area protected from the lachrymator spray stream. If removal of a swatch is not possible, swab the suspect and control areas with separate swabs wet with an appropriate solvent by following section C. steps 4. or 5. below. Record the physical location of these sampled areas on your worksheet.

4. When CS or CN is suspected, extract the suspect sample and control swatches with approximately 10 mL of methanol. If additional methanol is required it should be applied equally and sparingly to both the suspect sample and control swatches.

    a. Filter and concentrate the control and suspect methanol extracts with a stream of air and gently warming on hot plate to a volume of approximately 0.5 mL.

    b. Run control extract and analyze suspect extract for CS or CN by GC/MS (See Instrumentation, section A.1.).

    c. The extracts are placed in marked vials. The dried swatches and vials are returned with the evidence.

5. When capsaicin is suspected (typically a bright red-orange stain will be present on the garment or substrate) extract the stained sample and control swatches with approximately 5 to 10 mL of acetonitrile. If additional acetonitrile is required it should be applied equally and sparingly to both the suspect sample and control swatches.

    a. Filter and evaporate the control and suspect acetonitrile extract with a stream of air and gentle warming on a hot plate. If capsaicin is present, a viscous residue may remain.

    b. Add approximately 0.5 mL of methanol.

    c. Run control extract and analyze suspect extract for capsaicin by GC/MS (See Instrumentation, section A.1.).

    d. The extracts are placed in marked vials. The dried swatches and vials are returned with the evidence.

D. Alternative Extraction Procedure for Clothing or other Substrates

1. The solvent extractions of contaminated clothing in section C. steps 4. and 5. above may result in a complex matrix which may mask the target compound(s) during analysis. The alternate extraction procedures below may provide a "cleaner" sample by eliminating some of the matrix before subsequent analysis by GC/MS for the target compounds.

2. When substrate is suspected of containing capsaicin:

a. Extract sample and control swatches with approximately 10 mL of methanol.

b. Filter the control and suspect methanol extracts into individual separatory funnels.

c. Add approximately 10 mL of 2N NaOH to each funnel.

d. Mix by repeatedly inverting the funnel. Vent the funnel by opening the stopcock after each inversion. Use this technique for the subsequent mixing and extractions below.

e. Extract the control and suspect aqueous solutions twice with approximately 3 mL of chloroform. Remove and discard chloroform layer after each extraction.

f. Acidify and mix the control and suspect aqueous solutions with approximately 10 mL of 3N HCl.

g. Extract the control and suspect acidified solutions with approximately 3 mL of chloroform.

h. Retain the control and suspect chloroform extracts and concentrate with a stream of air to approximately 1 mL.

i. Run the control extract and analyze the suspect extract for capsaicin by GC/MS (See Instrumentation, section A.1.).

j. The extracts are placed in marked vials. The dried swatches and vials are returned with the evidence.

3. When substrate is suspected of containing CN or a mixture of CN and capsaicin:

Note: This procedure may consume the CN in analysis. If consumption of the evidence is an issue, see Command Directive EVH 32.

a. Run a heated headspace blank on two empty pint size paint cans (as per heated headspace procedure, MT-IA-2) by GC/MS (See Instrumentation, section A.1.).

b. Seal the suspect swatch and control swatch in the pint size paint cans that were run as blanks in step a. above.

c. Heat the can containing the suspect swatch in an oven for approximately 10 minutes at 70°C +/- 5˚C.

d. Sample headspace vapor (as per heated headspace procedure, MT-IA-2) and analyze for CN by GC/MS (See Instrumentation, section A.1.).

e. Repeat steps b. and c. above with the can containing the control swatch.

f. Cool cans completely and remove swatches.

g. The control and suspect swatches can be analyzed for capsaicin by following procedure in section C. step 5. or section D. step 2. above.

h. The extract is placed in a marked vial. The dried swatches and vial are returned with the evidence.

4. When substrate is suspected of containing CS or a mixture of CS and capsaicin:

a. Extract suspect and control swatches with approximately 10 mL hexane. Filter and equally divide the control swatch hexane extract into two separate beakers. Filter and equally divide the suspect swatch hexane extract into two separate beakers.

i. One portion of the suspect and control hexane extracts can be evaporated to dryness using a stream of air.

a) Add approximately 1 mL of methanol to each beaker.

b) Run control extract and analyze suspect extract for CS by GC/MS (See Instrumentation, section A.1.).

c) The extracts are placed in marked vials. The dried swatches and vials are returned with the evidence.

ii. The second portion of the control and suspect hexane extracts are placed into two separatory funnels.

a) Approximately 5 mL of 2N NaOH is added to each funnel.

b) Mix by repeatedly inverting the funnels. Vent the funnels by opening the stopcock after each inversion. Use this technique for subsequent mixing and extractions.

c) Remove and discard the hexane layer from the control and suspect funnels.

d) Acidify and mix the control and suspect aqueous layers with approximately 5 mL of 3N HCl.

e) Extract the control and suspect aqueous layers with approximately 3 mL of chloroform.

f) Retain the chloroform layers from the control and suspect and concentrate each with a stream of air to approximately 1 mL.

g) Run the control extract and analyze the suspect extract for capsaicin by GC/MS (See Instrumentation, section A.1.).

h) The extracts are placed in marked vials. The dried swatches and vials are returned with the evidence.

REPORT WORDING

See-Report Wording, APP I.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: General Unknowns

Method: Specialized Analysis

Procedure: Figures and Tables

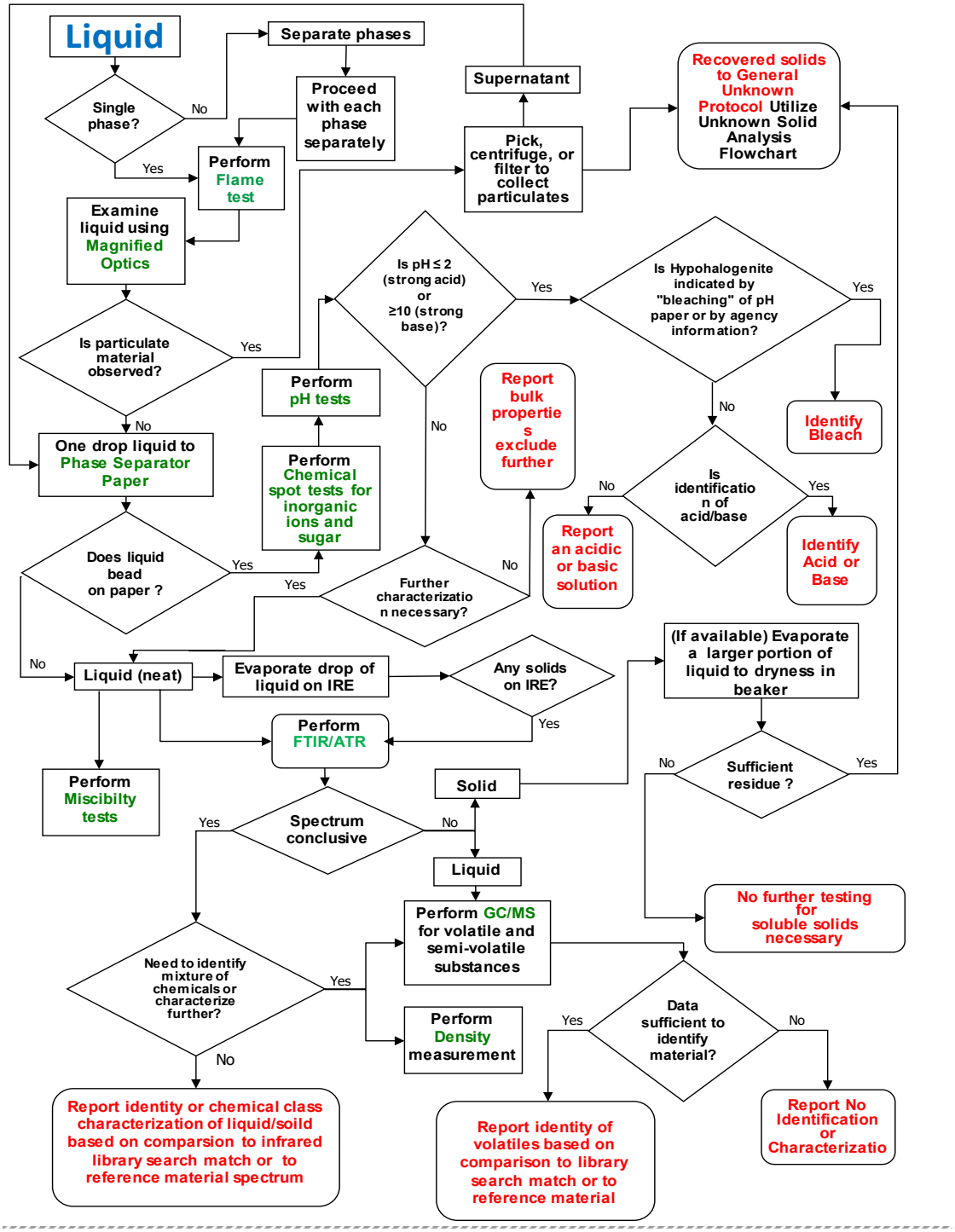
Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

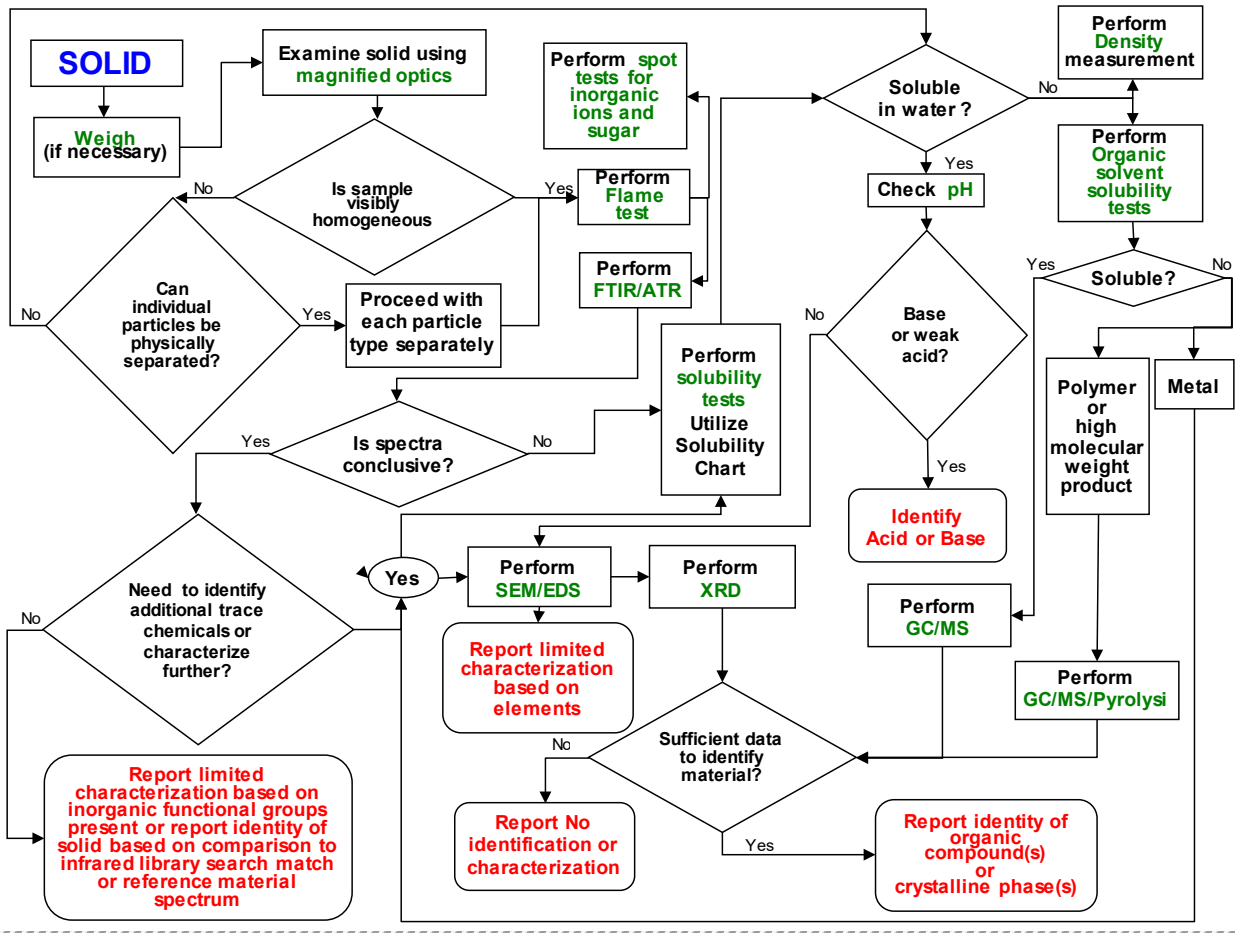
Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

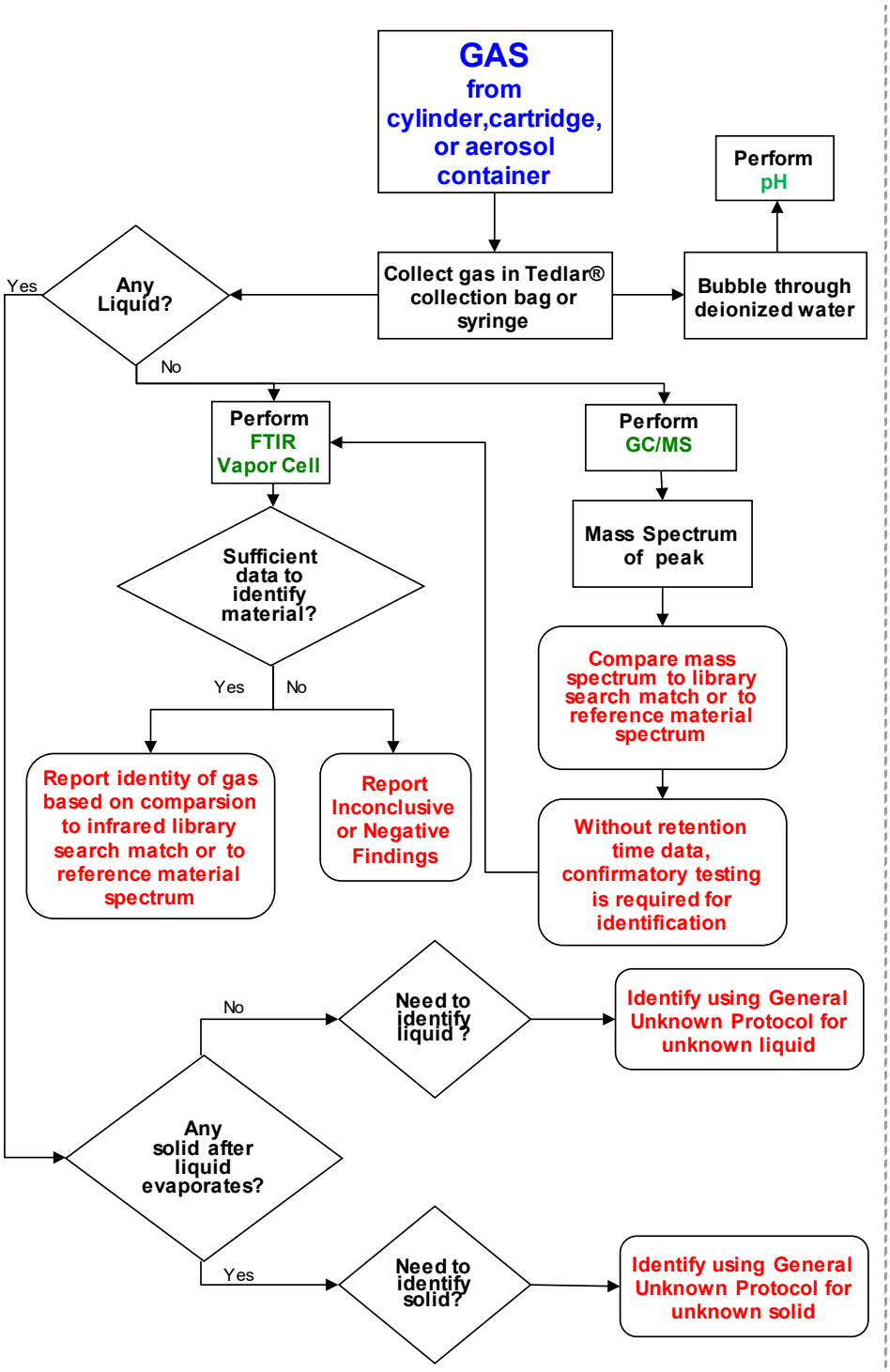
Unknown Liquid Analysis Flowchart
Figure 1



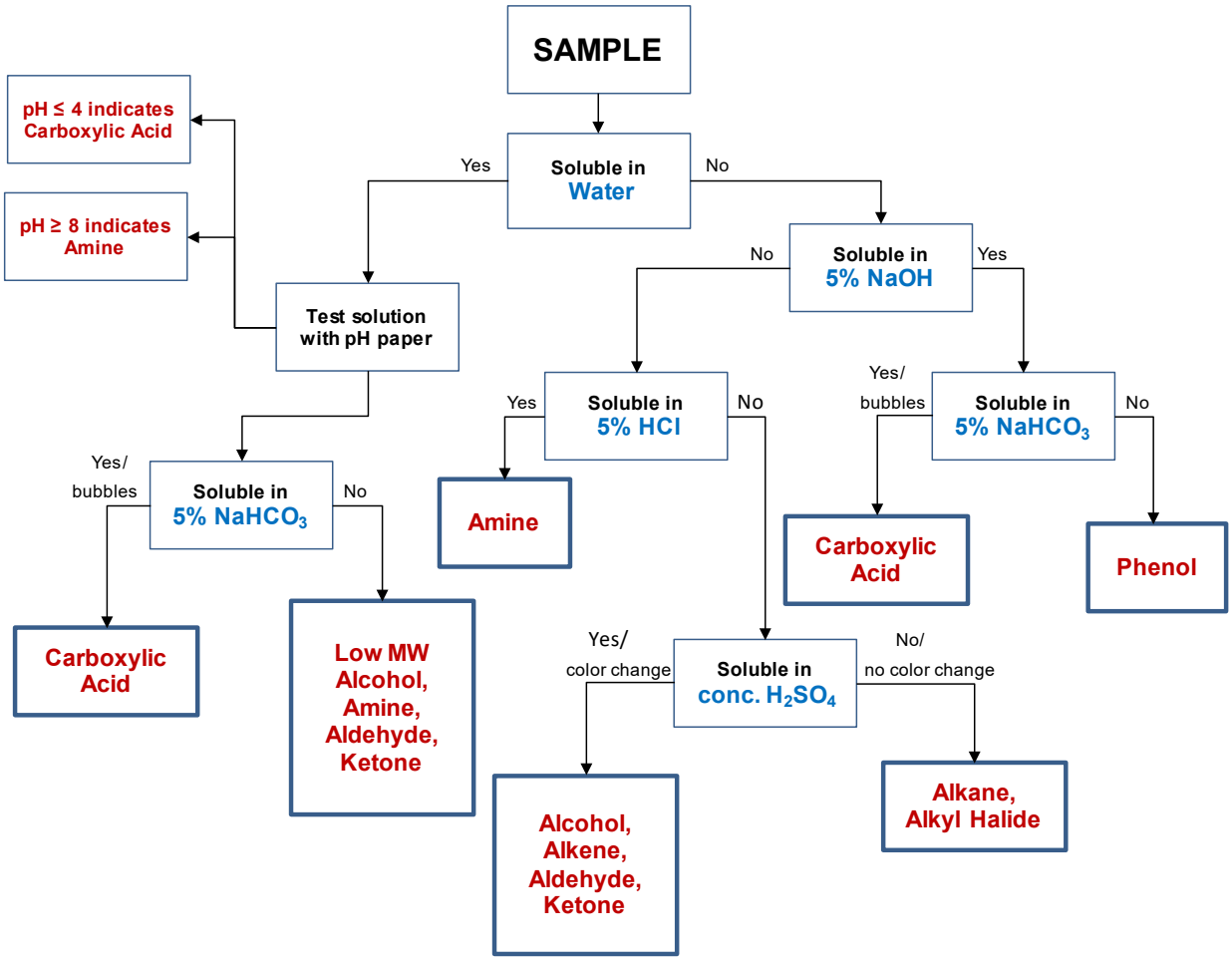
Unknown Solild Analysis Flowchart
Figure 2



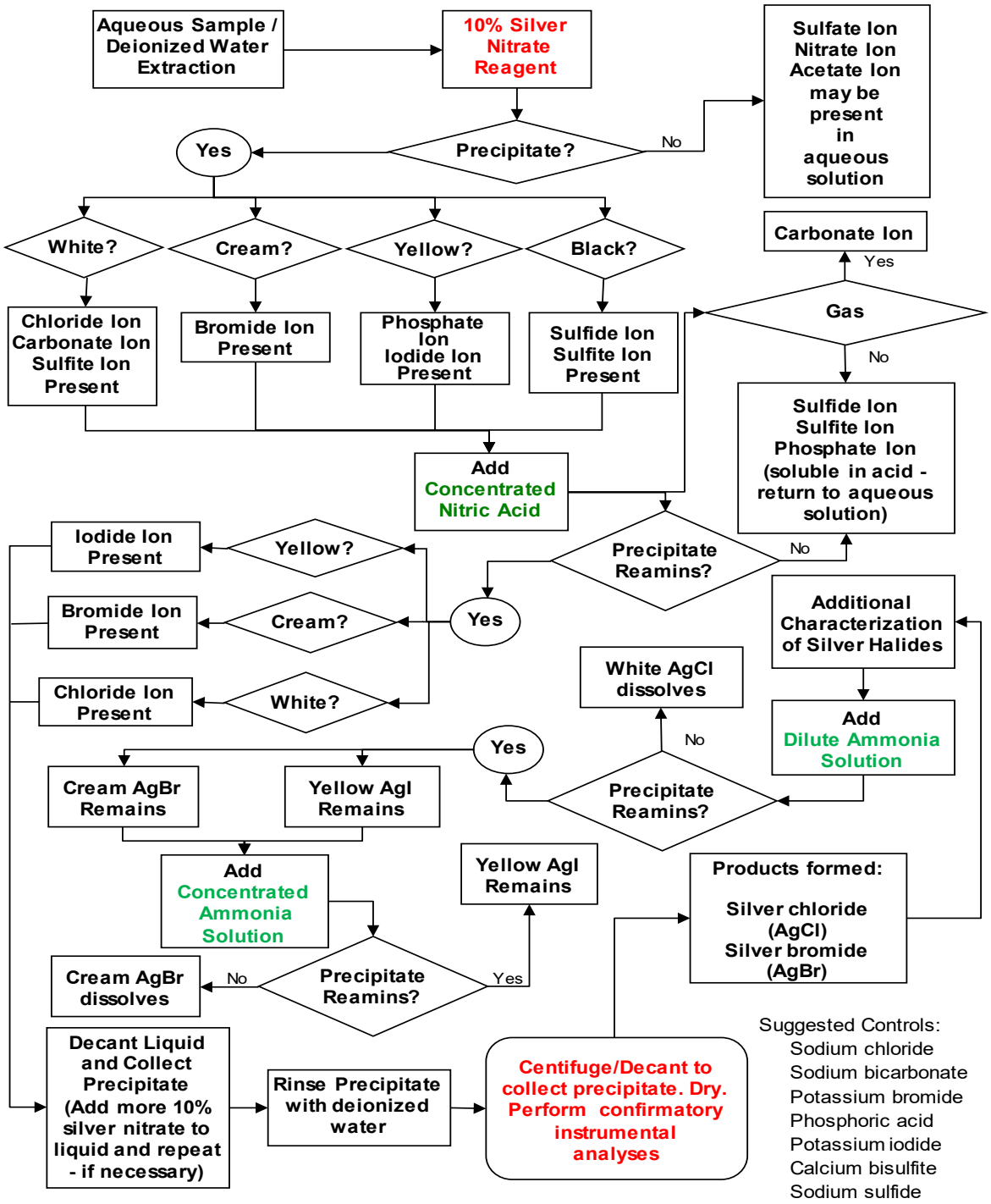
Unknown Gas Analysis Flowchart
Figure 3



Solubility Tests Flowchart
Figure 4

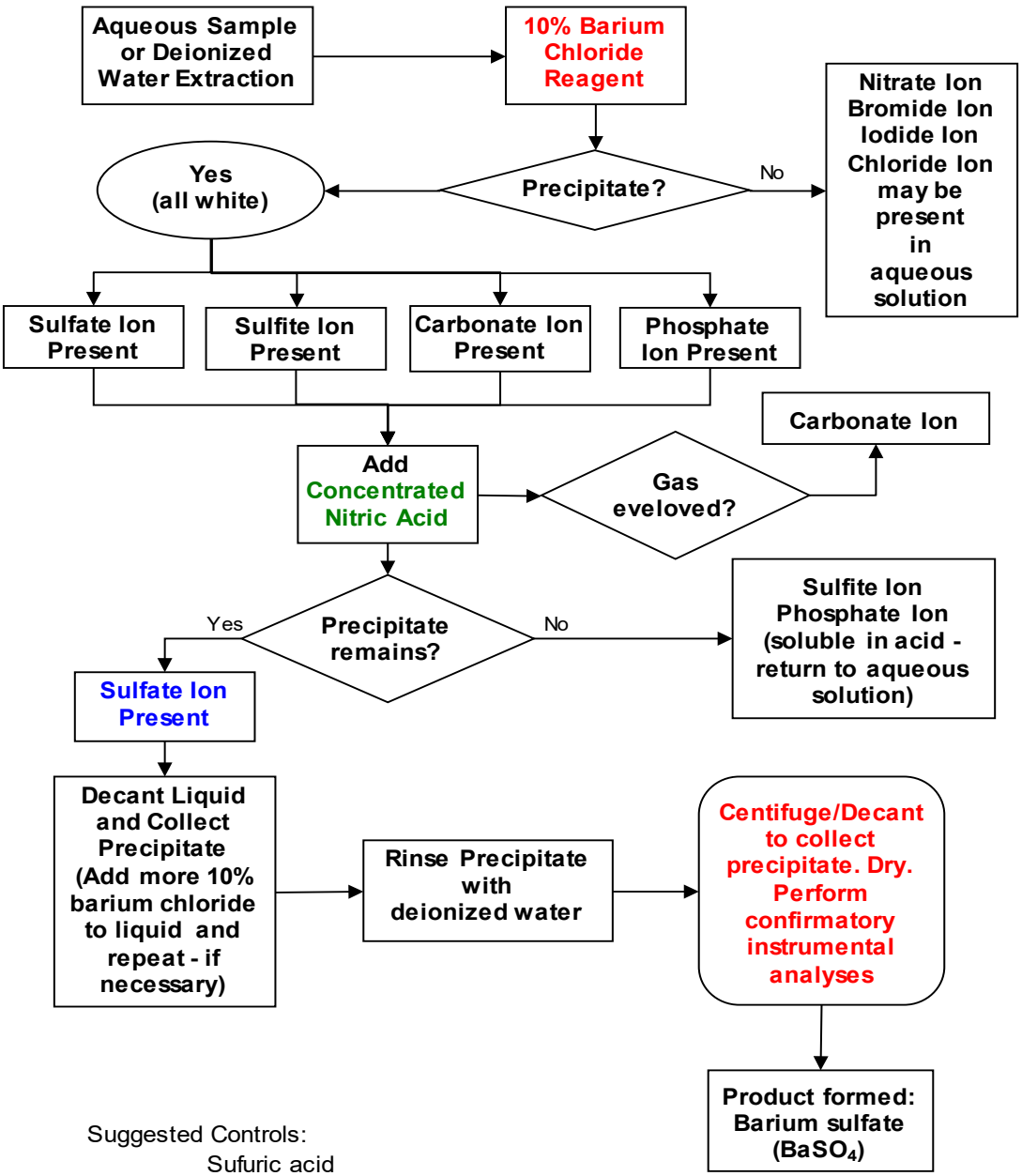


Silver Nitrate Reagent Chemical Reactivity Test Flowchart
Figure 5



Barium Chloride Reagent Chemical Reactivity Test Flowchart

Figure 6



Suggested Controls:

Sufuric acid
Sodium sulfite
Sidum Bicarbonate
Phosphoric acid

Flame Test Colors
Table 1

ELEMENT		COLOR OF FLAME
Al	Aluminum (metal flake or powder)	Bright white
As	Arsenic	Blue
B	Boron	Bright green
Ba	Barium	Pale/Yellow-green
Ca	Calcium	Orange-red
Cu^{1+}	Copper (I)	Green
Cu^{2+}	Copper (II) non-halide	Blue
Cu^{2+}	Copper (II) halide	Blue-green
Fe	Iron	Yellow to red-orange
Fe^{2+}	Iron (II)	Yellow-green
Fe^{3+}	Iron (III)	Orange-red
In	Indium	Blue
K	Potassium	Light purple to violet
Li	Lithium	Deep pink to crimson
Mg	Magnesium (metal flake or powder)	Bright white
Mn^{2+}	Manganese (II)	Pink
Mo	Molybdenum	Yellow-green
Na	Sodium	Bright yellow
P	Phosphorous	Pale blue-green
Pb^{3+}	Lead (III)	Blue
Rb	Rubidium	Red/Purple-red
Sb	Antimony	Pale green
Se	Selenium	Bright blue
Sr	Strontium	Crimson
Te	Tellurium	Pale green
Ti	Titanium (metal flake or powder)	Bright white
Tl	Thallium	Bright green
Zn	Zinc	Blue-green to pale green

Solubility of Ionic Compounds in Water
Table 2

Ions	Br^-	CH_3COO^-	ClO_3^-	ClO_4^-	CN^-	CO_3^{2-}	Cl^-	F^-	I^-	NO_3^-	OH^-	PO_4^{3-}	S^{2-}	SO_4^{2-}
Ag^+	N	Ss	N	N	N	N	N	S	N	S	N	N	N	Ss
Al^{3+}	S	Ss	S	S	?	–	S	Ss	S	S	N	N	N	S
Ba^{2+}	S	S	S	S	S	N	S	N	S	S	Ss	N	S	N
Be^{2+}	S	N	?	?	?	Ss	S	S	S	S	Ss	N	S	S
Ca^{2+}	S	S	S	S	S	N	S	N	S	S	Ss	N	S	Ss
Cd^{2+}	S	S	S	S	Ss	N	S	S	S	S	N	N	N	S
Co^{2+}	S	S	S	S	N	N	S	S	S	S	N	N	N	S
Cs^+	S	S	S	S_H	S	S	S	S	S	S	S	S	S	S
Cu^{2+}	S	S	S	S	N	N	S	S	–	S	N	N	N	S
Fe^{2+}	S	S	S	S	N	N	S	Ss	S	S	N	N	N	S
Fe^{3+}	S	S_C	S	S	?	N	S	N	–	S	N	N	–	S
H^+	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Hg^{2+}	Ss	S	S	S	S	–	N	N	N	S	–	N	N	D
Hg_2^{2+}	N	Ss	?	?	–	N	N	Ss	N	Ss	–	N	–	?
K^+	S	S	S	S_H	S	S	S	S	S	S	S	S	S	S
Li^+	S	S	S	S	S	S	S	Ss	S	S	S	S	S	S
Mg^{2+}	S	S	S	S	S	N	S	N	S	S	Ss	N	D	S
Mn^{2+}	S	N	?	S	N	N	S	S	S	S	N	N	N	S
NH_4^+	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Na^+	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ni^{2+}	S	S	?	S	N	N	S	S	S	S	N	N	N	S
Pb^{2+}	Ss	S	S	S_C	N	N	Ss	N	Ss	S	N	N	N	N
Rb^+	S	S	S	S_H	S	S	S	S	S	S	S	S	S	S
Sn^{2+}	N	D	S	?	–	–	N	S	Ss	S	N	N	N	S
Sr^{2+}	S	S	S	S	S	N	S	N	S	S	Ss	N	S	N
Zn^{2+}	S	S	S	S	N	N	S	Ss	S	S	N	N	N	S

Key for Table 2

S: soluble
S_C: more soluble in cold water
S_H: more soluble in hot water
Ss: Slightly soluble
N: Not soluble
D: Decomposes
[−] Does not exist

Summary of General Solubility Rules for Ionic Compounds in Water

- All salts of the group I elements (alkali metals = Li, Na, K, Cs, Rb) are *soluble.*
- All acetates ($CH3COO^-$), chlorates (ClO_3^-), perchlorates (ClO_4^- nitrates (NO_3^-) are soluble.
- All common compounds of hydrogen (H^+), ammonium (NH_4^+), potassium (K^+) and sodium (Na^+) are soluble.
- All chlorides (Cl^-), bromides (Br^-), iodides (I^-) are soluble except silver (Ag^+), mercury (Hg^+), and lead (Pb^{2+}).
- All sulfates (SO_4^{2-}) are soluble except lead (Pb^{2+}), barium (Ba^{2+}), strontium (Sr^{2+}). Calcium (Ca^{2+}) is slightly soluble.
- All carbonates (CO_3^{2-}) are insoluble except ammonium (NH_4^+), and those of Group 1 elements.
- All hydroxides (OH^-) are *insoluble* except those of Group 1 elements and ammonium (NH_4^+). Barium (Ba^{2+}), strontium (Sr^{2+}), and calcium (Ca^{2+})are slightly soluble.
- All sulfides (S^{2-}) are *insoluble* except those of Group 1 and Group 2 elements and ammonium (NH_4^+).
- All phosphates (PO_4^{-3}) are insoluble except ammonium (NH_4^+) and those of Group I elements.

pH Table
Table 3

Concentration of Hydrogen ions (compared to distilled water)	pH	Examples of solutions
1/10,000,000	14	Liquid drain cleaner
1/1,000,000	13	Bleach, oven cleaner
1/100.000	12	Soapy water
1/10,000	11	Household ammonia
1/1,000	10	Milk of magnesium
1/100	9	Toothpaste
1/10	8	Seawater, Baking soda
0	7	deionized water
10	6	Urine, Milk
100	5	Black coffee
1,000	4	Tomato juice
10,000	3	Soft drinks, Orange juice
100,000	2	Vinegar, Lemon juice
1,000,000	1	Gastric juice from stomach
10,000,000	0	Battery acid

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: General Unknowns

Method: Specialized Analysis

Procedure: Chemical Reactivity Tests, Microscopic Crystal Tests, and Reagents

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

CHEMICAL REACTIVITY and MICROSCOPIC CRYSTAL TESTS

Spot tests may be performed directly on particles for color observations, unless noted. The following color and microcrystalline tests are based on visual observations. A *Blank* and an appropriate *Control* sample must be run concurrently in order to observe the color or precipitate reactions. Suggested Control(s) appear with each test.

ALL SPOT TESTS SHOULD BE DONE IN A FUME HOOD!
SAFETY GLASSES MUST BE WORN!
CAUTION!!
(CAUSTIC, CORROSIVE, AND OXIDIZER MATERIALS IN USE)
DISPOSE OF REAGENTS PROPERLY

ANILINE SULFATE TEST: Place a small amount of solid sample or 5-10 drops of a deionized water extract of the sample into a test tube. Add an equal amount of aniline sulfate reagent solution to the tube. Vortex the tube. Tilt the tube slightly to the side and carefully add 2-4 drops of concentrated sulfuric acid down the side of the tube to form a separate layer. A blue ring will form at the interface of the acid and liquids indicating the presence of **chlorates**. Control: potassium chlorate.

BARIUM CHLORIDE TEST: Place a small amount of material into a test tube. Add approximately 2 mL. of deionized water. Vortex the vial to dissolve the material. Add two drops of barium chloride reagent. See Figure 6, barium chloride reagent chemical reactivity test flowchart for results.

CALCIUM CHLORIDE TEST: Into a test tube containing approximately 2 mL of calcium chloride reagent, add 5 drops of a **hydroxide** solution. A white precipitate forms.

CHLOROPLATINIC ACID TEST (Hanging drop verification): Dissolve a small amount of sample in a drop of deionized water on a microscope slide. Place a drop of platinic chloride reagent near the sample. Draw the drops together. If either **potassium or ammonium** is present, isotropic yellow/orange octahedral crystals are formed. To differentiate between the ions, use a **hanging drop procedure** with the same reagent. (Take a small amount of sample and place it in a spot well. Place a small drop of platinic chloride reagent on a microscope slide. Add 1 drop of 2N sodium hydroxide to the spot well and quickly cover the spot well with the reagent spotted microscope slide - reagent side down. If ammonium is present in the sample, ammonia vapor will transfer to the drop of reagent "hanging" on the underside of the microscope slide and isotropic yellow/orange octahedral crystals are formed. Potassium will form no crystals using this method. Control: Potassium nitrate, Ammonium nitrate.

COPPER CHLORIDE TEST: Into a test tube containing approximately 2 mL of copper chloride reagent, add 5 drops of a **hydroxide** solution. A blue precipitate forms.

DIPHENYLAMINE TEST: Place 1-2 drops of the diphenylamine reagent solution into a clean, dry spot well. Carefully add a small amount of sample. A blue color indicates the presence of **nitrates, nitrated compounds,** and **chlorates**. Control: potassium nitrate, potassium chlorate, nitrocellulose.

GRIESS TEST: Place a small amount of sample into a vial and add 5drops of reagent A and an equal amount of reagent B. A pink/red color indicates **nitrates**. Control: potassium nitrate.

IRON II CHLORIDE TEST: Into a test tube containing approximately 2 mL of iron II chloride reagent, add 5 drops of a **hydroxide** solution. A green precipitate forms.

IRON III CHLORIDE TEST: Into a test tube containing approximately 2 mL of iron III chloride reagent, add 5 drops of a **hydroxide** solution. A brown precipitate forms.

METHYLENE BLUE TEST: Place a small amount of sample or 5 -10 drops of deionized water extract of the sample into a test tube. Add 2-5 drops of zinc sulfate/potassium nitrate solution and 1-2 drops of methylene blue solution. The appearance of a blue/purple precipitate indicates the presence of **perchlorates**. Control: potassium perchlorate

MOLISCH'S TEST: Place approximately 0.5 mL a deionized water extract into a test tube. Add 2 drops of Molisch's test reagent. Vortex the tube. Tilt the tube to the side and carefully add approximately 1 mL concentrated sulfuric acid down the side of the tube to form a separate layer. After a few minutes, a red-violet color indicates the presence of **sugar**. Control: sucrose (table sugar).

NESSLER'S TEST: Place a small amount of sample or 1-3 drops of deionized water extract of the sample into a spot well. Add 2-3 drops of Nessler's reagent. An orange/brown color indicates the presence of **ammonium**. Control: ammonium nitrate.

NITRON TEST: Dissolve a small amount of sample in a small drop of deionized water on a microscope slide. Place a small drop of nitron reagent near the sample. Draw the drops together. If **nitrates** are present, long thin needles and imperfect radiates ("bow ties") appear showing high-order retardation colors. Control: Potassium nitrate.

POTASSIUM DICHROMATE TEST: Place 2-3 drops of a liquid sample into a spot plate. Add 2-3 drops of dichromate reagent. The dichromate reagent turns from orange to blue in the presence of **primary and secondary alcohols** or **aldehydes**. Control(s): ethanol, acetaldehyde

POTASSIUM HYDROXIDE / ETHANOL TEST: Place 2-3 drops of potassium hydroxide saturated ethanol reagent into a spot well. Add a small amount of the sample. A deep red color is

indicative of **TNT**. Control TNT.

PYRIDINE / 2N NAOH TEST: Place a small amount of sample into a test tube. Add 5-10 drops pyridine. Vortex the tube. Tilt the tube slightly to the side and add several drops of 2N sodium hydroxide down the side of the tube. A blue/green color indicates the presence of **sulfur**. Note: If concentration of sulfur in pyridine is too high, a dark brown color develops. The test should be re-run with less amount of sample. Control: sulfur.

SILVER NITRATE TEST: Place a small amount of material in a test tube. Add approximately 2 mL of deionized water. Vortex the tube to dissolve the material. Add two drops of silver nitrate reagent. See
Figure 5, silver nitrate reagent chemical reactivity test flowchart for results.

TOLLEN'S TEST: Place a small amount of the sample in 3-5 drops deionized water into a test tube. Add approximately 3-5 drops Tollen's reagent. Vortex the tube. The tube is immersed in a hot (75˚C ± 5˚C) water bath for approximately thirty seconds. A thin film of shiny metallic silver deposited on the inside wall of the test tube indicates the presence of an **aldehyde** or **sugar**. Control: acetaldehyde, sucrose (table sugar).

> **Tollens' Reagent Warning**: Ammoniacal silver solutions can be explosive. Do not allow reagent to dry out. Discard after use. Discard down drain followed by copious amounts of water.

THYMOL TEST: Place a small amount of sample into a spot well. Add 1-2 drops of Thymol reagent solution. Let dry and add 1 drop of concentrated sulfuric acid. A green color indicates the presence of **nitrates**. A orange/pink color indicates the presence of **RDX**. Controls: potassium nitrate, RDX.

ZINC CHLORIDE + STARCH TEST: Place 1 drop of zinc chloride reagent into a spot well. Add a few grans of starch. Add 1 drop of sample. A dark blue color indicates the presence of **OI^-**. If no color appears, add a few grains of potassium iodide. A blue color indicates the presence of **ClO^-** or **BrO^-**. Contols: NaOI, NaOBr, NaOCl.

ZINC CHLORIDE + FLUORESCEIN TEST: Place 1 drop of zinc chloride into a spot well. Add 1 drop of Fluorescein reagent. Add 1 drop of sample. A red color indicates the presence of **BrO^-**. If no color appears, add a few grains of potassium bromide. A salmon color indicates the presence of **ClO^-**. Controls: NaOCl, NaOBr.

REAGENTS

1) Aniline Sulfate Reagent (5%)
    a. 5 grams of aniline sulfate in 100 mL of deionized water
2) Barium Chloride Reagent (10%)
    a. 10 grams of Barium Chloride in 100 mL of deionized water
3) Calcium Chloride Reagent (10%)
    a. 10 grams of Calcium Chloride in 100 mL of deionized water
4) Copper Chloride Reagent (10%)
    a. 10 grams of Copper Chloride in 100 mL of deionized water
5) Diphenylamine (DPA) Reagent
    a. 0.25 grams of DPA in 100 mL of (80%) concentrated sulfuric acid
    b. 0.25 grams of DPA in 100 mL of ethanol
6) Fluorescein Reagent (saturated)
    a. Add sufficient fluorescein to 200 proof ethanol until fluorescein ceases to dissolve.
7) Greiss Test Reagents
    a. Solution A
        i. Dissolve 1grams of d-naphthylamine in 230 mL of boiling deionized water. Cool and decant the colorless supernatant liquid and mix with 100 mL of glacial acetic acid.
    b. Solution B
        i. 1 grams of sulfanilic acid in 100 m. of 30% acetic acid
8) Iron II Chloride Reagent (10%)
    a. 10 grams of Iron II Chloride in 100 mL of deionized water
9) Iron III Chloride Reagent (10%)
    a. 10 grams of Iron III Chloride in 100 mL of deionized water
10) Methylene Blue Reagent
    a. 0.03 grams of methylene blue in 100 mL of deionized water
11) Molisch's Reagent
    a. 2 grams of 1-naphthol in 50 mL of (95%) absolute ethanol

12) Nessler's Reagent

a. 8.15 grams of mercuric chloride in 90mL hot deionized water. Add the solution to a constantly stirring hot solution containing 9.96 grams potassium iodide in 30 mL of deionized water. Filter the red mercuric iodide precipitate with suction and wash twice using 30mL of deionized water each time. Stir the moist product into a hot solution containing 9.79 grams of potassium iodide in 8 mL of deionized water. Heat the solution in a hot water bath for 20 minutes. Stir occasionally, allowing about a fourth of the liquid to evaporate. Centrifuge or decant and discard any residue. Put the supernatant in an evaporating dish and store over calcium chloride in a desiccator. This will give a moist crystalline mass of tetraiodomecuriate (II) dihydrate, $K_2[HgI_4] \cdot 2 H_2O$. The moist crystalline mass can be dried further by pressing between sheets of filter paper and more desiccation over calcium chloride. The yellow crystalline product will last for years and is used to prepare Nessler's solution by dissolving a few yellow crystals in about 1mL of 3M potassium hydroxide just before testing for ammonia or the ammonium ion. As long as blanks and controls react appropriately the crystalline product can be used to prepare Nessler's reagent.

13) Nitron (1,4-Diphenyl-3-phenylamino-1,2,4-triazolium hydroxide) Reagent

a. 5 grams of Nitron in 10 mL of glacial acetic acid and 90 mL of deionized water (filter if necessary)

14) Platinic Chloride Reagent

a. 1 grams of platinic chloride in 15 mL of deionized water

15) Potassium Dichromate Reagent

a. 5 grams of potassium dichromate in 75 mL of concentrated sulfuric acid and 25 mL of deionized water

16) Potassium Hydroxide / Ethanol Reagent

a. 5 grams of potassium hydroxide in 100 mL of ethanol

17) Silver Nitrate Reagent (10%)

a. 10 grams of silver nitrate in 100 mL of deionized water

18) Sodium Hydroxide (2N or 2M solution)

a. 4 grams of sodium hydroxide in 50 mL of deionized water

19) Tollens' Reagent

a. To a 10 mL solution of 10% silver nitrate, add 10 mL of 10% sodium hydroxide. Silver oxide is precipitated out. The precipitate is then dissolved by the dropwise addition of a 1:1solution of ammonium hydroxide. This reagent should be freshly prepared. Discard after use following Safety Considerations instructions.

Tollens' Reagent Warning: Ammoniacal silver solutions can be explosive. Do not allow reagent to dry out. Discard after use. Discard down drain followed by copious amounts of water.

20) Thymol Reagent

a. 15 grams of Thymol in 100 mL of chloroform

21) Zinc Chloride Reagent (20%)

a. 20 grams of zinc chloride in 100 mL of deionized water

22) Zinc Sulfate / Potassium Nitrate Reagent

a. 5 grams of zinc sulfate and 4 grams of potassium nitrate in 20 mL of deionized water

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Microscopy

Method: Microscopy Techniques

Procedure: Using Optical Compensator Plates

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Compensator plates of known optical properties can be inserted into the optical path of a polarizing microscope to help determine optical information about a specimen. The compensator device is inserted into the accessory slot which is located between the objective lens and the analyzer of the polarizing microscope. The slot is oriented 45° away from the polarizer and analyzer and is not rotatable. The specimen orientation is changed by stage rotation.

There are five compensator plates commonly found in the Micro/Trace Section. They are:

A. The quarter-wave plate (¼λ) (140 nm □± 20 nm)

B. The full-wave plate (λ) (560 nm □± 25 nm)

C. The quartz wedge

D. The Berek compensator

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

PREPARATIONS

Köhler illumination.

MINIMUM STANDARDS & CONTROLS

A. Köhler illumination must be set up before using the compensator.
B. The polarizer, analyzer, and compensators must be in the proper orientation.
C. A performance check for the proper operation of the Berek Compensator will be made each time it is used. This will be done using a known man-made fiber. The range of acceptable birefringence values and performance check birefringence values for the fiber will be documented in .an appropriate log.
D. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Polarized Light microscope.

PROCEDURE OR ANALYSIS

The quarter-wave, full-wave, quartz wedge, and Berek compensators all work by aligning the slow direction of the compensator, indicated by an arrow on the device, with either the fast or slow direction of the specimen.

To add retardation, the slow direction of the specimen must be parallel to the slow direction of the compensator. The quarter- and full-wave plates add a known constant amount of retardation. The quartz wedge and Berek compensators add a variable amount of retardation.

To subtract retardation, the fast direction of the specimen must be parallel to the slow direction of the compensator. The quarter- and full-wave plates subtract a known constant amount of retardation. The quartz wedge and Berek compensators subtract a variable amount of retardation.

By observing the resultant colors when adding or subtracting a known amount of retardation, and relating these colors to retardation values, in nm, on the Michel-Lévy chart, one can often determine the retardation, in nanometers, of the specimen with more precision than without the use of a compensator.

If the fast or slow directions of the specimen are not known, they can be determined by deciding whether addition or subtraction of retardation has taken place when a compensator plate is inserted between the specimen and the analyzer.

The Quarter-Wave Plate (¼λ) (≈147nm):

The quarter-wave plate, composed of a sheet of mica, has a known retardation value of 147 nanometers. This wave plate is useful for retardation determination in the second to fourth orders of retardation, where the addition or subtraction of a quarter wavelength of retardation is easily distinguishable.

The Full-Wave Plate (λ) (□≈530nm):

The full-wave plate, composed of a piece of gypsum, has a known retardation of about 530 nanometers. This wave plate is useful in low orders of retardation (first to third) where the addition or subtraction of a full order is very distinctive.

The Quartz Wedge:

Composed of a thin wedge of quartz, this device allows for retardation additions or subtractions of up to 6 orders of interference (up to about 3,100 nanometers). This wave plate is most useful in a subtractive mode to determine the retardation of highly birefringent substances. By subtracting orders of retardation until the specimen appears black and counting the number of orders which pass through the background, the retardation of the specimen can be determined. The

amount of addition and subtraction is determined by specimen orientation and the thickness of the quartz through which the light must travel. The wedge thickness is determined by how far the device is inserted into the compensator slot. A scale from 1 to 6 orders is engraved on the device and can be read at the edge of the compensator slot.

The Berek Compensator:

This compensator is composed of a tilting plate of calcite which passes the light through an increasing thickness of calcite as the plate is tilted. The plate is used to make calculations of birefringence of the specimen by approximating the degree of tilt from a scale engraved on the device. Detailed instructions follow.

OLYMPUS BEREK COMPENSATOR

The Olympus Berek Compensator is primarily intended for:

A. approximating the measurement of retardation in double refracting media.

B. determining the optical character of the double refraction.

This compensator can be inserted into the slot of the intermediate polarizing tube for the Olympus Polarized Light Microscope.

A calcite plate incorporated in this compensator is designed to be perpendicular to the optical axis of the microscope at the 30 ^{o} division line for the insertion or removal of the compensator; otherwise the calcite edges might impinge against the slot of the intermediate tube and be damaged.

BEREK COMPENSATOR

I. INSTRUCTIONS FOR USE

A. Push in polarizer.
B. Cross polarizers so the field of view is at maximum darkness.
C. Remove present compensator, i.e. 530 nm.
D. Insert Berek compensator.
   One should now see either colors or a black cross in the field of view.
E. Place specimen fiber on stage and center on the intersection of the eye piece cross hairs.
F. Rotate stage until specimen is at a 45^{o} angle to the cross hairs.
G. Turn the knob on the Berek compensator and view the specimen fiber. One should see two dark black lines pass through the fiber. This represents the two maximum angles of extinction.

NOTE: If no dark black lines are observed rotate the specimen fiber 90º.

NOTE: If no dark black lines are visible but only washed out colors then it is not possible to use the Berek compensator to determine the birefringence of the specimen fiber.

H. Record the angles at which the two dark black lines pass through the cross hairs using the vernier scale on the Berek compensator. Always use the same location on the fiber to make each reading.
I. Remove the Berek compensator.
J. Remove the polarizer.
K. Determine the approximate diameter of the specimen fiber.
L. Repeat steps A - K for each specimen fiber.

II. CALCULATION OF RETARDATION (R)

A. Find the difference between the two maximum extinction angles.

a = 35.7º b = 24.5º

d = (35.7 - 24.5)

d = 11.2

B. Divide difference by 2 to find (i).

i = d/2

i = 11.2/2

i = 5.6

C. Using TABLE I, find the log f (i).

log f (i)

log (5.6) = 7.980

D. Use the optical constant for individual Berek compensator, (C).
log (C) = 3.907

E. Retardation, (R).

log (R) = [log f (i) + log (C)]

log (R) = (7.980 + 3.907)

log (R) = 11.887

F. The mantissa is 11
The index number is 887

G. Using TABLE II, the antilog of 887 is 771

H. The location of the decimal point is the mantissa minus ten plus one.

(11 - 10 + 1) = 2

I. Retardation

(R) = 77.1` nm

III. Retardation calculated by using TABLE III.

A. a = 35.7° b = 24.5 °

B. i = (35.7 - 24.55)/2 = 5.6

C. Using TABLE III

5.6 = 95.4

D. Constant for compensator

0.808

E. Retardation calculation

R = (95.4 x 0.808) = 77.1

F. Retardation is 77.1 nm

IV. Retardation using the Berek Compensator chart

The Berek Compensator may have been supplied with a chart from the manufacturer which references retardation values directly from the tilt angle measurements.

A. Determine the value of (i) using the preceding techniques from II A. + B, or III A. + B.
B. Use the Berek Compensaotr's chart to find the retardation value for the calculated (i).
C. For white light examinations, the e-line chart is used. Optical filters can be used, in which appropriate F-line, D line, or C-line charts are to be referenced.

V. Birefringence calculation

A. Retardation may also be expressed in fractions of the wavelength.

$$\Theta = \frac{R}{\lambda}$$

R = Retardation

λ = Wavelength of light being used

Θ = Birefringence or double refraction

B. Birefringence or double refraction may also be calculated when the thickness of the crystal is known.

N_e = velocity of extraordinary light wave

N_o = velocity of ordinary light wave

$$\Theta = N_e - N_o = \frac{R}{d}$$

d = approximate diameter of the fiber, in *nm* (or 1000d in *um*)

R = approximate retardation

Determination of Compensator Constants:

The compensator constants are found in the following manner: Using a monochromatic light, wavelength λ, the compensator is turned from zero or 30E position until the first dark line coincides with the intersecting cross hair of the eyepiece from either direction. Let there be (a_1) and (b_1), the angle of orientation (i_1) corresponding to a retardation of θ will be:

$$i_1 = \frac{a_1 - b_1}{2}$$

From Table I, find log f(i_1) and subtract log λ (wavelength used).

Hence, log C = log λ - log f(i_1) Any n^{th} pair of bands may be used for determining the value of log C.

In this case:

$$i_n = \frac{a_n - b_n}{2}$$

Hence, log C = log n + log λ - log f(i_n).

The value of log C determined by either formula will be identical.

Determination of Optical Character:

The amount of the compensator is marked for Z' (slow) and X' (fast) axes for determining the optical character of the direction of oscillation. Therefore, by turning the vernier scale of the compensator, the interference colors used for the distinction of the increase or decrease are varied within a range of 3 orders, for instance:

A. Orthoscopic Determination

With the crystal in the diagonal 45° to the plane of the polarizer or analyzer and parallel to the slow axis of the compensator, while turning the compensator drum, observe whether the interference color is increasing or decreasing. If increasing, it follows that the direction of oscillation is parallel to that of the compensator (additive case); if decreasing, the direction of oscillation is vertical to that of the compensator (subtractive case).

B. Conoscopic Observation

The drum of the compensator is turned to recognize the optical character by the directions of movement of the isochromates of the interference figure.

If the isochromatic figure moves toward the center of the interference figure, the oscillation is parallel to that of the Berek (additive position). If subtractive position, the isochromates of the interference figure will move outward-away from the center of the interference figure. Then the oscillation of the light wave is vertical to that of the Berek.

TABLE I
LOGARITHM f (i)

i	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
∞	—	4.484	5.086	5.438	5.688	5.882	6.040	6.174	6.290	6.392
1	6.484	6.566	6.642	6.712	6.776	6.836	6.892	6.945	6.994	7.041
2	7.086	7.128	7.169	7.207	6.244	7.280	7.314	7.346	7.378	7.408
3	7.438	7.466	7.494	7.521	7.547	7.572	7.596	7.620	7.643	7.666
4	7.688	7.709	7.730	7.750	7.770	7.790	7.809	7.828	7.846	7.864
5	7.881	7.898	7.915	7.932	7.948	7.964	7.980	7.995	8.010	8.025
6	8.039	8.054	8.068	8.082	8.095	8.109	8.122	8.135	8.148	8.161
7	8.173	8.185	8.198	8.210	8.221	8.233	8.244	8.256	8.267	8.278
8	8.289	8.300	8.310	8.321	8.331	8.341	8.352	8.361	8.371	8.381
9	8.391	8.400	8.410	8.419	8.429	8.438	8.447	8.456	8.465	8.473
10	8.482	8.491	8.499	8.508	8.516	8.524	8.532	8.541	8.549	8.557
11	8.564	8.572	8.580	8.588	8.595	8.603	8.610	8.618	8.625	8.632
12	8.640	8.647	8.654	8.661	8.668	8.675	8.682	8.689	8.695	8.702
13	8.709	8.715	8.722	8.728	8.735	8.741	8.748	8.754	8.760	8.766
14	8.773	8.779	8.785	8.791	8.797	8.803	8.809	8.815	8.820	8.826
15	8.832	8.838	8.843	8.849	8.855	8.860	8.866	8.871	8.877	9.882
16	8.888	8.893	8.898	8.904	8.909	8.914	8.919	8.924	8.929	8.935
17	8.940	8.945	8.950	8.955	8.960	8.965	8.969	8.974	8.979	8.984
18	8.989	8.993	8.998	9.003	9.007	9.012	9.017	9.021	9.026	9.030
19	9.035	9.039	9.044	9.048	9.053	9.057	9.062	9.066	9.070	9.075
20	9.079	9.083	9.087	9.092	9.096	9.100	9.104	9.108	9.112	9.116
21	9.120	9.124	9.128	9.132	9.136	9.140	9.144	9.148	9.152	9.156
22	9.160	9.164	9.168	9.172	9.175	9.179	9.183	9.187	9.190	9.194
23	9.198	9.201	9.205	9.209	9.212	9.216	9.220	9.223	9.227	9.230
24	9.234	9.237	9.241	9.244	9.248	9.251	9.255	9.258	9.262	9.265
25	9.268	9.272	9.275	9.278	9.282	9.285	9.288	9.292	9.295	9.298
26	9.301	9.305	9.308	9.311	9.314	9.318	9.321	9.324	9.327	9.330
27	9.333	9.336	9.339	9.343	9.346	9.349	9.352	9.355	9.358	9.361
28	9.364	9.367	9.370	9.373	9.376	9.379	9.382	9.384	9.387	9.390
29	9.393	9.396	9.399	9.402	9.405	9.407	9.410	9.413	9.416	9.419
30	9.421	9.424	9.427	9.430	9.432	9.435	9.438	9.441	9.443	9.446
31	9.448	9.451	9.454	9.456	9.459	9.462	9.464	9.467	9.469	9.472

TABLE II
Logarithm of the Natural Numbers

N	0	1	2	3	4	5	6	7	8	9
10	000	004	009	013	017	021	025	029	033	037
11	041	045	049	053	057	061	064	068	072	076
12	079	083	086	090	093	097	100	104	107	111
13	114	117	121	124	127	130	134	137	140	143
14	146	149	152	155	158	161	164	167	170	173
15	176	179	181	185	188	190	193	196	199	201
16	204	207	210	212	215	217	220	223	225	228
17	230	233	236	238	241	243	246	248	250	253
18	255	258	260	262	265	267	270	272	274	276
19	279	281	283	286	288	290	292	294	297	299
20	301	303	305	307	310	312	314	316	318	320
21	322	324	326	328	330	332	334	336	338	340
22	342	344	346	348	350	352	354	356	358	360
23	362	364	365	367	369	371	373	375	377	378
24	380	382	384	386	387	389	391	393	394	396
25	398	400	401	403	405	407	408	410	412	413
26	415	417	418	420	422	423	425	427	428	430
27	431	433	435	436	438	439	441	442	444	446
28	447	449	450	452	453	455	456	458	459	461
29	462	464	465	467	468	470	471	473	474	476
30	477	479	480	481	483	484	486	487	489	490
31	491	493	494	496	497	498	500	501	502	504
32	505	507	508	509	511	512	513	515	516	517
33	519	520	521	522	524	525	526	528	529	530
34	531	533	534	535	537	538	539	540	542	543
35	544	545	547	548	549	550	551	553	554	555
36	556	558	559	560	561	562	563	565	566	567
37	568	569	571	572	573	574	575	576	577	579
38	580	581	582	583	584	585	587	588	589	590
39	591	592	593	594	595	597	598	599	600	601
40	602	603	604	605	606	607	609	610	611	612
41	613	614	615	616	617	618	619	620	621	622

TABLE II
(Continued)

N	0	1	2	3	4	5	6	7	8	9
42	623	624	625	626	627	628	629	630	631	632
43	633	634	635	636	637	638	639	640	641	642
44	643	644	645	646	647	648	649	650	651	652
45	653	654	655	656	657	658	659	660	661	662
46	663	664	665	666	667	667	668	669	670	671
47	672	673	674	675	676	677	678	679	679	680
48	681	682	683	684	685	686	687	688	688	689
49	690	691	692	693	694	695	695	696	697	698
50	699	700	701	702	702	703	704	705	706	707
51	708	708	709	710	711	712	713	713	714	715
52	716	717	718	718	719	720	721	722	723	723
53	724	725	726	727	728	728	729	730	731	732
54	732	733	734	735	736	736	737	738	739	740
55	740	741	742	743	744	744	745	746	747	747
56	748	749	750	751	751	752	753	754	754	755
57	756	757	757	758	759	760	760	761	762	763
58	763	764	765	766	766	767	768	769	769	770
59	771	772	772	773	774	775	775	776	777	777
60	778	779	780	780	781	782	782	783	784	785
61	785	786	787	787	788	789	790	790	791	792
62	792	793	794	794	795	796	797	797	798	799
63	799	800	801	801	802	803	803	804	805	805
64	806	807	808	808	809	810	810	811	812	812
65	813	814	814	815	816	816	817	818	818	819
66	820	820	821	822	822	823	823	824	825	825
67	826	827	827	828	829	829	830	831	831	832
68	833	833	834	834	835	836	836	837	838	838
69	839	839	840	841	841	842	843	843	844	844
70	845	846	846	847	848	848	849	849	850	851
71	851	852	852	853	854	854	855	856	856	857
72	857	858	859	859	860	860	861	862	862	863
73	863	864	865	865	866	866	867	867	868	869

TABLE II
(Continued)

N	0	1	2	3	4	5	6	7	8	9
74	869	870	870	871	872	872	873	873	874	874
75	875	876	876	877	877	878	879	879	880	880
76	881	881	882	883	883	884	884	885	885	886
77	886	887	888	888	889	889	890	890	891	892
78	892	893	893	894	894	895	895	896	897	897
79	898	898	899	899	900	900	901	901	902	903
80	903	904	904	905	905	906	906	907	907	908
81	908	909	910	910	911	911	911	912	913	913
82	914	914	915	915	916	916	917	918	918	919
83	919	920	920	921	921	922	922	923	923	924
84	924	925	925	926	926	927	927	928	928	929
85	929	930	930	931	931	932	932	933	933	934
86	934	935	936	936	937	937	938	938	939	939
87	940	940	941	941	942	942	942	943	943	944
88	944	945	945	946	946	947	947	948	948	949
89	949	950	950	951	951	952	952	953	953	954
90	954	955	955	955	956	957	957	958	958	959
91	959	960	960	960	961	961	962	962	963	963
92	964	964	965	965	966	966	967	967	968	968
93	968	969	969	970	970	971	971	972	972	973
94	973	974	974	975	975	975	976	976	977	977
95	978	978	979	979	980	980	980	981	981	982
96	982	983	983	984	984	985	985	985	986	986
97	987	987	988	988	989	989	989	990	990	991
98	991	992	992	993	993	993	994	994	995	995
99	996	996	997	997	997	998	998	999	999	000

TABLE III
10,000 f (i)

i	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	0.0	0.0	0.1	0.3	0.5	0.8	1.1	1.5	1.9	2.5
1	3.0	3.7	4.4	5.1	6.0	6.9	7.8	8.8	9.9	11.0
2	12.2	13.4	14.7	16.1	17.5	19.0	20.6	22.2	23.9	25.6
3	27.4	29. 3	31.2	33.2	35.2	37.3	39.5	41.7	44.0	46.3
4	48.4	51.2	53.7	56.3	58.9	61.6	64.4	67.2	70.1	73.1
5	76.1	79.1	82.3	85.5	88.7	92.0	95.4	98.6	102.3	105.9
6	109.5	113.2	116.9	120.7	124.6	128.5	138.5	136.5	140.6	144.8
7	149.0	153.3	157.6	162.0	166.5	171.0	175.6	180.2	184.9	189.6
8	194.5	199.3	204.3	209.3	214.4	219.5	224.6	229.9	235.2	240.5
9	245.9	251.4	257.0	262.6	268.2	273.9	279.7	285.5	291.4	297.4
10	303.4	309.5	315.6	321.8	328.1	334.4	340.7	347.2	343.7	360.2
11	366.8	373.5	380.2	387.0	393.8	400.8	407.7	414.7	421.8	428.9
12	436.1	443.4	450.7	458.1	465.5	473.0	480.6	488.2	495.8	503.5
13	511	519	527	535	543	551	559	567	576	584
14	592	601	609	618	626	635	644	653	661	670
15	679	688	697	706	716	725	734	743	753	762
16	772	781	791	801	810	820	830	840	850	860
17	870	880	890	901	911	921	932	942	953	963
18	974	985	996	1006	1017	1028	1039	1050	1061	1072
19	1084	1095	1106	1118	1129	1141	1152	1164	1175	1187
20	1199	1211	1222	1234	1246	1258	1270	1283	1295	1307
21	1319	1332	1344	1357	1369	1382	1394	1407	1420	1432
22	1445	1458	1471	1484	1497	1510	1523	1537	1550	1563
23	1577	1590	1603	1617	1631	1644	1658	1672	1685	1699
24	1713	1727	1741	1755	1769	1783	1797	1812	1826	1840
25	1855	1869	1884	1898	1913	1927	1942	1957	1972	1987
26	2001	2016	2032	2046	2062	2077	2092	2107	2123	2138
27	2153	2169	2184	2200	2215	2231	2247	2262	2278	2294
28	2310	2326	2342	2358	2374	2390	2407	2422	2439	2455
29	2471	2488	2504	2521	2537	2554	2570	2587	2604	2620
30	2637	2654	2671	2688	2705	2722	2739	2756	2773	2791
31	2808	2825	2843	2860	2877	2895	2912	2930	2947	2965

REPORT WORDING

At their discretion, examiners can report the optical information derived from using a compensator.

REFERENCES

1. Bloss, F. D. *An Introduction to the Methods of Optical Crystallography*; Saunders College: Philadelphia, PA, 1961.

2. McCrone, W. C.; McCrone, L. B.; Delly, J. G. *Polarized Light Microscopy*; Ann Arbor Science: Ann Arbor, MI, 1978; pp 145-149.

3. Stroiber, R. E.; Morse, S. A. *Microscopic Identification of Crystals*; Robert E. Krieger, Ed.; Malabar, FL, 1981; pp 107-108.

ILLINOIS STATE POLICE
MICRO/TRACE
PROCEDURES MANUAL

Protocol: Microscopy

Method: Microscopy Techniques

Procedure: Photomicrography

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Photomicrography is photography through a microscope. Traditionally, it was accomplished by attaching a film camera to a microscope. Digital photography and image capture technologies have increased the speed and ease in which an analyst can document a representation of a microscopic view.

A record of a microscopic observation can be valuable in the examination of evidence. In order to document their original appearances, photomicrograph shall be taken of evidentiary items prior to being consumed or chemically or physically altered in a way that would prevent examination by another analyst. Microscopic side-by-side comparisons may be a critical component of physical match or fabric impression comparisons. If microscopic observations support associative physical match or fabric impression comparisons, photomicrographs shall be required. Photomicrographs may also be taken to enhance the descriptions of observations by analysts.

Equipment for photomicrography can range from highly specialized imaging systems to a simple camera set up on top of a microscope. This procedure is not intended as a step-by-step guide for operating the variety of photomicrographic equipment available. Neither can it replace the practice, time, and skill required by the analyst to achieve good photomicrographic images. Rather, this is an outline for the process of documentation by photomicrography as it applies to the Micro/Trace Section.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions

PREPARATIONS

Camera or imaging system has appropriate attachments and imaging supplies.
Samples to be photographed are prepared.
Microscope is in proper alignment for optimum illumination and resolution as needed.

INSTRUMENTATION

A. Camera or imaging system, and appropriate attachments.
B. Relevant microscope.

MINIMUM STANDARDS & CONTROLS

A. Photomicrographs shall be taken of evidentiary items prior to consumption or prior to alteration which would prevent examination by another analyst.
B. Photomicrographs shall be taken if microscopy is used to support technical conclusions such as in associative physical match or fabric impression comparisons. A detailed representative drawing or appropriately scaled photograph may suffice instead of a photomicrographic image.
C. Required images shall be stored as electronic data with unique file identifiers.
D. Photomicrographic images are to be appropriately identified with the the Date, Initials, Case number, and Item number ('DICI').

PROCEDURE OR ANALYSIS

1. Set up the microscope to achieve proper illumination and resolution for the sample. Kohler illumination is recommended for compound microscopes.
2. Determine the proper image recording format to be used. For this, the examiner shall consider what observations and what conditions need to be photographically recorded.
3. Position the sample in the microscope field of view.
4. Choose the appropriate settings for the camera or image capture system, and for the microscope. Adjustments shall be made and settings shall be optimized to create the best photographic record. Take images as needed.
5. Identify images and document magnification, if possible.

REPORT WORDING

None Required.

REFERENCES

1. Eastman Kodak, compiler *Photography Through the Microscope*, 8th ed.; Eastman Kodak Rochester, NY, 1985; Publication P-2.

2. Smith, R. F. *Microscopy and Photomicrography*, 2nd ed.; CRC: Boca Raton, FL, 1994.

3. Thomson, D. J.; Bradbury, S. *An Introduction to Photomicrography*; Oxford University: New York, 1987.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Hair

Method: Animal Hair Examination

Procedure: Species Determination

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The identification of animal hairs is best stated in Wildman's (1961) statement of the first page of his work.

> "First, although books and photographs are useful as guides, there are no reliable short-cut methods for identifying animal fibers by simply 'matching up' the microscopical appearance of an unknown fiber with a photomicrograph; the observer should have had experience in the examination and interpretation of diagnostic features of a variety of fibers. Secondly, and contrary to the suggestions made in some publications, no measurement method, such for example the measurement of distance between successive external scale margins or the measurement of fiber diameter, will itself reveal the precise origin of a fiber. Thirdly, chemical tests do not distinguish between animal fibers, since all animal fibers consist of the same substance, namely keratin. The only satisfactory procedure is to use the method of microscopy with a sound knowledge of fiber morphology and careful interpretations of the observations made."

Other Related Procedures:

Procedures 2 and 3 of this Method.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
Meltmounts	0-0-0
Permount	3-3-0
Xylenes	2-3-0

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. The examiner must be properly and thoroughly trained and have experience in animal hair examination. The examiner must have an animal hair reference collection characterized by animal species.
B. Animal hairs shall be identified as to species using transmitted light microscopy. The questioned and reference collection hairs shall be treated in the same manner. All of the features/characteristics of the questioned and reference collection hairs must be consistent without any unexplained differences to identify the genus or species of the questioned hair.
C. Case notes shall document microscopic characteristics which result in identification of an animal hair.
D. See Appendix II for Minimum Standards & Controls.

INSTRUMENTATION

Stereomicroscope
Brightfield Microscope

PROCEDURE OR ANALYSIS

An animal hair can be identified as to genus and sometimes species by the physical and microscopic examination of its length and diameter, scale patterns, medulla types, root shape, pigment location, shape, color, and color band pattern.

Scale casting should be used to identify the scale patterns and margin type. Whole mounts should be used to identify the remaining characteristics.

A combination of animal hair keys and examination of the characteristics of the questioned hair in comparison with the reference collection hairs is the best way to identify the genus or species of a questioned animal hair.

A combination of keys to animal hairs should be used since there is not any single key that covers all the mammal species that may be encountered in casework. There are a few common animal hairs that can be identified as to species by an experienced examiner without the use of keys. For example, deer family and antelope, dog, cat, swine, rabbit and chinchilla hairs are easily identified by an experienced examiner. In the vast majority of cases, fur hairs possess insufficient characteristics for species identification. Chinchilla hairs are an example of an exception to the proceeding statement.

REPORT WORDING

See Appendix I.

REFERENCES

1. Appleyard, H. M. *Guide to the Identification of Animal Fibers*; Wool Industries Research Association: Leeds, England, 1960.

2. Hausman, L. A. "The Microscopic Identification of Commercial Fur Hairs"; *Scientific Monthly* 1920, *10*, 70-78.

3. Hicks, John W. *Microscopy of Hair*; Federal Bureau of Investigation: Washington, DC, 1977.

4. Mayer, W. V. "The Hair of California Mammals with Keys to the Dorsal Guard Hairs of California"; *American Midland Naturalist* 1952, *38*, 480-512.

5. Moore, T. D.; Spence, L. E.; Dugnolle, C. E. *Identification of the Dorsal Guard Hairs of Some Mammals of Wyoming*; Wyoming Game and Fish Department: Cheyenne, WY, 1974.

6. Spence, L. E. *Study of Identifying Characteristics of Mammal Hair*; Wyoming Game and Fish Department: Cheyenne, WY, 1963.

7. Wildman, A. B. *The Microscopy of Animal Textile Fibers*; Wool Industries Research Association: Leeds, England, 1954.

8. Wildman, A. B. "The Identification of Animal Fibers"; *J. Forensic Science Soc.* 1961, *1*(2), 1-8.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Hair

Method: Animal Hair Examination

Procedure: Scale Casting

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Scale casting is used to duplicate the scale pattern of a hair and to make the cuticular pattern more discernible. Scale patterns are useful in discriminating different groups of animals; however, it is very seldom useful in discriminating hairs from different humans.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
Acetone	1-3-0
Fingernail Polish	1-3-0
Meltmount	0-0-0
Norland Optical Adhesive #65	2-1-1

PREPARATIONS

Make sure the hair is clean. If not clean, clean the hair in the appropriate manner.

MINIMUM STANDARDS AND CONTROLS

The use of cuticular pattern for species determination must consist of comparison of the scale cast of the questioned hair to scale cast of reference collection hairs or standard hairs submitted.

See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Standard Laboratory Microscopes.

PROCEDURE OR ANALYSIS

Three methods are suggested:

I. Clear Fingernail Polish Method

A. On a clean microscope slide, place a thin layer of clear fingernail polish which has a very low viscosity (diluted with acetone).

B. With fine forceps, place the hair onto the nail polish ensuring that the hair is completely imbedded in the casting material but not covered.

C. Allow the polish to dry overnight.

D. With fine forceps, pry the root end of the hair loose and gently peel the hair from the slide.

E. With a sharp scalpel, slice away the excess coating protruding above the flat surface of the scale cast. Be careful not to slice too deeply into the coating itself.

F. Observe the scale impression microscopically with transmitted illumination.

NOTE: Different nail polishes have somewhat different properties. Experiment to find which one works best for this method.

II. Additional methods for scale casting using Meltmount or Norland Optical Adhesive may be used. See References for information.

REPORT WORDING

See Appendix I.

REFERENCES

1. Carter, B.; Dilworth, T. "A Simple Technique for Revealing the Surface Pattern of Hair"; *American Midland Naturalist* **1971**, *85*, 260-262.

2. Ogle, R.; Mitosinka, G. "Rapid Method of Preparing Hair Cuticle Scale Casts"; *J. Forensic Sciences* **1973**, *18*, 82-83.

3. Petraco, N. "The Replication of Hair Cuticle Scale Patterns in Meltmount"; *Microscope* **1986**, *34*, 341-345.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Hair

Method: Human Hair Examination

Procedure: Preparation of Hair Roots for nDNA Submission and Hairs for mDNA Submission

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Reviewed by:

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Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

A probative hair may be recovered for Nuclear DNA (nDNA) or Mitochrondrial DNA (mDNA) analysis The proximal (root) end of a hair may be submitted for nDNA analysis, an entire hair may be submitted for mDNA. This procedure is considered a guideline for the analyst when recovering a hair root or hair.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
Permount	3-3-0
Xylene Substitute	2-3-0
Ethyl Alcohol	0-3-0
Bleach Solution	2-0-1
Water	0-0-0

PREPARATIONS

Standard Laboratory Practices.

INSTRUMENTATION

A. Stereomicroscope
B. Compound Microscope
C. Photomicrographic Equipment

MINIMUM STANDARDS & CONTROLS

A. Strict adherence to "Clean Technique Procedure for non-DNA Personnel" (MT-APP V) must be maintained.
B. The hair(s) to be processed and recovered for submission to the DNA Section must be probative evidence. The criteria for determining the suitability of submissions may be whether the hair(s) contains an anagen root or adhering tissue. An evaluation of the case and evidence should be reviewed by the Micro/Trace analyst and the DNA analyst (and/or other appropriate personnel) prior to analysis. If necessary, consumption documents are to be secured before proceeding with hair preparation.
C. Prepared hair specimens to be submitted to DNA may need to be subitemized if removed from a parent item of evidence.

D. Hair(s) to be submitted for mDNA must also be probative evidence. The hair(s) may be forwarded to the submitting agency or appropriate laboratory section as necessary. The same procedures apply to hairs for mDNA except the whole hair is processed.

PROCEDURE OR ANALYSIS

1. Hair(s) may be recovered from evidence items or from previously prepared slide mounts.

2. The appropriate hair(s) is selected for nDNA analysis by the Micro/Trace analyst in conjunction with DNA analysts or agency personnel.

3. If previous arrangements have been made for multiple hair roots to be processed as a single sample for nDNA analysis, the Micro/Trace analyst may combine the sample hair roots as a single -item or subitem.

4. The proximal end of the probative hair(s) may be photomicrographed.

5. The appropriate hair(s) can be recovered from mounted slide(s) using a few drops of xylene substitute to soften the mounting medium (usually Permount), removing or opening the cover slip, and recovering the probative hair(s).

6. The proximal end (approximately 2 cm) of each hair containing the root is cut, recovered, and washed in xylene substitute, if necessary to remove the mounting medium. The remaining distal end of the hair is to be preserved and identified. If the probative hair is less than or close to 2 cm in length, the entire hair may be recovered and submitted.

7. Alternatively, the probative hair root may be carefully cut and removed from a slide leaving the distal end of the hair in the original mounted position. This root section is washed in xylene substitute to remove the mounting medium.

8. The root end of the hair is placed in an appropriate UV'd tube or extraction vial which is supplied by the FB/DNA Section. Only one (1) hair is to be placed in each container unless previous arrangements have been made with the DNA Analyst.

9. The remaining evidence is repackaged and forwarded to the appropriate agency or location.

10. A case file and report is generated reflecting any actions and examinations performed.

REPORT WORDING

A report will be generated reflecting the actions of the Micro/Trace analyst. See MT-APP-I Report Wording.

REFERENCES

Forensic Biology/DNA Procedures Manual Procedure: Selection and Preparation of Hairs for DNA Analysis (IID-1).

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Fibers

Method: Sampling

Procedure: Obtaining Known Samples from Fabric

Reviewed by:

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Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

General Considerations

Fibers and the fabrics from which they originated may comprise some of the major pieces of evidence found during the crime scene investigation. Large numbers can be shed and transferred in accordance with the Locard exchange principle.

Fibers can be divided into two categories, natural and man-made. Each category contains a wide variety of generic classes and subclasses. The forensic scientist has to be not only knowledgeable in fiber classification but also knowledgeable in fiber transfers, fiber collection, fiber and fabric manufacture, and methods of fiber identification and comparison.

Comparison of fibers is a painstaking process. The examiner can approach the fiber comparison by setting out to show that the samples are not similar. The failure to detect any significant differences, after exhausting the methodology available to the examiner, necessitates the conclusion that the fibers could have the same origin. Often, white (colorless) and "indigo" blue cotton fibers are encountered which have little, if any, evidential value due to their prevalence. Fabric comparisons are more conclusive than a fiber comparison.

Physical and Chemical Properties

The properties which can be examined and compared are color and shade, longitudinal shape and size, internal characteristics, generic class and subclass, cross-sectional shape, refractive index/indices, birefringence, interference colors, pleochroism, sign of elongation, fluorescence, damage, common contaminants, and any processing. Along with those properties, fabrics can be examined for weave pattern, thread count, dye pattern, and thread twist direction.

These properties can be observed using magnifying glass, linen counter, vernier calipers, microspectrophotometers, cross-sectioning kit, FTIR, solubility schemes, melting point apparatus, refractive index liquids, and stereo, polarizing, comparison and epi-fluorescence microscopes.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.
Biohazard Precautions, if applicable.

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. Known samples should consist of fibers from the warp, weft, course, wale, and the stitching of the fabric. Several areas may have to be sampled due to fabric construction differences, fading, etc. Consideration should be given to collecting fibers from the victim's and the suspect's environments.

B. Fills, felted and non-woven fabrics need to be carefully sampled to obtain a representative known sample.

C. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

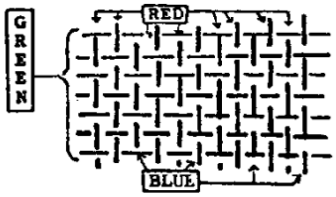
Stereomicroscope.

PROCEDURE OR ANALYSIS

Examine all yarns, (both directions) for any differences and sample the warp, weft and stitching, if applicable, of the fabric. Also, sample any faded areas.

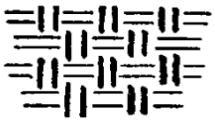
EXAMPLES: *(Warp = Horizontal direction in these diagrams)

1. YARNS OF DIFFERENT COLORS



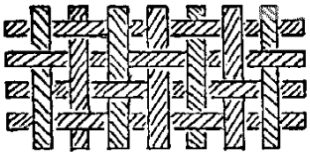
PLAIN WEAVE (O1 U1, by 1)
THREE-COLOR DESIGN
*WARP = GREEN ONLY
FILLING = 2 RED YARNS (INDIVIDUALLY WOVEN) ALTERNATELY WITH 1 BLUE YARN

2. YARNS OF DIFFERENT THICKNESS



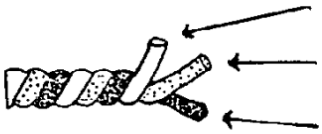
BASKET VARIATION OF THE PLAIN WEAVE
*WARP = A PAIR (2 WOVEN AS ONE) OF THIN YARNS ALTERNATELY OVER A PAIR OF WARP YARNS

3. YARNS OF DIFFERENT CONSTRUCTIONS



TWILL WEAVE, UNEVEN (02 U1, by 1)
*WARP - 2-PLY "S" YARNS ONLY (2S-1Z)
FILLING = ONE 1-PLY "S" YARN ALTERNATELY WITH ONE 1-PLY "Z" YARN

4. DIFFERENCES BETWEEN PLIES OF ONE YARN



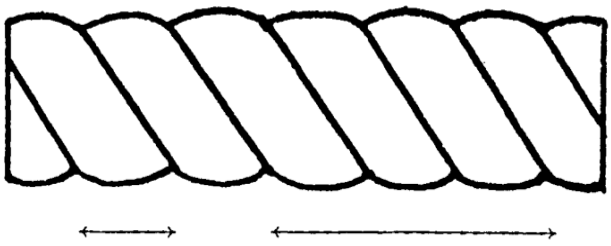
PLY WITH LUSTROUS (BRIGHT) FIBERS
PLY WITH SEMI-LUSTROUS (SEMI-DULL) FIBERS
PLY WITH HEAVILY DELUSTERED (DULL) FIBERS

3-PLY "S" YARN (3S-1Z) OF 100% NYLON 6.6 (EACH PLY CONTAINS MANY OF THE ABOVE INDICATED FIBERS TWISTED IN A "Z" DIRECTION)

Fibers of a 1-ply yarn or twine; piles of a 2-(or more) ply yarn or cordage; strands of a 2-(or more) strand ply:

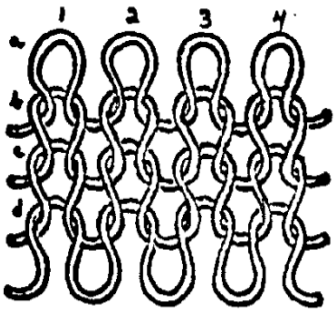
"S" TWIST "Z TWIST"

"CROWNS": TWISTED CORDAGE

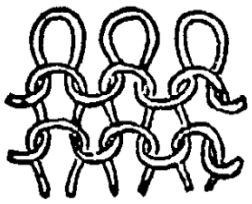


ONE "CROWN" ONE INCH (3 CROWNS PER INCH)

PLAIN KNIT (Jersey Stitch)



FACE



BACK

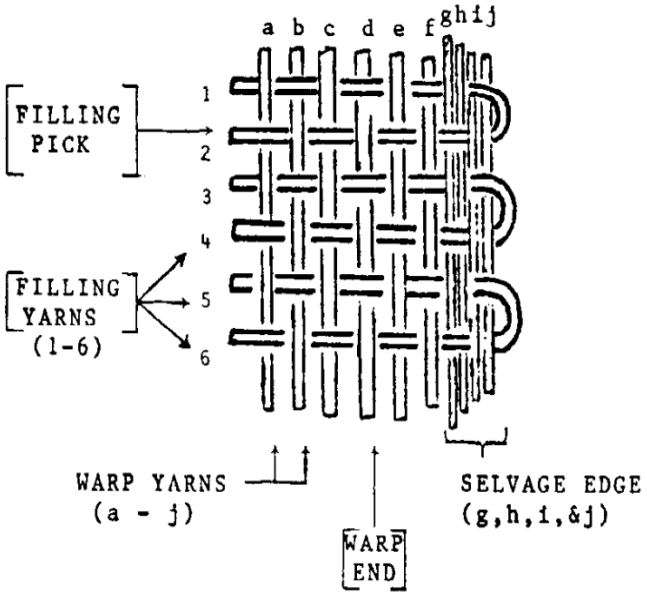
4 Main Types of Knit Stitches:

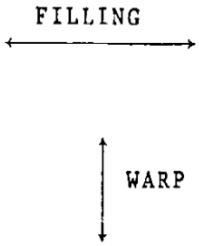
1. Knit Stitch
2. Purl Stitch
3. Miss Stitch
4. Tuck Stitch

COURSES = Rows of stitches running horizontally (Loops 1, 2, 3, & 4)

WALES = Rows of stitches running vertically (Loops a, b, c, & d)

PLAIN WEAVE -- O1 U1





3 Common Classes of Weave:

1. Plain Weave
2. Twill Weave
3. Satin Weave

NOTE:

The above listed yarns may or may not differ in composition from one yarn to another (within one fabric) or within a single yarn. The degree of delustering or dyeing may also differ. Therefore, "complete segments" of each yarn type within a fabric should be mounted (teased out) on a glass microscope slide to use as a "known" for comparisons with mounted questioned fibers.

REPORT WORDING

See Appendix I.

REFERENCES

Any good textile book dealing with the manufacturing of fibers and the construction of fabrics.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Sampling

Procedure: Comparison of Yarns to Fabrics

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The comparison of yarn to fabric requires the comparing of the physical dimensions of the questioned yarn to the physical dimensions of the yarn that composes the known fabric. If no significant differences are detected at that stage, then further examination and comparison of the fibers using the available procedures in this protocol are performed.

Other Related Procedures:

MT-VIIB and MT-VIIC

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.
Biohazard precautions, if applicable.

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. Known samples should consist of fibers from the warp, weft, course, wale, and the stitching of the fabric. Several areas may have to be sampled due to fabric construction differences, fading, etc. Consideration should be given to collecting fibers from the victim's and the suspect's environments.

B. Fills, felted and non-woven fabrics need to be carefully sampled to obtain a representative known sample.

C. Micrometers/calipers will be checked prior to use utilizing the appropriate gauge blocks to an established tolerance of +/- 0.003". A record of the check will be maintained in an appropriate log.

D. Fiber comparisons shall be required between questioned yarns and examplar fabrics if an association is established.

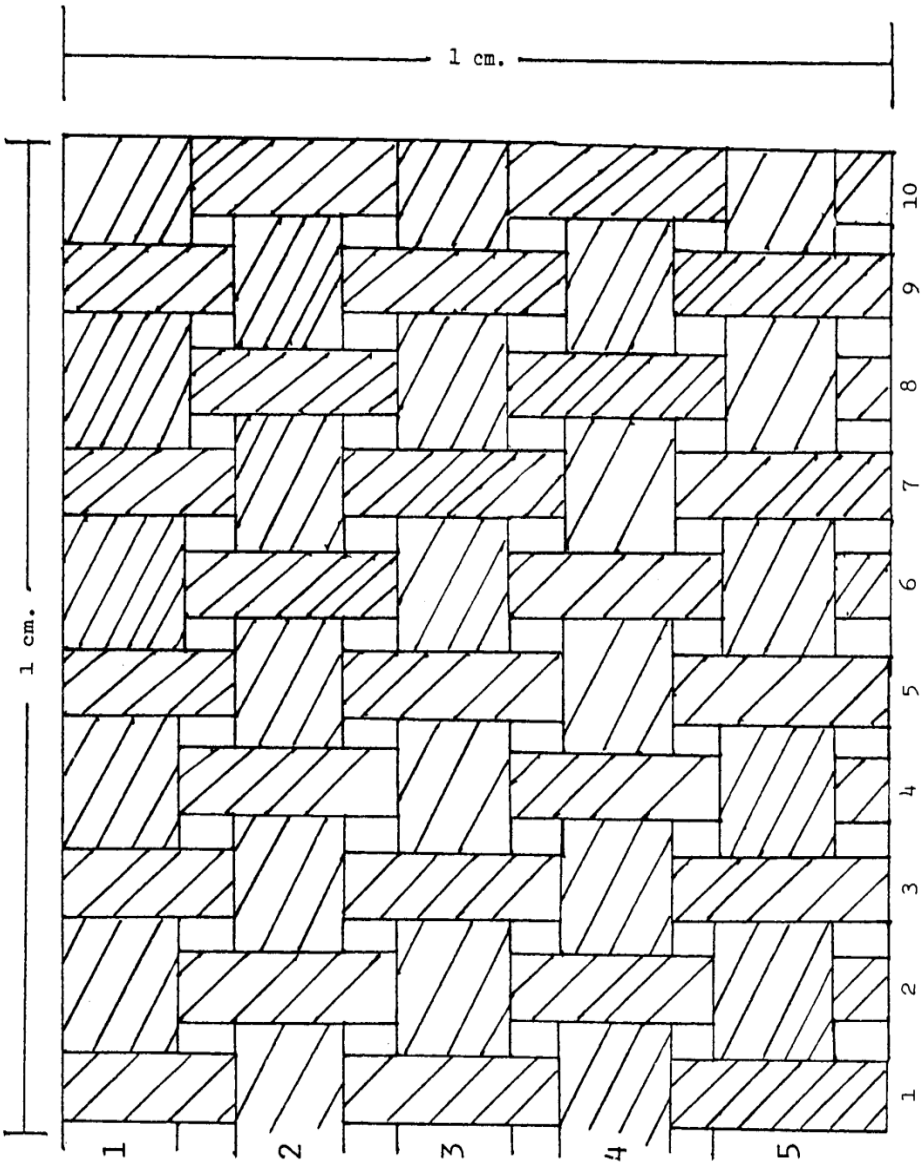
E. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

A. Stereomicroscope
B. Calipers
C. Micrometers

PROCEDURE OR ANALYSIS

These instructions will use a plain weave of over 1, under 1.



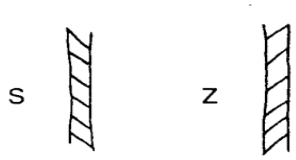
I. Count the number yarns/unit length in each direction.

A. In this example, there are ten yarns/cm. in the parallel position and five yarns/cm. in the perpendicular position.

B. There will usually be more warp yarns/unit length than filling yarns, i.e., in the example, there are ten warp yarns/cm. and five filling yarns/cm.

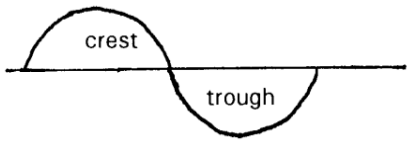
II. Remove the warp yarn and:

A. Determine the twist:



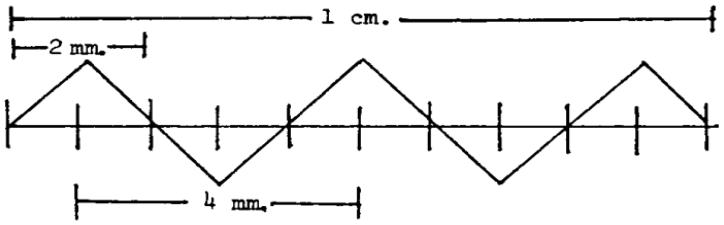
B. Count the number of crests and trough/unit length.

1.



2. On a simple over 1, under 1 weave, the number of crests and troughs in the warp yarn should equal the number of filling yarns, as determined in Step 1, in the same unit length.

3. In our example, there is a total of five crests and troughs/cm.



Calculations: $\frac{\text{unit lengths}}{\text{\# of crests and troughs}}$ = width of weave, (for a tight weave only)

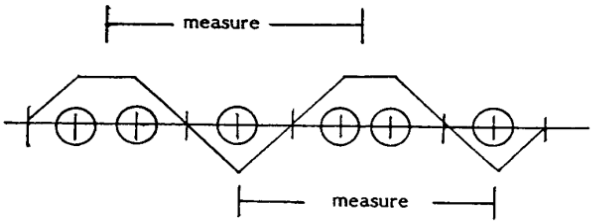
1cm/5 crests; each crest is 2 mm wide

peak to peak distance = (width of weave) x 2 = 4mm.

4. Note: Be careful of weaves other than over one and under one, or loose weaves.

   a. As mentioned above, one wave of the warp yarn should equal the width of a filling yarn in a tight weave. In a loose weave, the width of the weave may be much larger than the width of the filling yarn.

   b. In some cases where a weave goes over two or more fibers, it may be necessary to take two or more approximate measurements.



III. Repeat Step II for the filling yarn.

IV. Summary of determinations in the example.

	Warp Yarns
Yarns/cm.	10
Twist	Z
Total # of crests and troughs/cm.	5

Width of one weave $\frac{\text{unit length}}{\text{\# of crests and troughs}}$ $\frac{1\text{ cm}}{5}$ = 2mm

Peak to peak distance Width of weave x 2 = 2mm x 2 = 4mm

	Filling Yarns
Yarns/cm.	5
Twist	S
Total # of crests and troughs/cm.	10

Width of one weave $\frac{\text{unit length}}{\text{\# of crests and troughs}} = \frac{\text{cm}}{10} = 1\text{mm}$

Peak to peak distance Width of weave x 2 = 1mm x 2 = 2mm

V. Make approximate physical measurements on unknown.

A. If approximate measurements differ greatly - eliminate.

B. If approximate measurements are close - compare.

VI. Comparisons

A. If possible, count the number of fibers in the yarns.

1. Construction of a yarn.

a. Fibers make up a yarn.

b. Two or more yarns make up a strand.

2. The warp and filling may be composed of yarns and strands and should be broken down accordingly.

B. Determine the composition of the warp and filling yarns.

C. If you have the entire garment and you can follow the pattern, you may be able to determine where a yarn is missing and possibly match your questioned yarn to that area.

D. If similarities are detected between questioned yarn(s) and known fabric(s), further examination and comparison of fibers using procedures in this protocol are to be performed.

REPORT WORDING

See Appendix I.

REFERENCES

Any good textile book dealing with the manufacturing of fibers and the construction of fabrics.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Fibers

Method: Microscopic Examination and Comparison

Procedure: Generic Class Determination by Refractive Index

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The determination of the physical and optical properties of a fiber is sufficient to identify the natural fibers and is a preliminary step in identifying the man-made fibers.

Other Related Procedures:

MT-VIIB-2, 3 & 4
MT-VIIC-2 & 3

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
Cargille Liquids	3-2-2
Permount	3-3-0
Xylenes	2-3-0

PREPARATIONS

Standard Laboratory Practices.
Kohler Illuminations for Polarized Light Microscope

MINIMUM STANDARDS & CONTROLS

A. Animal fibers for comparison will be identified by the MT-VIA Procedure.

B. Vegetable fibers for comparison will be identified by the MT-VIIB-5 Procedure.

C. Man-made fibers for comparison shall be identified as to generic class using polarized light microscopy, and at least one more identification technique such as solubility tests, Berek Compensator or FTIR.

D. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Brightfield microscope.
Polarized light microscope.

PROCEDURE OR ANALYSIS

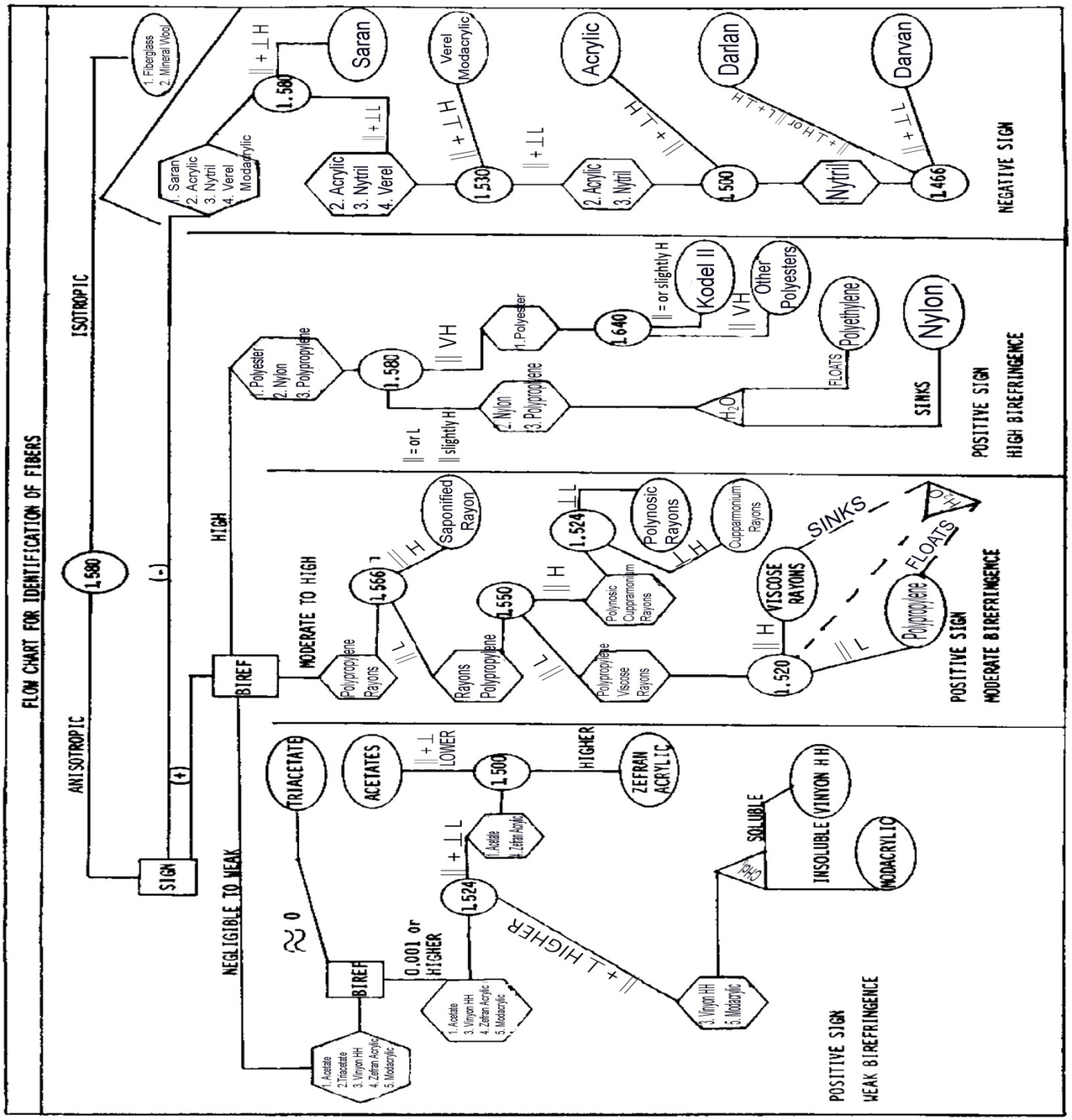
A. Relative Refractive Index Determination of a Fiber, Interference and Birefringence Estimation

An experienced microscopist may use the observations of optical properties to aid in characterization of a fiber. Relative refractive index and estimated birefringence determinations are insufficient procedures to determine fiber generic class.

1. Determine the direction of plane polarized light in the polarizing light microscope.
2. Mount the fiber in Permount, xylene, Meltmount, Cargille oil, or another suitable medium for which the refractive index can be estimated.
3. Focus the fiber on the microscope. Orient it parallel to the plane of polarized light. Perform Becke line observations.
4. Rotate the fiber to a position perpendicular to the plane of polarized light. Repeat Becke line observations.
5. The relative refractive indices (parallel and perpendicular) of the fiber compare to the mounting medium may be determined.
6. Insert the analyzer to cross the polars. Orient the fiber 45-degrees to the polarizer and analyzer. Observe the brightness, colors (interference colors) and extinction (positions of darkness) of the fiber as the microscope stage is rotated.
7. Fiber birefringence can be estimated from examination of the interference colors. A Michel-Levy color chart can be used as a reference for this.
8. Refractive indices and birefringence values will vary with fiber manufacturer, cross-sectional shapes, inclusions, and dyes. These values should be considered approximations for generic fiber identifications
9. The following charts may be used to reference generic fiber types based on relative refractive indices or birefringence determinations.

Fiber Type	N parallel	N perpendicular	Sign of Elongation	Birefringence
Acetate	1.478	1.473	+	0.005
Triacetate	1.469	1.469	+ or -	0 – 0.001
Acrylic	1.50-1.53	1.50-1.53	-	0.0020 – 0.005
Modacrylic	1.535	1.533	+	0.002
Vicose Rayon	1.535-1.555	1.515-1.535	+	0.020
Olefin/Polypropylene	1.530	1.496	+	0.034
Nylon/Polyamide	1.58	1.52	+	0.060
Polyester	1.71	1.53	+	0.175

Particle Atlas Edition Two
McCrone, Delly 1973 pp. 362-367



REPORT WORDING

See Appendix I.

REFERENCES

1. Catling, D.; Grayson, J. *Identification of Vegetable Fibers*; Chapman & Hall: New York, 1982.

2. Luniak, B. *The Identification of Textile Fibers*; Textile Book Service: Metuchen, NJ, 1953.

3. Mauersberger, H.R. *Matthew's Textile Fibers*, 6th ed.; John Wiley & Sons: New York, 1954, Chapters 1-9, 15, 19, and 20.

4. Robertson, J., Ed. *Forensic Examination of Fibers*; Ellis Horwood: New York, 1992.

5. Textile Institute. *Identification of Textile Materials*, 7th ed.; Textile Institute: Manchester, England, 1975.

6. Wildman, A.B. "The Identification of Animal Fibers"; J. Forensic Science Society 1961, 1, 1-18.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

PROTOCOL: Fibers

METHOD: Microscopic Examination and Comparison

PROCEDURE: Cross-Sectioning Fibers

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The cross-sectioning of fibers can give the examiner diagnostic information for identifying natural fibers and also give discriminating and diagnostic information for man-made fibers. Cross-sectioning can be carried out by two methods: optical cross-sectioning and physical cross-sectioning. These techniques can also be used to cross-section hairs.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.
Sharps Precautions.

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

Both the questioned and standard shall be treated in the same manner. A physical cross-section and photomicrography shall be used in determining the modification ratio of man-made fibers.

Jolliff method: Use packing threads of a contrasting color to the fiber being cross-sectioned. Do not over pack the plate.

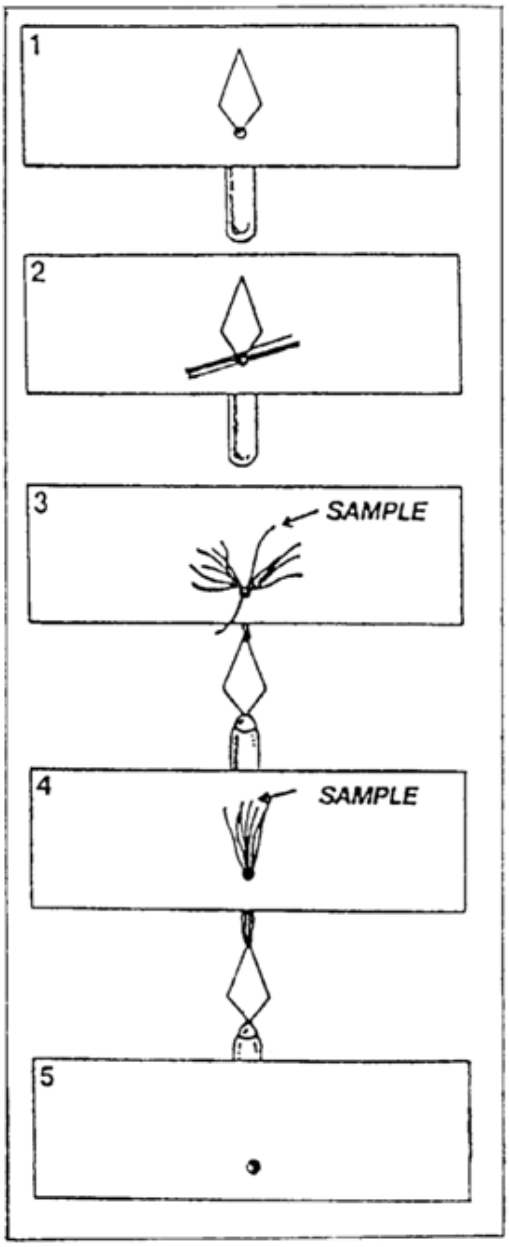
INSTRUMENTATION

Brightfield microscope.

PROCEDURE OR ANALYSIS

Technique 1: Jolliff Cross-Section Method.

CROSS-SECTIONING HAIRS AND FIBERS



Reference: Jolliff Cross-Section Kit, Industrial Analytical Laboratories, Fort Wayne, Indiana 46825

Procedure:

A. Push threader through hole in black cross-section plate.

B. Cut 4 2-inch lengths filler yarn of a color contrasting with the sample. Place these in the needle threader loop.

C. Pull the needle threader and filler yarn about 1/2 inch through the hole, leaving a tuft of filler yarn above the slide surface. Spread out the tuft and place a sample of the fiber to be examined across the tuft bundle.

D. Pull sample and filler yarn through the sample hole until the sample has partially passed through.

E. Cut filler and sample fiber above and below the slide with scissors. Cut filler and sample with a razor blade on both sides of the slide with a smooth even stroke.

F. A square around the cross-section can be cut out and mounted on a slide for permanent reference.

Additional cross-section slides available from Insulfab Plastics, Inc., 150 Union Avenue, East Rutherford, New Jersey 07073.

Note: Threader can be made by using a guitar string approximately 0.25 or 0.28 mm and a wall anchor.

Technique 2: Optical Sectioning

1. Mount the fiber longitudinally on a slide in a suitable mounting medium.

1. Using a compound microscope, focus on the fiber.

2. Slowly move the stage up and down to focus on the different planes of the fiber. The apparent cross section can be determined by which areas are in focus as the optical distance is changed.

Technique 3: Embedding in LR White

Materials and Equipment

Embedding mold tray	LR	White Resin (catalog #17411-500
Block Holder		LR White Accelerator (catalog #17413-10)
Fine Tip Tweezers		Polysciences Inc. 400 Valley Road
Microtome and Blades		Warrington PA 18976 (1-800-523-2575)

1. Prepare a suitable fiber sample that will be accommodated by the well size of embedding mold.

2. Pipette LR White Resin into the well of the embedding mold. For a 20 mold tray, about 2 mL of resin is needed for each sample well.

3. Add about 5 uL of LR White Accelerator to the well and mix thoroughly. The resin will begin to thicken in several seconds. The proportions of LR White Resin to Accelerator may be adjusted as needed.

4. Work quickly and use the tweezers to pick up the fiber sample and immerse it vertically into the resin. Push the sample all the way down to touch the bottom of the mold. The sample with stay upright if the resin has thickened enough. Hold the fiber vertically until the resin is set enough to hold it upright.

5. Quickly place a Block Holder on the prepared specimen so that the resin will set up with the holder in place.

6. Allow the preparation to dry. It should be solid in about 30 minutes.

7. Remove the preparation from the tray by inverting the tray and pushing on the underside of the well mold.

8. Fit the preparation onto the microtome by the block holder. A microtome and blade appropriate for sectioning plastics are required.
9. Section samples from the preparations as required. A fiber positioned vertically in the preparation will be cross-sectioned in the microtome.

10. Thin sections of about 10 to 50 µm may be mounted on microscope slides for examination.

11. Other embedding material may be substituted for LR White Resin. Manufacturer's direction for use are recommended.

Technique 4: Embedding in Polyethylene sheet material

Materials and Equipment

Glass slide
Fine tip tweezers
Hotplate
Scalpel or single-edge razor blade
Scissors

Polyetheylene sheet material
(such as found in a plastic bag)

1. Prepare a suitable fiber sample.

2. Turn on the hotplate to a medium heat setting.

3. Cut two portions of polyethylene sheet with scissors. They should be sized to fit on top of the glass slide without overhanging the edges.

4. Place one sheet on top of the glass slide. Using tweezers, place the fiber sample on top of the sheet. Place the second sheet on top of the fiber. This "sandwiches" the fiber between the two sheets. A few drops of xylene substitute may aid in the adhesion of the polyethylene sheets.

5. Place the glass slide and sample preparation on the hotplate. Allow the sheets to melt together. This will embed the fiber sample between the two sheets. Do not allow the sheet material to burn. Do not allow the sheet material to melt off the glass slide onto the hotplate.

6. Remove the glass slide with the preparation and allow to cool.

7. Using a scalpel or single-edge razor blade, cut the sample preparation into thin sections in a direction perpendicular to the length of the fiber. This will produce cross-sections of the embedded fiber.

8. These cross-sections may be mounted on microscope slides for examination.

9. Short polyethylene tubes, such as cut from a disposable pipet, may also be used to embed fibers. The fiber is placed in the tube center instead of "sandwiching" between two sheets. Follow steps 1-9, substituting the tube preparation for the polyethylene sheets.

REPORT WORDING

See Appendix I

REFERENCES

1. Skirius, Stephen, "Fiber Cross-Sections", Microscope, 34 (1), 26, 1986.

2. Welsh, Frank S., "A Polished Paint Layer Crossection in 30 Minutes", Microscope, 45 (2), 37-40, 1997.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Microscopic Examination and Comparison

Procedure: Comparison

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The comparison of questioned fibers with fibers from a known source is performed in every step of the examination of the fibers once the questioned fibers are recovered and the standard fibers are collected from a known source. The comparison process can be as simple as visual examination to as complex as an instrumental method.

The examiner can approach the fiber comparison by setting out to show that the samples are not similar. The failure to detect any significant differences, after exhausting the methodology available to the examiner, necessitates the conclusion that the fibers could have the same origin. Often, white (colorless) and "indigo" blue cotton fibers from blue jean material are encountered which have no evidential value due to their prevalence.

The examiner may state or imply that the questioned fiber exhibits the same microscopic characteristics and optical properties as the known sample, and accordingly, the questioned fiber is consistent with originating from the source of the known sample or from another item comprised of fibers that exhibit the same microscopic characteristics and optical properties. A fiber association is not a means of positive identification and the number of possible sources for a specific fiber is unknown. However, due to the variability in manufacturing, dyeing, and consumer use, one would not expect to encounter a fiber selected at random to be consistent with a particular source.

Other Related Procedures:

All Procedures in this Protocol.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. Fibers for comparison shall be identified by animal type or generic class as required by Appendix II Minimum Standards and Controls.
B. Fiber comparisons shall be conducted using a comparison microscope. The only exception shall be when fiber standards have been submitted and show significant visual or stereoscopic differences and the fibers do not represent a transfer. Microscopic dissimilarities may defer further testing.
C. Fiber comparisons shall include fluorescence microscopy observations.

D. Microspectrophotometry shall be used in the comparison of fibers that are dyed sufficiently to produce an adequate spectrum.
E. Fiber comparisons involving meaningful transfers shall be verified by a second qualified examiner. Fiber verifications shall be made by microscopic comparison of all standards and pertinent fibers. Verifications shall be documented by the verifying examiner.
F. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Stereomicroscope
Brightfield microscope
Polarized light Microscope
Comparison Microscope
Fluorescence Microscopy

PROCEDURE OR ANALYSIS

The comparison of fibers requires a combination of indirect and side-by-side comparison processes. The examiner should compare the questioned to the standard at all stages of the examination to determine if there are any unexplained (significant) differences. If these differences are observed at any point in the comparison process, the question fiber has to be considered as not originating from the source represented by the standard.

Fiber comparisons shall be conducted using a comparison microscope. In general, the microscopic characteristics which are compared include color; shade; fiber diameter and variations along its length; cross-sectional shape; surface features; internal characteristics; refractive index/indices; birefringence; pleochroism; interference; sign of elongation; fluorescence; and damage.

Instrumental analysis findings must also be consistent between question and standard fiber samples. Microspectrophotometry shall be used for comparison for suitable fibers.

All of these features/characteristics must be consistent in order for the conclusion that a fiber transfer has occurred.

REPORT WORDING

See Appendix I.

REFERENCES

1. Gaudette, B. D. In *Forensic Science Handbook*; Saferstein, R., Ed.; Prentice Hall: Englewood Cliffs, N.J., 1988; Vol. II, Chapter 5.

2. Robertson, J., Ed. *Forensic Examination of Fibers*; Ellis Horwood: New York, 1992.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Microscopic Examination and Comparison

Procedure: Solubility

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The solubility of man-made fibers in specific liquids and the fibers optical properties allows an examiner to identify the generic class of the fiber. Solubility tests seldom allow an examiner to identify the sub-generic class of a fiber and it also destroys the sample used in testing. For those two reasons, other non-destructive tests should be run if the sample is of limited size.

Other Related Procedures:

All Procedures in this Protocol.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
acetic acid, glacial	3-2-1
acetone	1-3-0
acetonitrile	2-3-0
chloroform	2-0-0
cyclohexanone	1-3-0
dimethyl formamide (DMF)	1-2-0
formic acid	3-2-0
hexafluoroisopropanol	3-0-0
Hydrochloric acid, conc.	3-0-0
nitric acid, conc.	3-0-0
sulfuric acid, conc. & 75%	3-0-2
water	0-0-0

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. All solvents and reagents used in solubility testing shall be checked by using known fibers from the reference collection before use on each case. The results of solubility reagent checks will be recorded in the case file.

B. The questioned and known fibers shall be examined in the same manner.

INSTRUMENTATION

Stereomicroscope or an old brightfield microscope dedicated to solubility testing.

PROCEDURE OR ANALYSIS

A. Select one of the following solubility charts.
B. Starting at the top and working downward, conduct solubility tests in the following manner. The fiber's optical properties may eliminate some steps.

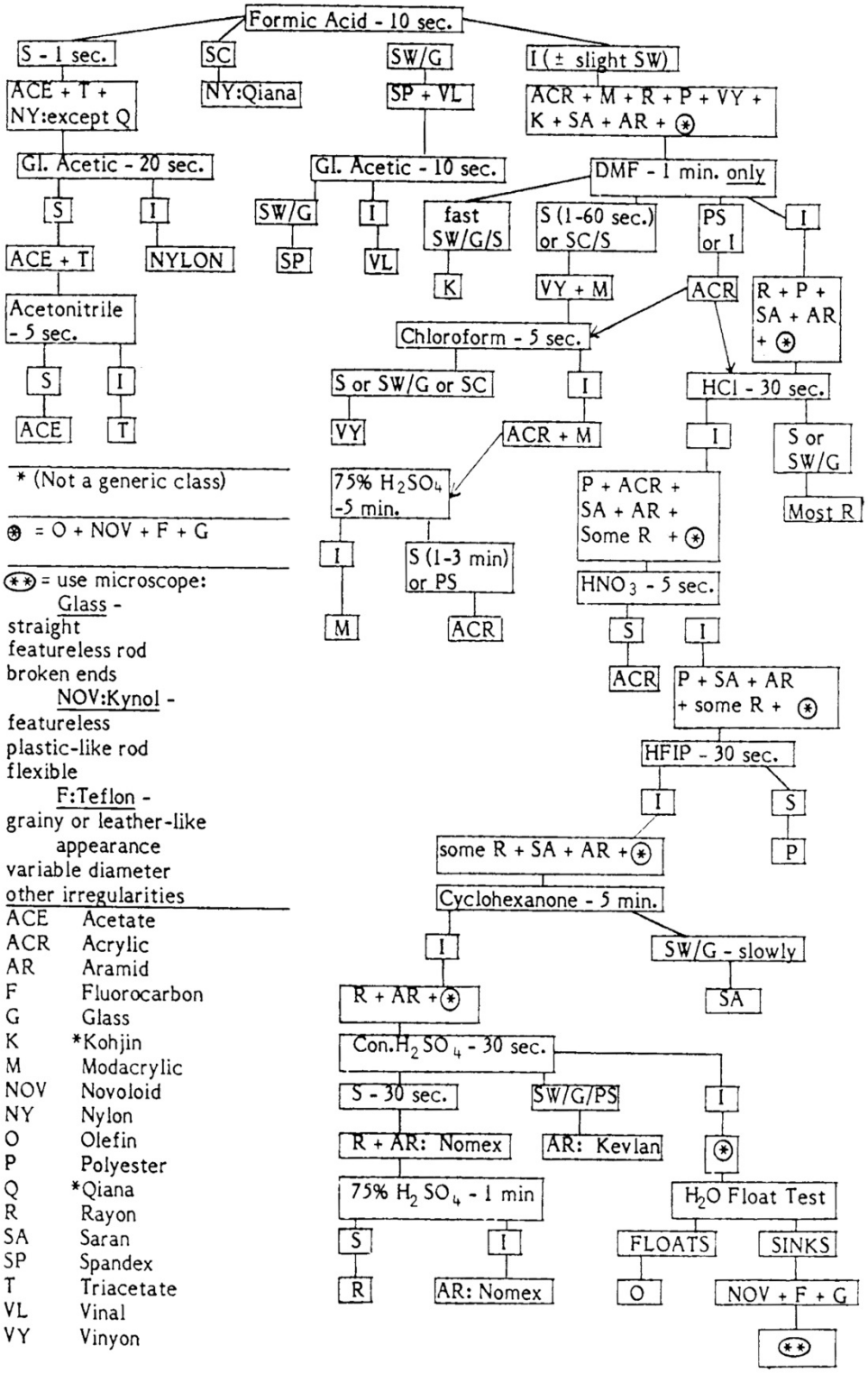
1. Place a small piece of the fiber on a microscope slide.
2. Cover with a coverslip and place on a microscope.
3. Focus on the fiber.
4. Allow the selected reagent to run under the coverslip and react with the fiber.
5. Observe whether the fiber is soluble, insoluble, swells, gels, or shrinks.
6. Compare results with the selected chart and proceed to the next step.
7. Continue until fiber is identified.

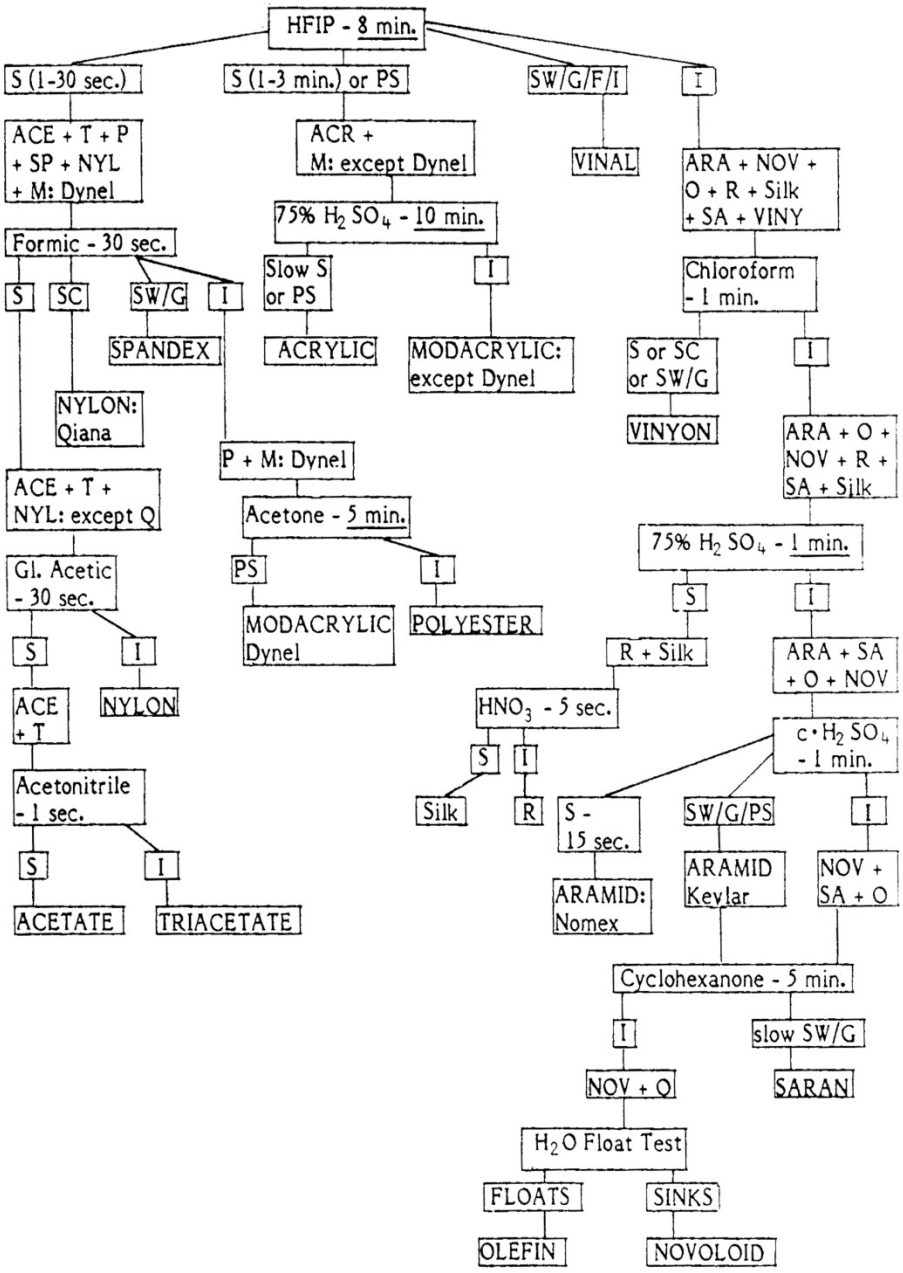
Key to Solubility Reactions:

S	=	Soluble (fades, splinters, or breaks apart and goes into solution).
SW	=	Swells
G	=	Gels (Plasticizes)
P or PS	=	Partly Soluble (not all portions of a fiber or not all fibers within a group of like fibers are soluble within 5 minutes).
F or Fan	=	Ends "fan" or "mushroom" (damaged areas also swell).
B	=	"Balloons" (forms large swellings with unswollen bands between the swellings).

SC = "Spinal Column" (a fast SW/G into a form which resembles the series of small bones forming a human "backbone").

I = Insoluble (no reaction).





These generic fiber classes are included in this scheme:

ACETATE	NYLON	SPANDEX
ACRYLIC	OLEFIN	TRIACETATE
ARAMID	POLYESTER	VINAL
MODACRYLIC	RAYON	VINYON
NOVOLOID	SARAN	(Silk is also included)

REPORT WORDING

See Appendix I.

REFERENCES

1. Gaudette, B. D. In *Forensic Science Handbook*; Saferstein, R., Ed.; Prentice Hall: Englewood Cliffs, N.J., 1988; Vol. II, Chapter 5.

2. Robertson, J., Ed. *Forensic Examination of Fibers*; Ellis Horwood: New York, 1992.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Microscopic Examination and Comparison

Procedure: Natural Fiber Identification

Reviewed by:

_____
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Micro/Trace Command Advisory Board

Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

For this procedure, natural fibers consist of commercial vegetable fibers, silk, and asbestos. Animal hair used in textiles will be identified using hair procedures. The principle component of vegetable fibers is cellulose; therefore, these fibers may be referred to as cellulosic fibers.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
acetic acid, glacial	3-2-1
Cargille liquids	3-2-2
chloroform	2-0-0
glycerine	1-1-0
hexafluoroisopropanol	3-0-0
hydrogen peroxide, 30%	2-0-1 (strong oxidizer)
nitric acid, conc.	3-0-0
Permount	3-3-0
sulfuric acid, 75%	3-0-2
xylenes	2-3-0

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. All natural fibers will be examined by a minimum of brightfield microscopy. All vegetable fibers, except cotton, will be crossed sectioned for identification.
B. All natural fibers, except cotton, will be compared for identification to reference collection fibers.
C. Distinguishing characteristics must be present for identification. Case notes shall document characteristics which resulted in identifications.

INSTRUMENTATION

Stereomicroscope
Brightfield microscope
Polarized light microscope
Dispersion staining microscope

Transmission electron microscope (TEM)

PROCEDURE OR ANALYSIS

Drawings and/or photomicrographs can be found in the references of this procedure.

CAUTION: Dislocations, by themselves, are not significant in any fibers for a definitive identification and the drying-twist technique is not always diagnostic.

1. Visual examination of the fiber.
2. Stereoscopic examination of the fiber.
3. Brightfield microscope examination of the fiber.

Depending on the type of fiber, the appropriate additional possible steps can be used:

4. Cross-section
5. Polarized light microscopy
6. Dispersion staining
7. Transmission electron microscope (TEM)
8. Refractive index determination
9. Solubility
10. Techniques A – D in this procedure

Cotton (Gossypium)

Distinguishing characteristics:

Flattened, ribbon-like, convoluted surface (twisted) with reversal in direction. Bright irrespective of its position between crossed polars.

Supporting characteristics:

Varying lumen size and shape. Irregular dye take-up.

Coir (Cocos nucifera L.)

Distinguishing characteristics:

Cross-section is a bundle of thick wall cells with irregular shaped lumen.

Supporting characteristics:

Lens-shaped stegmata in ash. Ultimates range from 0.01 to 0.04" long and 12 to 24 µm in diameter. Pits in fibers.

Flax (Linum usitatissimum)

Distinguishing characteristics:

Irregular polygonal cross-section, pentagonal or hexagonal slightly rounded in outline, thick walls. X-shaped cross hatches. Smooth surface except where ringed with transverse nodes.

Supporting characteristics:

Small, well-defined lumen disappearing near the ends, except in immature fibers. Ultimates . 3mm long and 15-20 µm in diameter with pointed ends. High luster. Dislocations. Occasionally, cells show faint cross-checking. No crystals or convolutions.

Hemp (Cannabis sativa)

Distinguishing characteristics:

Irregular cross-section. Longitudinal fractures, dislocations are frequent and pronounced. No nodes.

Supporting characteristics:

Cell ends are blunt and irregular to tapering pointed ends. Ultimates are 0.5 - 5 cm long and 15-50 µm in diameter. Cluster crystals in short chains, 3-4 long. Single cluster crystals. Very occasional cubic, rhombic and small acicular crystals.

Jute (Corchorus capularis or Corchorus olitorius)

Distinguishing characteristics:

Polygonal or rounded polygonal, thick wall cross-section with large lumen. Smooth longitudinal surface with only occasional markings.

Supporting characteristics:

Spindle shaped ultimates, 1-6mm long, 15-25 µm in diameter. Pitted cell walls. Regular dislocations, but not numerous. Cubic crystals in chains and occasional cluster crystals. Usually light brown in color.

Kapok (Bombax malabaricum or Eriodendron anfractuosum)

Distinguishing characteristics:

Hollow, smooth cylindrical, thin walled (wide lumen, very thin walls).

Supporting characteristics:

Frequently bent over itself. Tapers to point at one end, slightly bulbous base at other end. Ultimates . 18mm long and 20-30 µm in diameter.

Manila or **Abaca** (Musa textilis)

Distinguishing characteristics:

Irregular round or oval cross-section, irregular sizes, thin walls and large empty lumen. Stegmata in ash.

Supporting characteristics:

3-12 mm long and 16-32 µm in diameter ultimates. Fiber cells have smooth walls and sharp or pointed ends. Silica bodies.

Ramie (Boehmeria nivea)

Distinguishing characteristics:

Elongated cells, thick walls and well defined lumen in cross-section. Small node-like ridges and striations longitudinally. Undyed fibers are very white with silk-like luster.

Supporting characteristics:

Ultimates are 2.5-30 cm long and 40-75 µm in diameter. Cross markings. Crystals in unprocessed fibers. Cluster crystals in chains and single cluster crystals.

Sisal (Agave sisalana)

Distinguishing characteristics:

Spiral elements. Broad lumen. Fiber ends are broad, blunt and sometimes forked. Polygonal cross-section, sometimes with rounded edges.

Supporting characteristics:

Ultimates are 3-7 mm long with an average 24 µm diameter. Large acicular crystals.

Silk

Distinguishing characteristics:

Fine diameter, 9-12 µm average. RI . 1.50 to 1.55. Irregular appearance due to cross-section which is triangular with rounded corners. No delusterants. Structureless surface with cross-over markings on some. Solubility scheme can be used for identification.

Asbestos

Distinguishing characteristics:

Elemental composition. Currently, the scientific community criterion for identification is transmission electron microscopy (TEM). Use dispersion staining or polarized light microscopy for tentative identification and use the phrase "asbestos or asbestos-like" in report. See "References."

TECHNIQUES

A. Removal of air in fiber bundles

Equipment: Hot plate and a beaker.

1. Boil in water to remove the air.
2. Mount in appropriate mounting medium.

B. Maceration (Produces fiber ultimates)

Equipment: Hot plate and a flask.

1. Place sample in a flask with 6% hydrogen peroxide-glacial acetic acid (2:1) and reflux for one hour at 100E C.
2. Transfer fiber sample to distilled water and shake to separate fiber strands into individual cells.
3. Mount in appropriate mounting medium. Temporary mountant - 50% glycerin. Permanent mountant - Permount or Meltmount.

C. Ashing (Caution: destructive technique.)

Equipment: Muffle furnace (a Bunsen burner may be substituted) and porcelain crucible.

1. Place sample in a small porcelain crucible, with lid, and heat in muffle furnace at 600E C for 3-4 hours. Cool.
2. Mount ash in proper mountant, moving the ash as little as possible.

D. Drying-twist Test

Principle: If a wet flax fiber is held at one end and allowed to dry, the free end, directed at the observer, will be seen to move in a clockwise direction. Hemp fiber under similar conditions follows a counterclockwise movement.

Equipment: A pair of tweezers with fine tips meeting accurately.
A hot plate. A dark background is helpful.

1. Soak the fibers for a few minutes in water.
2. Using the tweezers, remove the fibers.
3. Hold fibers over the hot plate (low temperature setting) and observe the direction of twist on drying.

REPORT WORDING

See Appendix I. For asbestos, also refer to "PROCEDURE OR ANALYSIS" section of this procedure.

REFERENCES

1. Catling, D.; Grayson, J. *Identification of Vegetable Fibers*; Chapman & Hall: New York, 1982.

2. Mauersberger, H. R. *Matthew's Textile Fibers*, 6th ed.; John Wiley & Sons: New York, 1954, (appropriate chapters).

3. McCrone, W. C.; McCrone, L. B.; Delly, J. G. *Particle Atlas*; Ann Arbor Science: Ann Arbor, MI, 1973; Vol. II, pg. 373-374, Vol. V, pg. 1369-1378, and Vol. VI, pg. 1644-1651, (**for asbestos**).

4. Robertson, J., Ed. *Forensic Examination of Fibers*; Ellis Horwood: New York, 1992, (appropriate chapters).

5. Textile Institute *Identification of Textile Materials*, 7th ed.; Textile Institute: Manchester, England, 1975, (appropriate sections).

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Instrumental Examination and Comparison

Procedure: Microspectrophotometer

Reviewed by:

_____
Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Spectrophotometric techniques are based on the ability of substances to interact with characteristic frequencies of radiation. Since each isolated species of ion, atom, or molecule will exhibit an individual set of definite energy levels, it can absorb only the frequencies that correspond to excitation from one level to another.

The microspectrophotometer is used in forensic work to eliminate the possibility of a metameric pair. A metameric pair is two colors that appear to match visually in one type of illumination but have different wavelength absorption (or transmission) value(s). Examples of this would be known paint or fibers samples which microscopically match the color of the unknown sample, however, the UV-visible spectra of the known and unknown would be different.

A match of the color between the unknown and the standard can be mathematically described by using a modification of Beer's Law.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Caution: Light from the deuterium and mercury lamps is hazardous to your eyes. Take the necessary precautions.

PREPARATIONS

Standard Laboratory Practices.
Holmium Oxide, didymium, and photometric reference materials will be stored in the manufacturer supplied container.

MINIMUM STANDARDS & CONTROLS

A. The questioned and known samples shall be examined in the same manner.
   1. Mercury and deuterium lamps shall be turned on 30 minutes prior to use and allowed to stabilize.
   2. Integration time shall be optimized for each analysis.
   3. Precise fluorescence spectral measurements require the use of quartz slides and cover slips.

B. Performance checks shall be conducted each day prior to running samples.
   1. A wavelength performance check using holmium oxide and didymium and a photometric performance check using ND 0.1, ND 0.5, and ND 1.0 shall be conducted.

2. The ranges of acceptability of wavelength and photometric performance will be assured according to Appendix II Minimum Standards and Controls.
3. A fluorescence performance check using a known fluorescent material shall be conducted. Results will be checked for reproducibility.
4. Documentation of performance checks shall be maintained in an appropriate log and the case file.

C. A blank or background will be run for each sample.

D. Tests performed and their results are to be documented.
1. Relevant parameters shall be preserved in the casefile. Minimum parameters for microspectrophotometry are the sample preparation and mode (absorbance/transmittance/reflectance). For fluorescence, the filter cubes shall be recorded.
2. Spectra and spectra enhancements shall include the Date, Initials, Case number, and Item number ('DICI').

E. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Microspectrophotometer

PROCEDURE OR ANALYSIS

Method of sample preparation will be documented.

Since dye intensity can vary along a fiber's length (particularly with natural fibers) and because of the effects of irregular cross sections, several spectra should be obtained from each fiber sample. The exact number of runs required shall depend on the homogeneity of the questioned fiber and of the known sample. The analyst should plot mean (averaged) and/or standard deviation spectra calculated from multiple spectra of an item.

Comparisons of visible and UV transmission or absorption spectra are to be performed by graphical overlays of standard and questioned fiber spectral data. For a questioned fiber and a fiber standard to be considered consistent or similar, with each other, the spectra of the questioned fiber should fit within a range of the spectra of the fiber standard and have the same shape and appearance.

Comparison of fluorescence spectra begins with examination of entire spectral range and filter cube data, followed by examination of specific detected peaks. Comparison should include peak shape, maxima, minima, inflection points, and relative intensities.

If spectral differences are observed between questioned and known fibers, and these differences cannot be explained (e.g. sample contamination, condition, or damage), then the questioned fiber has to be considered as not originating from the known standard.

Parameters for sample visualization, data acquisition, and data display must be optimized. Documentation of relevant parameters shall be included in the case file.

REPORT WORDING

See Appendix I.

REFERENCES

1. Gaudette, B. D. In *Forensic Science Handbook*; Saferstein, R., Ed.; Prentice Hall: Englewood Cliffs, N.J., 1988; Vol. II, Chapter 5.

2. Robertson, J., Ed. *Forensic Examination of Fibers*; Ellis Horwood: New York, 1992.

3. Willard, H. H.; Merritt, L. L. Jr.; Dean, J. A.; Settle, F. A. Jr. *Instrumental Methods of Analysis*, 7th ed.; Wadsworth: Belmont, CA, 1988.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Instrumental Examination and Comparison

Procedure: FTIR Microscope

Reviewed by:

_____

Forensic Scientist Ellen Chapman, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____

Bureau Chief Timothy A. Tripp
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Forensic Sciences Command

INTRODUCTION

The generic classification of man-made fibers has historically been determined by a combination of optical properties by polarized light microscopy, solubility testing and melting-point determinations. These methods may become less reliable because of rapid advances in fiber technology. Infrared analysis of fibers can provide information about fiber composition to supplement that obtained by the aforementioned methods. The American Society for Testing and Materials (ASTM) lists IR spectroscopy as the preferred method of analysis for identifying man-made fibers (ASTM D276.00a (2008)).

The spectra and spectra enhancements shall include the Date, Initials, Case number, and Item number ('DICI').

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Liquid nitrogen precaution - extreme cold. NFPA is 3-0-0

PREPARATIONS

Standard Laboratory Practices.

A. The dewar must be filled with liquid nitrogen a minimum of thirty minutes before the first sample run.

B. Care should be taken to eliminate or lessen the effects of interference fringes, contaminants, and pressure effects due to sample preparation.

MINIMUM STANDARDS & CONTROLS

A. The questioned and known samples shall be examined in the same manner.
B. Identification made by comparison to other than reference collection fibers must be noted. Comparison data shall be preserved in the case file.
C. Identification made via computer search must be confirmed by visual comparison to the computer reference file and noted in the casefile.
D. See Appendix II for Minimum Standards & Controls.
E. See Method "Fourier Transform Infrared Spectroscopy" (MT-IXA)

INSTRUMENTATION

Fourier transform infrared microscope.

PROCEDURE OR ANALYSIS

FTIR Sample Preparation

These are examples of how to prepare fiber evidence for analysis on the FTIR. Due to the difference in crystallinity among fiber generic classes, not all techniques will give the best results with each type of fiber. Fibers like Acrylic, Acetate, and Triacetate crush easily and should not be pressed. Spandex, plasticized man-made fibers, and irregular shaped fibers need to be compressed in order to hold their flattened shape. A diamond cell or 2 salt plates in a compression cell can be used when analyzing these types of fibers.

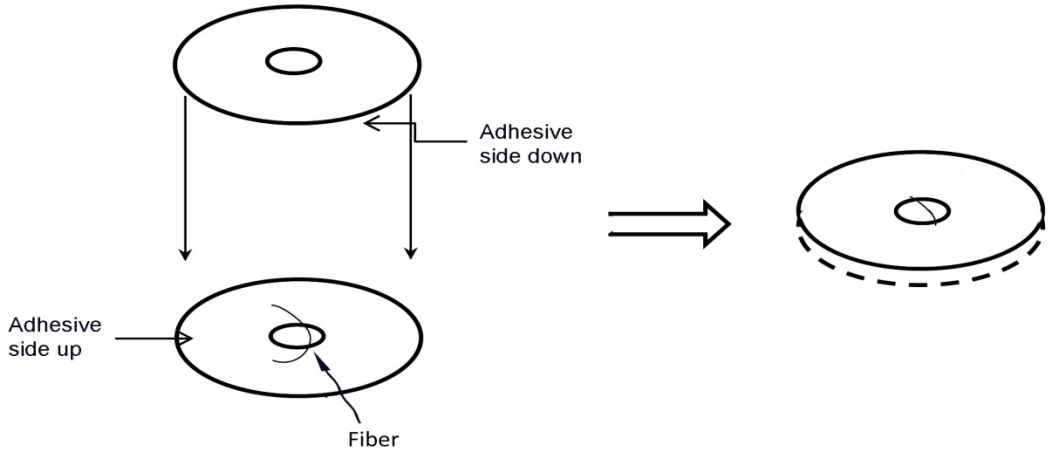
It is a good idea to very gently roll the fiber on a frosted glass slide to scratch the surface of the fiber. This prevents reflection from the fiber that will show up as fringing within the spectra. For optimum spectra results a fiber thickness between 5 - 15 µms is ideal.

Always prepare your standard first!

A. ROLLER

- Cut piece of fiber.
- Flatten fiber on frosted glass slide with roller by pressing firmly and pushing in one direction.
  Repeated rolls back and forth can result in fiber adhering to roller surface
- Repeat until fiber is flattened.
- Place flattened fiber in a holder or place fiber directly on KBr plate.
- A holder can be made from adhesive "dot" labels:
  - Position 2 1/2" dot labels (still on the backing) so that they align.
  - Use the 1/8" hole punch to make holes in the center of each label.
  - Remove one label from the backing, stretch the fiber over the hole of the adhesive side of the label with the tweezers.
  - Make a "sandwich" by placing a second label over the first with the fiber in the center (see bottom). Be sure the adhesive sides are pressed together.
  - Flatten the fiber using the micro press.

- Run the sample making sure to close the diaphragm on the microscope down around the fiber.



B. MICRO PRESS / HAND PRESS
- Fibers should already be placed between adhesive labels - Put label on press, center press on fibers.
- Press
- Run sample

C. DIE PRESS
- Remove internal cells and place fibers on small cell.
- Put die casing on top of smaller cell and reassemble die.
- Using a 10 ton press, press at approximately 2 tons.
- Take caution when removing cells because flattened fiber can adhere to either side of cell surface.
- Run sample

D. COMPRESSION CELL

1. Salt Plates
   - Use 2mm diameter KBr or NaCl salt plates.
   - Insert one plate in the cell, sandwich fiber between both plates.
     - Tighten clamp
     - Run sample
2. Diamond Cell
   - Insert diamond cell into compression cell with the flat side face up.
   - Place fiber and a small amount of KBr powder on the diamond cell.
     Put other diamond cell on top so fiber is between both flat sides of diamond cells.
   - Tighten clamp
   - Run sample
     As an option you can remove the top diamond cell after the fiber has already been compressed and omit the KBr. Due to air spaces between the diamond

cells, the spectra will show an increased amount of fringing.

FTIR OPERATING CONDITIONS

The following are suggested parameters for fibers which may be modified for the different FTIR models. Any method must meet the minimum standard of analysis.

A. Collect Date: View/change parameters

Resolution	4cm^{-1}
Number of scans, SAMPLE	20 to 200
Number of scans, REFERENCE	20 to 200
Number of scans, BACKGROUND	20 to 200
Detector gain	1

B. Display Date: View/change parameters

First X wave numbers	4000.00
Last X wave numbers	600.00
First Y absorbance	0.00
Last Y absorbance	120.00
or	
First Y transmittance	0.00%
Last Y transmittance	100.00%

REPORT WORDING

See Appendix I.

REFERENCES

1. Bartick, E. G.; Tungol, M. W. In *Forensic Science Handbook*; Saferstein, R., Ed.; Prentice Hall: Englewood Cliffs, N.J., 1993; Vol. III, Chapter 4.

2. Robertson, J., Ed. *Forensic Examination of Fibers*; Ellis Horwood: New York, 1992.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Instrumental Examination and Comparison

Procedure: FTIR Attenuated Total Reflection (ATR)

Reviewed by:

_____
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Approved by:

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Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

The purpose of Internal Reflection Spectroscopy is to provide a non-destructive procedure for the analysis and identification of organic and inorganic material. Internal Reflection Spectroscopy produces Attenuated Total Reflection (ATR) spectra. This is accomplished by placing the sample in optical contact with the surface of an internal reflecting element (IRE). These elements have a high refractive index and will resist mechanical damage and chemical attack. The most commonly used IRE is the diamond. The same considerations in determining and comparing spectra are used with the ATR accessory.

RELATED PROCEDURES

Fourier Transform Infrared Spectroscopy (MT-VIIC-2)

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.
ATRs and FTIRs use a laser beam to align the mirrors during the operation of the instrument. Do not look directly into the beam as damage to the eyes can result.

PREPARATIONS

Standard Laboratory Practices.
No preparation of the sample is necessary. The sample can be used directly on the IRE.

INSTRUMENTATION

Fourier Transform Infrared Spectrometer
Attenuated Total Reflection Accessory
Reference: Operator's Manual

MINIMUM STANDARDS & CONTROLS

A. The questioned and known samples shall be examined in the same manner.

B. Identification made by comparison to other than reference collection fibers must be noted. Comparison data shall be preserved in the case file.

C. Identification made via computer search must be confirmed by visual comparison to the computer reference file and noted in the casefile.

D. See Appendix II for Minimum Standards & Controls.

E. See Method "Fourier Transform Infrared Spectroscopy (MT-IXA).

PROCEDURE

A background spectrum must be collected at the time of the analysis.

A sample is placed on the IRE mount area and positioned to optimal area as viewed on the monitor. The clamp is secured (hand-tightened) to insure good contact between the sample and the IRE as indicated on the instrument. Spectra of all question and known samples shall be collected in the same manner.

The IRE should be cleaned and visually inspected between samples.

Question and known samples are to be analyzed in the same manner. Computer reference libraries may be used as an aid in identification.

Spectra will be maintained in the case file.

The following are suggested method parameters:
Number of Scans = 32
Resolution = 8 cm-1
Gain = 8
Apodization = Happ-Genzel
Starting wave number = 4000cm-1
Ending wave number = 400cm-1

Parameters may be adjusted to provide sufficient intensity and resolution for spectra comparison and/or identification.

REPORT WORDING

See Appendix I.

REFERENCES

1. Harrick, N.J.; *Internal Reflection Spectroscopy*; Interscience Publishers: New York 1967.

2. Graf, R.T.; Kownig, J.L.; and Ishida, J.; *In Fourier Transmission Infrared Characterization of Polymers*; H. Ishida, Ed.; Plenum: New York, 1987.

3. Neil, J.E. and Bibby, A.; *Improvements in the use of Attenuated Total Reflection Fourier Transform Infrared Dichroism for Measuring Surface Orientation in Polymers*; Applied Spectroscopy. Vol. 51 Number 8, 1997. Pages 1083-1091.

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Fabric Impressions

Procedure: Fabric Impressions

Reviewed by:

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INTRODUCTION

Among the types of physical evidence that may connect a suspect to a crime scene are fabric impressions. Although not normally as conclusive as a fingerprint, it may provide investigative information that, combined with other evidence, can be sufficient to aid in the successful prosecution of a criminal case. If recognized, properly collected, and submitted to the laboratory, such evidence will be helpful in an investigation whether it may be a vehicle/pedestrian accident, burglary, or homicide. These impressions might be on paint, metal, glass, plastic, dust, soil, or in blood.

A. Types of Fabric Impression Examinations:

1. Examination of evidence to determine if a fabric impression exists and is it suitable for comparison with suspect's or victim's clothing.

2. Elimination of the standard as having made a particular fabric impression.

3. If a fabric impression exhibits similar class characteristics as the standard.

4. If a fabric impression was positively made by the standard.

Fabric impression comparison involves the physical similarity between the unknown and known based upon the probability of random occurrence of individual characteristics. Such characteristics are formed during the use of the item.

Characteristics may be divided into two general categories: (1) overall design which limits origin to the same class group and (2) specific details which eliminate others in the same class group. The degree of elimination is dependent upon the nature of the individual characteristics and their quality. No minimum number of characteristic agreements are required to effect an identification.

Formation of the individual characteristics created by use are generally the result of cuts, rips, burns, or tears to the fabric. The shape and size of the accidental damage are dependent upon surfaces and objects encountered during wear.

In general, fabric impressions are hard to observe and once detected should not be disturbed except for photographing for record shots. These impressions are either two-dimensional impressions on hard substrates or three-dimensional impressions in softer substrates. The impression should be preserved and submitted to the laboratory because minute fibers may be present and can easily be lost due to any processing at the scene. Casting at the scene may be necessary on three-dimensional impressions.

Fabric impression comparison is a procedure requiring observation of characteristics according to size, shape, and spatial relationship. The evaluation of any impression is the determination of class and individual characteristics which are present. Due to a variety of overall design, fabric impressions suitable for comparison include those, which may eliminate only or possess similarity only. The presence of class characteristics alone is sufficient to render an impression suitable for comparison purposes.

Comparison requires a known standard which is restricted to the victim's/suspect's clothes or other fabric. The most useful test impressions are those which attempt to duplicate the conditions of the contact transference. These conditions include the amount of force of the impact, possible distortion of the fabric, folds in the fabric, if the impression was left by rolling the fabric, the nature of the substrate, and environmental conditions at time of contact. As a general guide, however, prolonged wear will diminish the likelihood of conclusive examination results.

Casts, lifts, and test impressions produced in the course of examination are returned to the submitting agency.

Other Related Procedures: None

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

Chemical	NFPA
acetic acid, glacial	3-2-1
acetone	1-3-0
amido black	2-0-0
ammonium thiocyanate	3-0-2
antimony trichloride	3-0-3
7,8-benzoflavone	1-1-1
Biofoam	0-0-0
chloroform	2-0-0
clay, modeling	0-0-0
collodion	2-4-1
coomassie brilliant blue R250	2-0-0
crocein scarlet 7B	2-0-0
dental stone	0-0-0
ethanol	0-3-0
ethyl ether	2-4-1

fingerprint ink	0-0-0
fingerprint powder	0-0-0
8-hydroxyquinoline	3-0-1
iodine	1-0-1
luminol	2-0-1
margarine	0-0-0
methanol	1-3-0
molybdenum disulfide	2-1-0
ninhydrin	2-0-1
nitric acid	3-0-0
paraffin	0-1-0
petroleum ether	1-4-1
plaster of paris	1-0-0
potassium thiocyanate	3-0-2
sodium acetate	1-0-0
sodium carbonate	1-0-0
sodium perborate	1-0-1
5-sulfosalicylic acid	4-0-2
sulfuric acid	3-0-2
talc powder	1-0-0
Tergitol 7	1-1-1
tetramethylbenzidine	2-0-0
trichloroacetic acid	2-0-0
water, deionized	0-0-0
water, distilled	0-0-0

PREPARATIONS

Standard Laboratory Practices.

Ordinarily, there is no single best process, since results depend to such a great extent upon the particular items and conditions of the specific case. Since the various techniques are non-destructive and not sequence dependent, the examiner may choose one by personal preference, and continue to apply additional techniques as necessary to maximize results. These variations in processing techniques typically do not influence the validity of the test procedure.

MINIMUM STANDARDS & CONTROLS

A. Collection and preservation of any fibers that adhere to the impressions substrate must be performed before any fabric impression examination is begun. Fiber comparisons shall also be required between standards and the fibers adhering to questioned fabric impressions if a previous association has been established.

B. Fabric impressions shall be examined and compared visually, physically, and with magnification. Representative photographs/photomicrographs shall be required of fabric impression comparisons that result in meaningful associations.
C. Fabric impression comparisons involving meaningful transfers shall be verified by a second Microscopist. Fabric impression verifications shall be compared by examination of all standards or exemplars and pertinent impressions. Verifications shall be documented by the verifying examiner.
D. Fabric impression test impressions should be used for comparison in most two-dimensional questioned impression cases when the known item cannot readily be eliminated.
E. Fabric impression test impressions should be produced in a manner similar to the question impression(s), if at all possible. Adequate exemplars of the fabric must be made while attempting to duplicate any distortion due to force.
F. Micrometers and calipers will be checked prior to use utilizing the appropriate gauge blocks to an established tolerance of +/- 0.003". A record of the check will be maintained in an appropriate log.
G. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Caliper or micrometer as needed.

PROCEDURE OR ANALYSIS

The scene impression should be first examined for any adhering fibers, and, if present, they should be removed for further examination and comparison. Questioned impressions will be evaluated to determine suitability for comparison purposes. Standard impressions are then compared with the question fabric impressions, specifically searching for a correspondence in class characteristics and individual characteristics. The impression should also be examined for weave/knit pattern, thread direction and any indication of folds or distortion. Any unexplained differences between the impression and the fabric at this point would eliminate that fabric as making that impression.

The two-dimensional crime scene impression is a reverse positive image of the fabric. Comparing the crime scene impression directly to the fabric is difficult. Not only will accidental characteristics be reversed, many minute characteristics which might be recorded will not be visible. The test impression provides a medium which may be compared against the crime scene impression.

Every effort should be made to obtain an impression which closely resembles the crime scene impression. This can be usually accomplished by: (1) taking a number of test impressions, and (2) duplicating the way the scene impression was made.

There are a number of ways to make a test impression. Where one method might work well on one case, another method may have to be used on the next case. The fabric impression examiner should be familiar with these different techniques. When appropriate, the examiner should consider limiting the potential of damaging the fabric in the selection of the method to be used.

Procedures listed in the Footwear/Tiretrack Procedures Manual may be considered as suitable for fabric impression enhancement.

Visible Fabric Impressions

Visible fabric impressions must be examined to determine if they are suitable for comparison or elimination purposes. The impressions should either be examined directly or photographically (1:1 reproductions).

- Fabric impressions must contain sufficient discernible class characteristics to effect a comparison.

- Fabric standards must be submitted to the laboratory to effect a complete comparison.

Submitted Photographs of Fabric Impressions

Photographs which are submitted as the evidence which records the questioned fabric impressions are visually examined to determine if they are suitable for comparison or elimination purposes.

- In most cases, the photographs of the fabric impressions must be 1:1 reproductions or the photographic negatives must have the capability of being photographically printed 1:1. (There are instances when photographs are not 1:1 reproductions, however, they contain a sufficient amount of class characteristics, therefore they may be utilized for elimination purposes.)

- The photographs must also contain sufficient discernible class characteristics to effect a comparison.

- Standard fabric must be submitted to the laboratory to effect a fabric comparison.

The absence of a scaling device which makes a 1:1 reproduction impossible usually renders a photograph unsuitable for a complete and accurate comparison.

Cast Impressions

Unknown cast impressions are examined and processed in the following manner:

- Casts are carefully cleaned in an attempt to remove excess soil and dirt.

- Casts are visually examined to determine the suitability for comparison and identification purposes. This examination can include:

    - Determination of fabric construction design

    - Determination of spacing of the weave or knit yarns

    - Determination of defects

    - Determination of wear characteristics

    - Determination of individual characteristics

Casts which contain a sufficient amount of class and individual characteristics and are deemed suitable will be examined and compared with known fabric submitted by the requesting agency.

OTHER TEST IMPRESSION METHODS

A. Fingerprint ink and paper

Spread a small amount of fingerprint ink over a piece of glass with an ink roller. Press the fabric against the inked glass. Press the fabric against white bond paper supported by sheets of newspaper or butcher paper. Fingerprint ink may cause very minute characteristics to become filled in and not be observed clearly.

B. Talc powder - carbon paper - fingerprint powder.

1. Talcum powder is spread over a surface such as a sheet of butcher paper. The talc is then shaken off the paper leaving a very fine coating. A sheet of carbon paper is placed on a few sheets of newspaper or butcher paper which are placed underneath to prevent extraneous particles recording false details. Press the fabric against the talc covered paper and then onto the carbon paper, a test impression recording minute detail is visible.

2. Another version of this method is to dust the fabric with a fine coat of gray fingerprint powder then press the fabric on a piece of carbon paper supported by a few sheets of newspaper or butcher paper. The results will be similar to B.1.
As a receiving surface, carbon paper presents one problem, it may develop crease marks or wrinkles.

C. Fingerprint powder - plastic - glass - talc powder.

This procedure is similar to method number B.2., except that glass does not require support. Plastic will require some protective base to prevent extraneous debris from causing false detail. The advantage of glass or plastic over carbon paper is that creases will not form and the impression can be made either black or white through photography depending on the background and lighting procedures. A black background will show white powder in its natural state. Using a back light (impression lit from the rear) and a white background, the white powder will appear black. This form of test impression is very fragile and should be photographed immediately.

D. Margarine and Magna brush.

A minute amount of margarine is rubbed into the palm of the hand and then against the fabric. A test impression is obtained by pressing against a piece of glass, good quality white bond paper or clear plastic supported by paper. The receiving medium is then dusted with a Magna brush developing a clear image. Spray PAM can be substituted for the margarine.

E. Fingerprint lifting tape - fingerprint powder.

A further method: dust the fabric with the desired color of fingerprint powder and lift the impression with fingerprint tape. In this procedure apply the tape by hand or roller. In order to maintain proper laterality and not obtain a mirror image, place the lifted impression on a clear acetate sheet and view it from the reverse side. This method is impractical for full size clothing but may be useful when trying to record a specific area. (Source: Michael Cassidy, Royal Canadian Mounted Police)

For impressions that are three-dimensional, the test impressions can be made in clay, a lead sheet or other suitable materials.

Force can be applied for making test impressions on material that is not fragile by using a rubber mallet and wood against the fabric. Approximate measurements of the impressions can be made using a linen tester (thread counter) and/or vernier caliper.

Finally, it must be remembered in comparing fabric and fabric impressions that there are many common types of weaves and knits, and that the duplication of a fabric impression is in itself not proof that the standard fabric made the impression because many pieces of fabric have the same method of construction. Individualizing characteristics in both the impression and fabric necessitates an identification. The presence of a fabric impression on hard paint of an automobile is almost conclusive proof that the vehicle has struck fabric forcibly. Do not forget that an impression in dust or grease may not have been made forcibly.

REPORT WORDING

See Appendix I.

REFERENCES

Any good textile book dealing with the construction of fabrics.

1. Bodziak, William J. *Footwear Impression Evidence*; Elsevier Science Publishing Co. Inc.; New York, 1990.

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ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Fasteners

Procedure: Buttons, Fasteners and Attaching Threads

Reviewed by:

_____
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Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

A button is a knob or disk used as a fastener or as a decoration on clothing. Other fasteners include snaps and hooks and eyes. Buttons are made in various sizes and of various materials and the attachment method varies.

The attaching threads may be made from a homogeneous material or a mixture of textile fibers.

Other Related Procedures:

Methods A, B and C of this Protocol (MT-VIIA, B, and C).

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. For buttons and fasteners, a three-sided drawing, photograph, or photocopy (top, bottom and side) with measurements shall be made if a transfer is detected.
B. For attaching thread, fiber comparison shall be performed between these questioned fibers and submitted standards, fibers, or fabric if a transfer is detected.
C. Micrometers and calipers will be checked prior to use utilizing the appropriate gauge blocks to an established tolerance of +/- 0.003". A record of the check will be maintained in an appropriate log.
D. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Caliper or micrometer as needed.

PROCEDURE OR ANALYSIS

A. Examine the clothing to determine if any buttons or fasteners are missing. If any are missing, proceed with the examination.

B. Compare the buttons or fasteners on the clothing to the questioned item visually and stereoscopically. Record the pertinent dimensions, including diameter, thickness, hole size and shank length, if applicable. Drawings may be helpful for demonstrative aids.

Caution: smaller buttons can be found at the neck and sometimes on the cuff.

C. Compare any attaching threads to the attaching threads from the clothing. Use the procedures in this protocol that deal with the identification and comparison of fibers to complete the examination and comparison of the attaching threads.

D. The questioned item is consistent with originating from the piece of clothing if there is no discernible difference between them.

REPORT WORDING

See Appendix I.

REFERENCES

None.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Fibers

Method: Rope

Procedure: Rope, Cordage and Knots

Reviewed by:

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INTRODUCTION

All ties of fastenings that use rope or cordage fall into three general classifications: knots, hitches, and bends. All are "knots" in the full sense of the term, and there are some ties which have more than one classification, their purposes being interchangeable.

A basic understanding of rope/cordage construction is necessary before any examination/comparison can be performed. The following are the basic definitions to understand ropes, cordage and knots.

Bend - a knot which ties the ends of the two free ropes together, or the action of knotting two ropes together (same origin as the word "bind").

Braided - laid rope manufactured by twisting fibers together into yarns, yarns into strands, and strands into rope. The lay of the rope is the spiral twist of the strands. This rope resembles plaited rope in construction except that it is made up of a great number of yarns instead of strands. Braided rope is balanced by having an equal number of left-hand and right-hand turns. This is actually two ropes, one inside the other. The outer rope is called the cover or sheath and the inner one, the core; both are braided.

Cordage - a comprehensive word for any line, whatever its size or material, which has no special purpose.

Fiber (or filament) - the smallest element of material forming the individual fibers of rope.

Heart (or core) - the loosely twisted strand, or bundle of parallel yarns or filaments, running the length of larger ropes to form an inner heart or filler. Found in ropes of more than three strands and in most braided lines, it may be simply a cheap filler of weak stuff; or it could have a specific role as a stiffener or reinforcer.

Hitch - a knot that secures a rope to a post, ring, spar or rail, etc., or to another rope which takes no part in tying the knot. A hitch won't keep its shape on its own. In climbers' jargon, a "hitch" is often just a temporary fastening.

Knot - in the restricted sense, is a tie made in a rope and usually requires the manipulation of only one end.

Lay - the direction, either left-handed or right-handed, of twist in the strands of rope; also the nature (tight, medium or loose) of that twist.

Line - a general label for most cordage with no special purpose. Line is also used to refer to rope that does have a purpose, such as clothesline, heaving line, fishing line.

Plaited - an eight-strand rope is made up of four right-hand and four left-hand turned strands, laid in pairs. This type of construction resists rotation and is used for flag halyards, clothes lines, etc.

Rope - any cordage over .4 inch in diameter.

Staple - graded fibers or chopped filaments for ropemaking (rope made from staple has a fibrous or fuzzy surface due to all the ends).

Strand - yarns twisted together in the opposite direction to that of the yarn itself; a major element in the complete rope. Rope made with strands--not braided--is "laid line".

Yarn - any number of individual fibers or filaments twisted together as the first stage in ropemaking.

Knots have been with mankind since the caveman. To quote Walter Gibson, "At least 99 percent of the population knows how to tie a knot of some sort; and of those, at least 99 percent do it blindly or by rote, unless they have had some instruction or have made a study of rope work." The identification of a knot, hitch or bend requires more than a basic layman's knowledge of knots.

Other Related Procedures:

Methods A, B and C of this Protocol (MT-VIIA, B, and C).

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

PREPARATIONS

Standard Laboratory Practices.

MINIMUM STANDARDS & CONTROLS

A. A drawing, photograph, or photocopy of the rope or a photocopy of a rope of the same type of physical construction, along with all the pertinent measurements shall be included in the notes.
B. Fiber comparisons shall also be required between standards and the fibers in questioned ropes if a previous association has been established.
C. Micrometers and calipers will be checked prior to use utilizing the appropriate gauge blocks to an established tolerance of +/- 0.003". A record of the check will be maintained in an appropriate log.
D. See Appendix II for Minimum Standards and Controls.

INSTRUMENTATION

Caliper or micrometer as needed.

PROCEDURE OR ANALYSIS

A. If the rope has any knots, hitches or bends, those should be identified if they are germane to the case. This identification should not be attempted unless the examiner has more than a layman's knowledge about this subject.

    1. Identification of the knot, hitch or bend will be made by determining how the questioned item is tied and comparing it to photographs or drawing in reputable text. A photocopy of the photograph or drawing shall be included in the examiner's notes. The examiner's report shall identify the knot, etc., by its proper name.

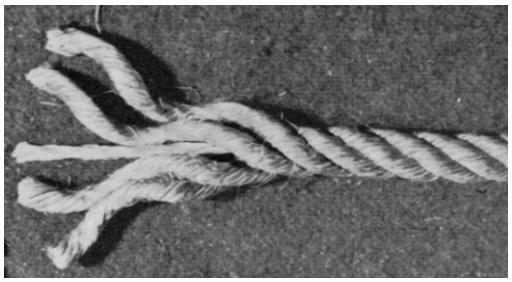
B. The full identification of the rope's construction shall be done by identifying the physical construction and identifying the materials of which the rope is composed.

    1. When comparing a questioned rope to a submitted known rope, an elimination of a common source is reached when any difference in physical construction or materials is observed.

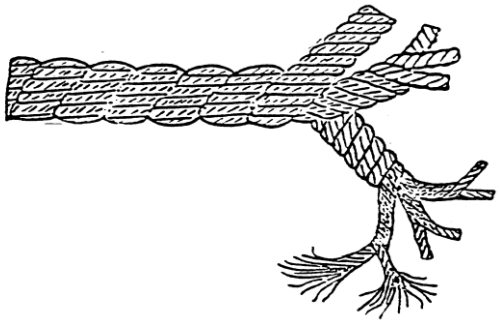
    2. The examiner's notes shall reflect the physical construction, including crowns per inch, and the identity of the fibers/filaments that compose the rope.

    3. Identification of the fibers/filaments shall be reached by following the appropriate procedures in Methods A, B and C of the Protocol (MT-VIIA, B, and C).

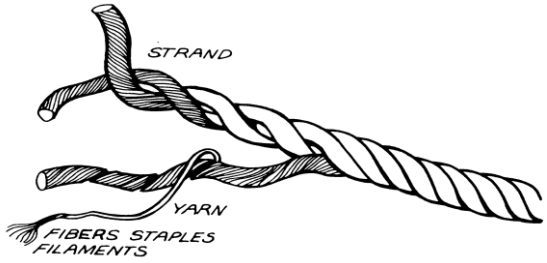
The following are examples of rope construction.



Shroud-laid rope has four strands laid up right-handed around a heart or core.



In this example, three plies are twisted together in a "S" direction. Each ply contains three strands twisted together in a "Z" direction. Each yarn contains fibers twisted together in a "Z" direction. The multiple filaments are usually twisted in the opposite direction of the strand.



Cable-laid rope has three strands, each of which is a plain-laid rope, laid up left-handed.

REPORT WORDING

See Appendix I.

REFERENCES

1. Ashley, C. W. *The Ashley Book of Knots*; Doubleday: New York, 1944.

2. Budworth, G. *The Knot Book*; Sterling: New York, 1985.

3. Day, C. L. *The Art of Knotting and Splicing*, 3rd ed.; Naval Institute Press: Annapolis, MD, 1981.

4. Gibson, W. *Fell's Official Guide to Knots and How to Tie Them*, rev. ed.; Fell: Hollywood, FL, 1989.

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

Protocol: Pressure Sensitive Tape

Method: Pressure Sensitive Tape Comparison

Procedure: Tape Construction and Component Analysis

Reviewed by:

_____
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Approved by:

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INTRODUCTION

Pressure sensitive tapes may be used by criminals in several ways, therefore, it is not uncommon that tapes become evidence in investigations. Pressure sensitive tapes are available to consumers in a variety of types, such as duct tape, electrical tape, and packaging tape. The comparison of tapes may provide valuable associations between victim, scene, and suspect.

When tapes are submitted as evidence, analyses by multiple forensic sections must be considered. An evaluation of which exams are probative, such as for microscopic trace materials, latent fingerprints, biology, or physical match, should be done for every case. Analysis of microscopic trace materials such as hairs and fibers are already documented in the Micro/Trace Procedures Manual. Examinations of physical matches are likewise documented in the Physical Match Procedures. The procedure in this section involves the comparison of tape constructions and compositions.

Some types of tapes exhibit more variability than others. It should be noted that although potentially probative, this analysis is limited to the comparisons of class characteristics. To conclude an association, no unexplained differences should be observed between probative tape specimens.

The type of tape submitted will best dictate the analytical scheme for comparison. The analyst should review the literature references in this procedure for information. This procedure serves as a guideline for comparative analysis.

SAFETY CONSIDERATIONS

A. Analysts are to consult their respective safety manual for the proper safety considerations necessary to handle potential biohazard materials.
B. Standard Laboratory Practices should be followed due to potential exposure to Latent Print residue and chemical reagents.

Chemical	NFPA	Chemical	NFPA
Liquid nitrogen	3-0-0	Acetone	1-3-0
Xylenes	2-3-0	Hexanes	1-3-0
Chloroform	2-0-0	Methanol	1-3-0
Xylene substitutes	2-3-0		

PREPARATIONS

A. Requests for examination should be considered on a case-by-case basis to determine which analyses are probative. The order of examinations should be initially determined to preserve transient evidence and provide the most valuable information.
B. Because subsequent analytical scheme may be a multi-unit endeavor, the proper handling and collection of evidence is essential by all involved sections.
C. If questioned and known tapes can be physically matched at tape ends, further examination may not be necessary.

MINIMUM STANDARDS & CONTROLS

A. A reference collection for various tapes shall be maintained.
B. The use of equipment and instruments shall include performance checks and their required documentation as stated in MT-APP-II.
C. Case notes shall document characteristics and test results which resulted in comparisons. Associations shall show no unexplained differences between probative tape specimens.
D. The minimum requirements for associations are:
    a. Results from macroscopic observations and physical measurements.
    b. Fiber analysis results of reinforcement material, if present.
    c. FTIR test results of adhesive and backing materials.
    d. Elemental test results (SEM/EDX or XRD) of adhesive materials.
E. Report conclusions shall be based on the tests performed.

INSTRUMENTATION

Stereomicroscope
Transmitted light microscope
Polarized light microscope
Fluorescence microscope
Calipers
Fourier Transform Infrared Spectrometer (may have Microscope or ATR attachment)
Scanning Electron Microscope with Energy Dispersive X-ray Detector
Pyrolysis Gas Chromatograph/Mass Selective Detector
X-ray Diffraction Spectrometer

PROCEDURE OR ANALYSIS

A. The initial examination of tape may be only to recover trace materials from the backing and adhesive surfaces. Do not cut or alter the tape ends. If further submission to the Latent Print section is requested, consider whether the tape should

be untangled or separated. Comparison of the recovered trace materials shall follow appropriate procedures listed in this manual.

B. If items are submitted for Physical Match comparison to the tape ends, the Physical Match Procedure should be performed prior to comparison of tape components.

C. Comparison of tape construction and components:
Side-by-side comparisons are to be performed on probative tape specimens. A stereomicroscope may be helpful in visual examinations.

1. Macroscopic examination/physical characteristics measurements
   a. Used and tangled tapes may be separated with freezing in liquid nitrogen, solvents, or gentle heat.
   b. Make note of the following physical characteristics for each probative tape specimen:
      Markings, stria, and inclusions to the backing
      Adhesive color and texture
      Scrim (yarn) count – count warp and fill yarns per unit area
      Film backing color, texture, and weave
      Tape width and thickness
   c. Examine tape samples with UV light for similarities or differences.
2. Tape components of backing, fibers if present, and adhesives maybe separated mechanically (forceps) or with solvents such as hexane, xylene, or xylene substitutes. This may be helpful in macroscopic observations as well as microscopic and instrumental analyses. Tape specimens may be dirty or have residue from previous examinations. Attempt to remove adhering material from prepared samples prior to analysis. Alternately, test representative samples recovered from underlying clean portions of the item if possible.
3. Microscopic examination
   a. Examine fiber reinforcement material, if present. Document weave, yard description, and scrim count, if present, and identify fiber types. Refer to Fiber Procedures (MT-VIIA-C) for identification and comparisons.
   b. Examine backing material by placing it on glass microscope slides. The PLM optical characteristics such as angle of extinction relative to the tape edge, the direction of film orientation (MOPP, BOPP), and retardation of clear packing tape film backing are found to be discriminatory.
   c. Disperse softened or dissolved adhesive material onto glass slides. Examine for fillers or pigments.
   d. Tape cross-sections can be made by freezing sections with liquid nitrogen and cutting with a sharp blade. Examine cross-sections microscopically.

e. Fluorescence microscopy can be used in conjunction with the above examinations.

4. FTIR (including Micro-FTIR and ATR)
   a. The adhesive and, if desired, the film backing material are to be examined by FTIR separately and compared. For "clean" specimens, the ATR may be used without sample preparation.
   b. If the tapes are vinyl (such as black electrical tape), examine the plasticizer from the surface of backing. To extract the plasticizer, fold the tape, backing side up, to make a trough. Add chloroform dropwise into the trough and collect the solvent into a suitable dish. Evaporate the solvent. The remaining residue is to be analyzed by FTIR.
5. Elemental (SEM/EDX or XRD)
   a. Sample preparation should consider the condition and cleanliness of the specimen, its attachment to the mount, the need for a conductive coating, etc. It is recommended that each probative tape specimen has at least one sample of the backing and adhesive mounted separately.
   b. SEM images of backings may yield information too small for light microscopic observations and are to be used for comparison. Tape cross-sections may also be mounted and observed.
   c. Elemental analysis of the backing and adhesive materials are to be examined separately and compared.
6. Other techniques (such as Pyrolysis GC/MS)
   Literature references may be consulted for the application and procedure for additional comparative analyses.

REPORT WORDING

See Appendix I

REFERENCES

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Instrumentation

Method: Fourier Transform Infrared Spectroscopy (FTIR)

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Approved by:

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INTRODUCTION

Infrared spectroscopy is a valuable method for the identification and comparison of various items commonly analyzed by a Micro/Trace analyst. This technique can provide molecular information regarding major organic and inorganic components of the item being examined and can be used while comparing two or more samples.

FTIR data may be acquired by multiple methods. Two of the methods employed by ISP include transmission and internal reflection. Transmission spectra are produced by measuring the energy transmitted through a sample when a single-path infrared beam passes through it. Sample preparation may require chemical extraction or thin slicing of the sample and mounting the slices onto a KBr or NaCl crystal.

For FTIR using internal reflection, a multiple-path infrared beam is passed through an internal reflecting element (IRE) with a high refractive index at a certain angle. The most common IRE is a diamond, and acquisition is usually via an attenuated total reflection (ATR) instrument accessory. The internal reflection creates an evanescent wave which extends above the crystal IRE. When a sample is held in contact with the IRE, the sample absorbs energy and the evanescent wave is altered, or attenuated. This attenuated energy is passed back into the infrared beam. A detector measures the changes in the beam and produces an infrared spectrum.

Identifications made using FTIR rely on comparisons of peak positions and relative peak intensities between sample and standard spectra. This is true whether data is acquired via transmission or internal reflection methods. While transmission and internal reflection spectra of the same substance will show distinct similarities and may provide useful information as to the identification of a specific substance, care must be taken to ensure that identifications are made based on comparisons of spectra acquired in the same manner (i.e. transmission or internal reflection). When used for spectral comparisons, the objective is to determine whether any meaningful differences exist between the samples.

For various reasons, components in lesser amounts are typically more difficult to identify unequivocally. Reasons for this include interference of the absorption bands of the major components with the less intense bands of minor components and sensitivity issues whereby the minor components are present at concentrations below the detection limits of the instrument.

Related Procedures

A. Paint Analysis Fourier Transform Infrared Spectrometer (FTIR) Analysis (MT-IIB-3)
B. Paint Analysis FTIR Attenuated Total Reflection (ATR) (MT-IIB-5)
C. General Unknowns Identification (MT-VI)
D. General Unknowns Common Acids, Bases, and Bleaches Identification (MT-IVA-1)
E. General Unknowns Intoxicating Compounds Identification (MT-IVA-2)
F. General Unknowns Lachrymators: Extraction and Identification (MT-IVA-3)
G. Fibers FTIR Microscope (MT-VIIC-2)

H. Fibers FTIR Attenuated Total Reflection (ATR) (MT-VIIC-3)
I. Pressure Sensitive Tape (MT-VIIIA-1)

Terminology

A. Attenuated total reflection (ATR) – a method of spectrophotometric analysis based on the reflection of energy at the interface of two media which have different refractive indices and are in intimate contact with each other.
B. Mid-infrared – pertaining to the IR region of the electromagnetic spectrum with wavelength range from approximately 2.5 to 25 Um (wavenumber range approximately 4000 to 400 cm^{-1}.
C. Internal reflection element (IRE) – ATR crystal with a high refractive index and excellent IR transmitting properties, such as a diamond.

Safety

A. A laser beam is used to align the mirrors during the operation of the instrument. Do not look directly into the beam as damage to the eyes can result.
B. If the FTIR microscope is used, use personal protective equipment to cover eyes and skin while working with liquid nitrogen.
    a. Liquid nitrogen precaution – extreme cold. NFPA is 3-0-0

Preparations

A. KBr or NaCl crystals
B. Diamond anvil cell
C. Scalpels

Instrumentation

A. Fourier Transform Infrared Spectrometer equipped with both a deuterated triglycine sulfate (DTGS) and mercury cadmium telluride (MCT) detectors
B. ATR Accessory
C. Microscope Accessory
D. Beam Condenser
E. Gas Purge system

Minimum Standards and Controls

The following are requirements to meet the minimum standards and controls for Fourier Transform Infrared Spectroscopy.

A. Documentation

1. Documentation of performance checks shall be maintained in an appropriate log and in the case file.

2. If the ATR accessory is being used, the peak to peak (P2P) value shall be recorded in the case file.

3. If the accessory being used requires a blank prior to a sample, a copy of that blank will be included in the case file.

4. A copy of the IR spectrum will be included in the case file. IR spectra must include case number, item number, date, analyst's initials, resolution, number of scans, frequency range, and mode (absorbance or %T).

5. Digital copies used for spectra enhancement must include case number, item number, date, and analyst's initials.

6. Work notes shall include the method of acquisition used (i.e. ATR, microscope, bench transmission).

7. Any reference used to make a conclusion, must be included in the case file.

8. The worksheet will indicate the result(s) of the IR analysis and any comparison(s).

B. Function Checks

1. A suitable polystyrene reference material will be run each day the instrument is in use. Compare peak locations (wavenumbers) to defined ranges of acceptability from the manufacturer's ValPro Qualification Report listed below:

Wavenumber	High Limit	Low Limit
3060.0	3061.0	3059.0
2849.5	2850.5	2848.5
1942.9	1943.9	1941.9
1601.2	1602.2	1600.2
1583.0	1584.0	1582.0
1154.5	1155.5	1153.5
1028.3	1029.3	1027.3

If these checks, even after being repeated, are outside of the acceptability ranges, repair service will be required, and the instrument will be placed "not in service/do not use" until repaired.

2. A check of peak to peak (P2P) ratio value will be done each day prior to using the ATR accessory. A value of 2.00 (or 10% of the original energy level) or more is

necessary. If a minimum value of 2.00 (or a minimum of 10% of the original energy level) is not achieved, an alignment of the ATR will be performed. If, after alignment, the minimum value of 2.00 (or a minimum of 10% of the original energy level) is not achieved, repair service will be required, and the instrument will be placed "not in service/do not use" until repaired.

3. A background spectrum shall be run for each analysis and stored into the computer. It is advisable to collect a background spectrum before beginning analysis as well as every 5-10 minutes to minimize the impact of environmental factors on analytical spectra. Infrared data are collected from both the sample and a previously stored or newly acquired background. Taking the ratio of the sample spectrum to the background enables removal of absorptions from the cell or support material (i.e. diamond absorptions) or from the atmosphere (i.e. carbon dioxide and water vapor), or both. The latter absorptions can be minimized by purging with dried and filtered air, desiccant packs, or nitrogen gas. The number of scans acquired for each specimen can vary depending on sample type and size.

4. Blanks – if a background is not acquired immediately before or after the sample, a blank of the cell or FTIR card used will be run before each item.

C. Comparisons
   1. The questioned and known samples shall be examined in the same manner.

   2. Comparisons of known and questioned evidence can be conducted with both spectra displayed in either transmittance or absorbance.

   3. There are several factors to consider when assessing whether or not spectra can be distinguished from one another: the presence or absence of absorption bands, their positions, shapes, and relative intensities.
      a. For spectra that cannot be distinguished from one another, characteristic absorption bands observed in one spectrum are also present in the comparison spectrum. The position of the absorption bands should have reasonable agreement with each other and is somewhat dependent on the shape of the absorption band. The positions of significant peaks should be within +/- 4 cm^{-1}. Additionally, absorption bands should have comparable relative intensities and shapes for the spectra being compared.
      b. If subtle differences are noted between questioned and known items, where possible, collect additional spectra to demonstrate whether the differences are repeatable and therefore meaningful. The number of additional spectra collected is predicated by several things: the amount of sample present, the hetero-/homogeneity of the material, typical spectral variation observed in similar materials, etc. Therefore, the number of replicates shall be determined on a case-by-case basis.

c. Note: transmission and internal reflectance methods should produce similar spectra, but corresponding wavenumbers may differ by more than +/- 4 cm^{-1}. If a reference is available that was acquired by a different mode than the sample spectrum, the data may still be used to support a conclusion, given the differences in the two spectra can be explained.

4. Spectra are dissimilar if they contain one or more meaningful differences (for example, absence or presence of constituents, reproducible intensity differences).

5. Spectra cannot be distinguished if they contain no meaningful differences.

6. A spectral comparison is inconclusive if no determination can be made as to whether observed differences are meaningful (for example, peaks are not well resolved, sample condition).

Procedure or Analysis

A. Transmission methods generally require more extensive sample preparation. The sample shall be thin enough not to over-absorb. For transmission data viewed in % transmittance, spectral peaks optimally should not fall below 10% T. For spectra displayed in absorbance, the maximum absorbance optimally should be 1.0 or less. This typically requires a sample thickness of approximately 5-10 um.

B. A resolution of 4 cm^{-1} should be used for most accessories and sample types but the number of scans can vary depending on the sample size. However, the same instrumental parameters, including the number of scans, should be acquired for the background as for the sample.

C. Diamond absorbs infrared radiation in the 2300 to 1900 cm^{-1} region; therefore, sample absorptions in this region can be obscured if the accessory being used has a diamond IRE.

D. Main Bench

1. Transmission: Beam Condenser with Diamond Anvil Cell
   a. For small samples, a diamond cell with a beam condenser is preferred.
   b. Prior to analysis, a background spectrum of the empty diamond cell is collected. The same background spectrum can be used for multiple samples or a new one can be collected for each sample. If a background is not collected immediately prior to the sample, a blank of the diamond cell should be analyzed.
   c. The sample is placed on the clean diamond anvil cell and compressed between the windows to a desired thickness.
      i. Sample compression should be done under a stereomicroscope to ensure uniform coverage of the diamond.
   d. The cell is placed into the sample holder in the main bench of the instrument.

e. Close the compartment and allow the instrument to equilibrate.
f. Collect the spectrum.

2. Transmission: FTIR Card
    a. Used for liquid samples
    b. Collect a background with an empty sample compartment.
    c. Put the FTIR card that will be used for the Item into the sample compartment and collect a blank spectrum.
    d. Put drops of the sample onto the FTIR card and analyze.

3. ATR:
    a. In some instances, such as when an ATR accessory is used, there is no sample preparation required. The sample may be used directly (run neat) or extracted.
    b. However, since ATR is a surface technique it is necessary to remove any extraneous material from the area to be examined prior to analysis.
    c. Be aware that the force applied when using the ATR accessory can deform the sample.
    d. Make sure the Durascope tab is selected in the Omnic software.
    e. Prior to analysis, a background spectrum is collected with the pressure arm raised. The background spectrum can be used for multiple samples or a new one can be collected for each sample.
    f. If a background is not collected immediately prior to the sample, a blank with the pressure arm lowered should be analyzed.
    g. Place the sample on the diamond mount (IRE) area and secure the clamp by applying pressure until good contact is maintained between the sample and the diamond. Take care to not over tighten because the IRE can be damaged if too much force is used in this process.
        i. When working with a liquid, it should be dropped directly onto the IRE. If the sample of interest is in a solvent, allow the solvent to evaporate completely prior to applying pressure and collecting a spectrum. The pressure arm will not be used for liquid samples.
    h. Clean the IRE after analysis by wiping with a kim wipe or gently wiping with methanol.
        i. *Do NOT use compressed air around the FTIR. This will result in damage to the beamsplitter.*

4. Gas Cell:
    a. Attach the gas cell stopcock to a vacuum pump with flexible tubing and open the stopcock.
    b. Evacuate the gas cell for approximately 2 minutes and close the stopcock.
    c. Collect and save a background on the FTIR
    d. Run the evacuated gas cell as a blank on the FTIR.
    e. Sampling

i. Collect approximately 3 mL of the headspace vapor sample in a gas-tight syringe.
ii. Attach the syringe directly to the gas cell inlet and open the stopcock. Release the vapor/gas from the syringe into the gas cell. The volume of vapor/gas required for analysis will be dependent upon the concentration of the vapor/gas and the volume of the specific gas cell.
iii. Run the vapor/gas in the gas cell on the FTIR.
iv. Complete a library search or compare spectrum obtained to reference material run under the same parameters.

E. Microscope Accessory:

1. The use of a microscope accessory is preferred for very small samples since spectra can be obtained by using this technique on samples as small as 10 to 20 um in diameter.

2. The microscope attachment permits the analysis of multiple samples placed on an appropriate support material.

3. The method affords the advantage of viewing the sample optically and choosing the most appropriate area for analysis.

4. Spectral measurements using an FTIR microscope can be obtained in transmission, reflection, or ATR mode.
   a. Transmission mode
      i. Transmission measurements are commonly used because they generate spectra with fewer artifacts than other sample modes. However, transmission methods generally entail more sample preparation than reflection techniques.
      ii. Samples can be mounted onto a NaCl or KBr crystal for analysis.
         a) Generally, it is desirable to press a sample after sectioning and mounting onto a crystal to produce a wider width for each layer and to produce a more uniform thickness.
         b) The sample shall be rendered thin enough not to over-absorb.
      iii. Fill the dewer with liquid nitrogen and allow the instrument to equilibrate for at least 30 minutes prior to analyzing any samples.
      iv. Make sure the Continuum – transmission tab is selected in the Omnic software.
      v. Turn on the reflex aperture illumination, reflection illumination and transmission illumination and ensure both field irises are fully open.
      vi. Set the condenser refractive index compensation value to 0.
      vii. Place the polystyrene film on the stage and focus.
      viii. Focus and center the condenser.
      ix. Analyze the polystyrene.

x. The software will ask to analyze the background so remove the polystyrene and select start.
xi. See Minimum Standards and Controls in this Method to ensure acceptability of polystyrene system check.
xii. Mount the sample holder onto the microscope stage.
xiii. If a KBr crystal or NaCl crystal are used to mount the sample, change the condenser refractive index compensation value to about 1.5 to account for the refractive index of these crystals.
xiv. Focus on your sample and adjust the aperture.
    a) The aperture controls the area and location of the infrared beam striking the sample and the transmitted light reaching the detector.
    b) Apertures also block unwanted radiation originating outside of the area of interest. If stray light is allowed to reach the detector, absorption intensity is reduced so the aperture should be chosen so that its edges are as close as practicable to the edges of the sample being analyzed to minimize the amount of stray light produced.
xv. Collect an infrared spectrum of the sample.
xvi. The Continuum microscope requires a background of the sampling media to be collected after every sample. To do this, move the stage to an unused area of the crystal and collect a spectrum using the same sample aperture configuration as used for the sample.
xvii. If sample size is limited, the resulting spectrum can be noisy. To increase the signal to noise ratio (S/N), the number of scans can be increased. It is important to collect spectra with high S/N to permit visualization of fine detail such as small sharp peaks or shoulders in the resultant spectrum.

b. Reflection mode
i. If samples are analyzed directly on an infrared light reflecting surface (i.e. low e-glass or gold mirror), the reflection mode can be used to produce spectra mimicking double-pass transmission spectra.
ii. The sample is viewed and the area to be analyzed is centered in the field of view.
iii. The area of interest is isolated from the remainder of the field of view with an aperture.
iv. Collect an infrared spectrum of the sample.
v. The background spectrum is collected from an unused area of the reflective support using the same aperture configuration and number of scans as used for the sample.

c. ATR mode
i. Applying consistent pressure to each sample can mitigate spectra variations.
ii. Intra-sample variations can result from sample heterogeneity; therefore, multiple samplings shall be conducted as feasible.

iii. Since ATR is a surface technique, smears on samples can be analyzed using this method without much sample prep.
iv. View the sample and center the area to be analyzed in the field of view.
v. Place the crystal in direct contact with the area of interest.
vi. Collect a spectrum of the sample.

F. Spectral Searching

1. Spectral searching is the process whereby a spectrum of an unknown material is evaluated against a library (database) of digitally recorded reference spectra with the purpose of classification or identification of the unknown material.

2. Computer programs can be very helpful for comparing unknown spectral scans to those of known materials, but a skilled FTIR analyst is needed to examine the computer selected spectral matches to ensure that sample identifications are both accurate and complete.
    a. The analyst shall not rely on the Hit Quality Index value of a library match generated by the search function of the FTIR software as the sole basis of a component identification but rather as a tool during FTIR data analysis. Computer matching programs have difficulties with subtle differences that can be critically important.

3. The results of a spectral search can be affected by several factors: baselines, sample purity, absorbance linearity (Beer's Law), sample thickness, sample technique and preparation, physical state of the sample, wavenumber range, spectral resolution, and choice of algorithm.

Report Wording

See Appendix I.

References

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ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Instrumentation

Method: Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS)

Reviewed by:

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INTRODUCTION

Scanning electron microscopy (SEM) provides detailed high-resolution images of a sample by scanning the surface with a focused beam of electrons. This beam causes secondary electrons, backscattered electrons, and x-rays to be emitted from the sample. Backscattered electrons provide information about the differences in the atomic number of the elements present in a sample while secondary electrons produce topographic information.

An energy dispersive x-ray spectrometer (EDS) detects the x-rays emitted and these x-rays provide elemental identification and compositional information. When the electron beam hits the inner shell of an atom, it knocks off an electron from the shell, leaving a positively charged electron hole. When the electron is displaced, it attracts another electron from an outer shell to fill the vacancy. As the electron moves from a higher-energy shell to a lower-energy shell of the atom, this energy difference can be released in the form of an x-ray. This x-ray is unique to the specific element and transition that released it. Therefore, by detecting the emitted x-rays from a sample the elements present can be identified.

SEM/EDS is a non-destructive technique.

Related Procedures

A. Paint Analysis Scanning Electron Microscope/Energy Dispersive X-Ray (MT-IIB-3)
B. Primer Gunshot Reside Analysis (PGSR) (MT-IIIA)
C. General Unknowns Common Acids, Bases, and Bleaches Identification (MT-IVA-1)
D. Pressure Sensitive Tape (MT-VIIIA-1)

Terminology

A. Accelerating voltage – the energy of the electrons in the focused primary SEM electron beam.
B. Backscattered electron (BE) imaging – a technique that uses high energy electrons that originate from the primary electron beam of the SEM and are elastically reflected by the specimen to create an image of the sample. The probability of backscattering is proportional to atomic number.
C. Charging – a negative charge accumulation on either a nonconductive sample or a sample that is not properly grounded. This effect can interfere with image formation and x-ray analysis because of beam deflection. It can usually be eliminated by the application of a conductive coating or by the use of a low vacuum system.
D. Compton scattering – occurs with low atomic weight elements when low energy is used. Low energy causes an Auger electron (an electron from the outer shell) to be ejected. The result is a Compton peak occurring at approximately 200eV before the element's peak.
E. Continuum (Bremsstrahlung x-rays) – the background on which characteristic x-ray peaks sit on.

F. Energy dispersive x-ray spectroscopy (EDS) – x-ray spectroscopy based on the simultaneous measurement of the energies of x-rays emitted by a sample.
G. Escape peak – a peak resulting from incomplete deposition of the energy of an x-ray entering the energy dispersive x-ray spectrometer detector. This peak is produced when an incoming x-ray excites a silicon atom within the detector crystal, and the resulting Si Kα fluorescence x-ray exits the detector crystal. It occurs at the principal peak energy minus the energy of the Si Kα fluorescence x-ray (1.74 keV). The escape peak intensity is about 1-2 percent of the parent peak.
H. Live time – the time over which the energy dispersive x-ray spectroscopy electronics are available to accept and process incoming x-rays. Live time is often expressed as a percentage of real time.
I. Major peak – element whose main peak height is greater than 1/3 of the peak height of the strongest peak in the spectrum.
J. Minor peak – element whose main peak height is between 1/10 and 1/3 of the peak height of the strongest peak in the spectrum.
K. Pulse processor time – operator-selected value for the time designated to record a response by the detector. A higher value (longer time) results in a more accurate determination of the detector amplifier pulse height (better spectral resolution). A lower value results in a higher count rate but with reduced spectral resolution.
L. Raster – the pattern scanned by the electron beam on a sample. The raster dimensions change inversely with magnification.
M. Sample (representative sample) – a representative portion of the specimen selected and prepared for analysis that is expected to exhibit all the elemental characteristics of the parent specimen.
N. Scanning electron microscopy (SEM) – a type of electron microscope in which a focused electron beam is scanned in a raster on a solid sample surface. The term can also include the analytical technique of energy dispersive x-ray spectroscopy.
O. Secondary electron (SE) imaging – imaging using low-energy electrons produced from the interaction of beam electrons and conduction band electrons of atoms within the interaction volume, with only those near the surface having sufficient energy to escape.
P. Spectral artifacts – spectral peaks other than characteristic peaks, produced during the energy dispersive x-ray spectrum detection process. Examples include escape peaks and sum peaks.
Q. Spectral resolution – a measure of the ability to distinguish between adjacent peaks in an x-ray spectroscopy detection process. It is usually determined by measuring peak width at half the maximum value of the peak height or full-width-half-maximum (FWHM).
R. Sum peak – a peak resulting from the simultaneous detection of two photons. This is manifested as a peak at the combined energy of line(s) for the specific element(s) involved.
S. System peaks (stray radiation) – peaks that can occur in the x-ray spectrum as a result of interaction of the electron beam or fluorescent radiation with components of the scanning electron microscope itself.
T. Trace peak – element whose main peak height is less than 1/10 of the peak height of the strongest peak in the spectrum.

U. Variable pressure mode – the mode that allows some SEMs to operate at varying chamber pressures. The need for application of a conductive coating is minimized when using variable pressure mode; however, EDS can be complicated because of the electron beam spread experienced at higher operating pressures.
V. X-ray mapping – scan a specific area to determine the distribution and relative proportion (intensity) of previously defined elements.

Safety

A. X-ray radiation is generated by the equipment.
B. High levels of heat are generated in the column during analysis. Allow system to cool before working inside the column.

Preparations

A. Carbon Coater
B. Carbon tape, discs, or colloidal suspensions for mounting sample
C. Copper and Aluminum for instrument performance check
D. Sample stubs for SEM
E. SEM sample gripper

Instrumentation

A. Scanning Electron Microscope with an Energy Dispersive X-Ray detector
B. Backscatter Detector
C. Secondary Electron Detector

Minimum Standards and Controls

The following are requirements to meet the minimum standards and controls for Scanning Electron Microscopy with an Energy Dispersive X-Ray Detector:

A. Imaging
    1. The detector type (Secondary Electron imaging or Backscatter Electron imaging) and magnification will depend on the specimen. Contrast, brightness, stigmation, and focus are adjusted to provide the most information for each sample. If items are being compared, consider using the same detector and magnification for all the samples.

B. Documentation
    1. Digital copies from EDS will include accelerating voltage, date, peaks identified, case number, item number, and analyst's initials. Method of generation must be included in the case file.
    2. Copies of the copper-aluminum reference check will be included in the case file.

C. Function Checks

1. A performance check, with a copper-aluminum reference material, will be run each day the instrument is in use. Compare peak energies and ranges to defined ranges of acceptability as listed below. If checks, even after being repeated, are outside of the acceptability ranges, repair service will be required, and the instrument will be placed "not in service/do not use" until repaired.

PERFORMANCE CHECK – Copper/Aluminum
ASPEX Explorer

Elemental X-Ray Line KeV	Accepted Range (+/- 10 eV) KeV
Cu (La1) 0.928	Cu (La1) 0.918 - 0.938
Al (Ka1) 1.487	Al (Ka1) 1.477 – 1.497
Cu (Ka1) 8.047	Cu (Ka1) 8.037 – 8.057
Cu (KB1) 8.904	Cu (KB1) 8.894 – 8.914

Reference

Aspex Corporation (2012)

PERFORMANCE CHECK – Copper/Aluminum
Phenom SEM

Elemental X-Ray Line KeV	Accepted Range (+/- 10 eV) KeV
Cu (La1) 0.930	Cu (La1) 0.920 -0 .940
Al (Ka1) 1.486	Al (Ka1) 1.476 – 1.496
Cu (Ka1) 8.039	Cu (Ka1) 8.029 – 8.049
Cu (KB1) 8.904	Cu (KB1) 8.894 – 8.914

Reference

Nanoscience Instruments Inc. (2020, October 15)

D. Comparison of EDS spectra – for questioned and known samples

1. Compare the elements detected and their relative abundances from the spectra.
2. For spectra that cannot be distinguished from one another, characteristic peaks in one spectrum are also present in the comparison spectrum in the same relative abundances.
3. Additional spectra may be collected if subtle differences are noted between items to demonstrate whether the differences are repeatable and therefore exclusionary.
4. Comparison findings of *Similar/Indistinguishable, Dissimilar/ Distinguishable,* and *Inconclusive* can be made using the criteria outlined in the Minimum Standards and Controls of this Procedure Manual.
5. Document the reasoning supporting any comparisons.

Procedure or Analysis

A. Sample Preparation
    1. Mount samples on adhesive stub (preferably carbon tape). Ensure that the sample is flat and adhered onto the adhesive.
    2. Carbon coat all sample stubs.
        a. Place the adhesive stub onto the carbon coater table.
        b. Check the condition of the moving carbon rod, sharpen and/or replace if needed.
        c. Close and press down the top-plate to ensure a good seal.
        d. Pump the chamber to better than 0.05mb by turning the power on.
        e. Ensure the voltage knob is turned on.
        f. Choose which method to use: Manual with Pulse, or Automatic Using Preset Voltage and Time.
            i. Manual with Pulse – select "Manual" and pulse the "Start/Stop" switch to obtain short bursts of evaporation.
            ii. Automatic – select "Auto" and press "Start/Stop" to heat the rods for 6 seconds.
        g. Continue until an adequate amount of carbon film has been built up on the stub. This can be checked by putting an unused glass slide or aluminum stub in the evaporation chamber along the sample stub and observing the film buildup.
        h. Switch the unit off when an adequate carbon film has been determined.
        i. After the evaporation chamber has returned to atmospheric pressure, lift the top-plate to remove the sample.
        j. If the carbon coating is not adequate for viewing in the SEM, repeat the above procedure.
        k. CAUTION: After evaporation, the carbon evaporation source will be hot. Exercise care when handling.
    3. Sample Identification
        a. Mark sample stubs as needed for identification and/or orientation in the SEM sample stage.
        b. Insert sample onto sample stage and note location.
        c. Evacuate chamber.
    4. Document the type of substrate; sample preparation, including any coating applied; and the position of samples on the stub.

B. Instruments
    1. Aspex Explorer SEM
        a. Turn on beam.
        b. Move to copper location.
        c. Select secondary detector.
        d. Saturate beam.
            i. Move to copper tape.
            ii. Adjust Filament Drive until copper tape is observed.

iii. Go to Source Image mode.
iv. Adjust Filament Drive until a defined round beam image is observed.
v. Ensure beam is centered. If not, center beam with screws on gun cap.
vi. Go to Line Scan mode.
vii. Adjust Filament Drive until Line Scan peak is maximized. Reduce peak slightly with Filament Drive.
viii. Adjust Emission Control (under Advanced Filament) until Line Scan peak is maximized. Reduce peak slightly with Emission Control.
ix. Go to Source Image mode.

e. Parameters of SEM:
i. Accelerating Voltage: 25kV
ii. Spot Size: Based upon beam saturation
iii. Backscatter Detector: Set to operational threshold (Line Scan)
iv. Vacuum Mode: High

f. Perform performance check.
i. Performance check can be performed on either secondary or backscatter detector.
ii. If EDS is out of calibration, check beam saturation, sample area location, SEM parameters, sample focus, and manually adjust gain and offset. Perform performance check again before adjusting gain and offset.

2. Phenom XL SEM
a. Turn on beam.
b. Parameters of SEM:
i. Accelerating Voltage: 20kV
ii. Spot Size: 75%
iii. Backscatter Detector: Set to operational threshold (Line Scan)
iv. Vacuum Mode: High
c. Adjust operational threshold (Line Scan).
d. Perform performance check using copper aluminum recipe.
i. If the EDS is out of calibration, check sample area location, SEM parameters, sample focus. Perform performance check again before calibrating EDS detector.

C. Sample Analysis and Imaging (Independent of Instrument)
1. The detector type (Secondary Electron imaging or Backscatter Electron imaging) and magnification will depend on the specimen. If items are being compared, consider using the same detector and magnification for all the samples.
2. Select an appropriate detector for imaging
a. Secondary electron imaging generally provides topographic information about a sample.

b. Backscatter electron imaging provides image contrast that is generally proportional to the average atomic number.

3. Determine area of analysis or imaging based on sample size, presence of adjacent layers, and sample heterogeneity.
4. Position a sample in the field of view and center it under the electron beam.
5. Determine magnification and focus of sample area. Contrast, brightness and beam stigmation can also be adjusted on the Aspex Explorer if needed. Adjust these parameters to provide the most information for each sample. Typically, the sample focus and beam stigmation adjustments are made while viewing the sample at a magnification greater than that to be used for analysis or imaging. Adjust the magnification and move the sample to select the area to be imaged.
6. Evaluate sample for charging. If charging is detected, mitigate it by using one of the following strategies:
    a. Move to a different area of the sample.
    b. Adjust the beam current. Analysis time of sample area will need to be increased.
        i. NOTE: Performance check will need to be reanalyzed.
    c. Change to the lowest chamber pressure that will neutralize sample charging. A degraded image quality and increased EDS analytical volume is directly proportional to the chamber pressure selected.
        i. NOTE: Performance check will need to be reanalyzed.
    d. Carbon coat the sample.
7. Collect as many images as needed to provide adequate support for documenting the physical characteristics of the sample and any conclusions that are drawn.

D. EDS Spectrum Collection

1. Collect a spectrum to obtain elemental information about a sample, layer, or inclusion.
2. Determine how the sample will be collected by using one of the following methods:
    a. Spot. A spot is collected by positioning the electron beam at a fixed position on a sample to provide localized elemental information.
    b. Area analysis. A rastered spectrum is collected by defining the area over which the electron beam will be scanned and collecting elemental information for that area.
    c. Spectral image (i.e., elemental maps) – Phenom only. Spectral datasets, in which each pixel is associated with an EDS spectrum, are collected from an analyst-defined area. Spectral imaging can be used to obtain both elemental composition and distribution from a sampled area.
3. Determine the area in the sample that will be analyzed through consideration of analysis goals, sample size, the presence of adjacent layers, and sample heterogeneity.
4. Collect replicate analyses, if necessary, to evaluate variation in the sample.

E. Spectrum Interpretation

1. Peak detection. A peak is generally considered to be detected when the signal is greater than three times the noise (where noise is the square root of the background). Actual detection limits are material dependent, but in bulk materials is generally accepted to be about 0.1 wt%.
2. Peak assignment. Assign peaks in a spectrum to an element (or elements) or another spectral feature (e.g. escape peak, sum peak, system peak) using X-ray peak centroid energy value markers. EDS software packages can detect and label element symbols on the spectrum; however, it is the analyst's responsibility to inspect each peak to ensure it corresponds to the proper characteristic energy.
3. An element is considered to be present if a distinctive set of lines is produced or when a single peak occurs at an energy which cannot be mistaken for another element or spectral artifact. Identification of an element is not always possible such as when an element is present at low levels, or the lines required for confirmation are overlapped with the lines of other elements.
4. Overlapping elements may be differentiated by determining that the intensities of each element's characteristic peaks are in the proper ratios. Common overlapping element X-rays include:
    - Ti Kβ / V Kα
    - V Kβ / Cr Kα
    - Cr Kβ / Mn Kα
    - Mn Kβ / Fe Kα
    - Fe Kβ / Co Kα
    - Pb Mα / S Kα / Mo Lα
    - Ba Lα / Ti Kα
    - K Kβ / Ca Kα
    - Zn Lα / Na Kα
    - P Kα / Zr Lα
    - Al Kα /Br Lα
    - Sb Lα /Ca Kα (partial overlapping)
    - Sn Lβ/Sb Lα (partial overlapping)

5. Elements detected. Assess the elements detected in a sample to determine if they are consistent with those anticipated in the material being analyzed.
6. Compound classification. The elements detected in an analysis can permit an analyst to draw conclusions about the presence of a compound. However, the following must be considered:
    a. EDS is an elemental technique and does not provide information concerning chemical bonds or crystal structures. Therefore, certain compounds in a sample can be indicated but not specifically identified.
    b. Document the reasons supporting the above determinations.
7. Label each identified peak with the corresponding elemental symbol. Denote if the identification is tentative.

Report Wording

See Appendix I.

References

A. ASTM E1588-17 “Standard Practice for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry”, ASTM International, West Conshohocken, PA, 2017, www.astm.org.

B. ASTM E2809-13 “Standard Guide for Using Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy in Forensic Polymer Examinations”, ASTM International, West Conshohocken, PA, 2013, www.astm.org.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Instrumentation

Method: Gas Chromatography/Mass Spectrometry (GC/MS)

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

Gas chromatography/mass spectrometry is an instrument used in several Micro/Trace disciplines. Gas chromatography is a separation technique capable of separating highly complex mixtures based primarily upon differences of boiling point and polarity of the sample. A GC operates by introducing a sample into the instrument by means of an injection port and carrying that sample through a column where the sample is separated into its individual components. The separated components leave the column and enter the mass spectrometer where they are ionized, fragmented, sorted and detected. The fragmentation pattern of a specific compound is unique and represented in a mass spectrum. By combining the retention time data from the gas chromatogram and the fragmentation pattern from a mass spectrum and comparing that data to a known reference, the identity of the original compound can be determined.

Pyrolysis gas chromatography/mass spectrometry (PyGC/MS) is a destructive technique that uses pyrolytic breakdown products to compare one sample to another. Pyrograms, the chromatograms of the pyrolytic products, are influenced by numerous sample characteristics and instrumental parameters. These can include sample size, shape and condition, ramping rates, final pyrolytic temperature, type of capillary column(s), gas flow rates, temperature programs, and detector type(s). The resulting patterns of peaks in the known and questioned sample pyrograms are used for comparison purposes.

Related Procedures

A. Fire Debris Analysis (MT-I)
B. Paint Analysis Scanning Electron Microscope/Energy Dispersive X-Ray (MT-IIB-3)
C. General Unknowns Common Acids, Bases, and Bleaches Identification (MT-IVA-1)

Terminology

A. Base peak – the largest peak in the mass spectra and has a relative abundance of 100%.
B. Extracted ion profile (EIP) – a group of ions that, together, represents a class of chemical compounds that are presented as a single chromatogram that is representative of that particular class.
C. Molecular ion – an ion formed by the removal of an electron without fragmentation of the molecular structure, therefore, the ion is equal to the molecular weight of the compound.
D. Mass to charge ratio (m/z) – a ratio of an ion's mass to its molecular charge.
E. Resolution – a measure of the ability of a column to separate two peaks (baseline to baseline) through a combination of column efficiency and selectivity. Resolution is expressed as the ratio distance between two peak maxima to the mean value of the peak width at the baseline.
F. Retention time – the time it takes from the moment of injection until a compound elutes and is detected at the peak maximum.
G. Total Ion Chromatogram (TIC) – represents the summed intensity across the entire range of masses being detected at every point in the analysis.

Safety

A. Standard Laboratory Practices
B. The inlet is hot. Take proper precautions when doing any inlet maintenance when it has not been cooled.

Preparations

A. Syringe
B. Pyrolyzer cup
C. Cup removal tool

Instrumentation

A. Gas Chromatograph with a Mass Spectrometer with a column suitable for the type of analysis being conducted
B. Pyrolyzer (if needed)
C. Helium tanks
D. Autosampler (if desired)

Minimum Standards and Controls

The following are requirements to meet the minimum standards and controls for Gas Chromatography/Mass Spectrometry:

A. Documentation
    1. A copy of the total ion chromatogram(s) and any mass spectra used for identification will be preserved in the case file.
        a. The chromatograms must be traceable to the GC/MS from which they were generated.
    2. Pertinent instrument parameters, including a column description, carrier gas, injection temperature, injection volume, split ratio, oven temperature, oven ramps, oven hold times, solvent delay, detector temperature, scan range will be recorded.
    3. The case number, analyst's initials, date, and item number will be recorded on all copies. Item number is optional on references.
    4. A copy of any blanks run will be included in the case file.

B. Function Checks
    1. A record of the instrument maintenance and function checks will be kept in LAM.
    2. A tune will be run each day a case is worked. A tune is an internal quality assurance mechanism using Perfluorotributylamine (PFTBA), to check ranges of acceptability.
    3. Criteria for Mass Spectrometer Tune Report

a. For PFTBA mass assignments of 69, 219, 502 are +/-0.2 amu.
b. The isotopic ratios (relative abundances of the naturally occurring isotopes) must fall within the acceptable range of the theoretical values:

ISO RATIO	RANGE	THEORETICAL VALUE
70/69	0.5−1.6%	1.08%
220/219	3.2−5.4%	4.32%
503/502	7.9−12.3%	10.09%

4. If these checks, even after being repeated, are outside of the acceptable range, repair service will be required, and the instrument will be placed "not in service/do not use" until repairs are made.
5. A control sample will be run daily, prior to casework. The control is a reference material run under the same conditions on the same method as used for the case samples. Before accepting a control, the chromatogram must be compared to a previously accepted control and examined for peak shape, height, and retention time reproducibility (this comparison does not need to be included in the case file).
6. Blanks
    a. Blanks will be run under the same conditions as the samples.
        i. Pyrolyzer - a blank of the cleaned sample cup will be run immediately prior to each item.
        ii. Standard inlet – a blank of the needle and/or solvent will be run immediately prior to each item.

C. Comparisons
1. Pattern comparison
    a. The injection volume and concentration of a sample can change the overall pattern of the chromatogram (i.e. a chromatogram from an overloaded or weak sample may look different than one injected with the proper concentration). Therefore, if possible, ensure the amount of sample being injected is similar in the known and unknown samples.
    b. Ensure the relative abundance and retention times of major peaks and the overall chromatogram are similar.
        i. Heterogeneous samples may cause variations from one chromatogram to another, therefore, it may be necessary to analyze a sample multiple times to determine its variability.
        ii. Any major differences observed should be explained in the notes.
    c. Chromatograms are dissimilar if they contain one or more meaningful differences (for example, absence or presence of peaks, reproducible intensity differences).
    d. Chromatograms cannot be distinguished if they contain no meaningful differences.

e. A chromatographic comparison is inconclusive if no determination can be made as to whether observed differences are meaningful (insufficient sample for multiple analyses, slight differences in minor peaks from samples shown to be heterogeneous).

2. Retention times
    a. Acceptance Criteria
        i. Any qualitative comparison(s) will be made to in-house references after the data has been assessed for suitability (see MT App III).
        ii. The references will be run on the same instrument and under the same conditions, including the column, carrier gas, and oven temperatures, oven ramps, and oven hold times. (Injection parameters are excluded as they relate to sample introduction, not separation.)
        iii. In order to be considered a positive result or indication, the retention time of the apex of the sample peak must be within +/- 2% of the retention time of the apex of the appropriate reference. This will be determined using the following equation: [RT (K) – RT (Q)]/RT (K) x 100%.
        iv. The reference for comparison must be included in the case file.
3. Mass Spectrometry
    a. Acceptance Criteria
        i. The mass spectrum shall be compared to an in-house reference or instrument library.
        ii. Positive identification of an unknown sample is determined by visual comparison of fragmentation patterns. Full scan mass spectral identification is not rigidly defined; rigid criteria may lead to misidentification or under-identification. The analyst should base identification on several factors: unique ions, ion abundance, S/N and available reference collections.
        iii. Spectra should contain the base peak and all major ions unique to the analyte with a fragmentation pattern consistent to a reference library or reference spectrum unless variations have been documented and deemed acceptable by the laboratory.
        iv. Ensure the spectra have the same molecular ion, when observed.
            a) Certain chemical compounds do not produce an observable molecular ion under EI mass spectral conditions. In such cases, comparisons can still be conducted based on the fragment ions present in the spectra.
        v. Consideration of the following factors when making an identification:
            a) Extraneous ions may indicate co-eluting components, background noise, column bleed, septum bleed, and carrier gas impurities.

b) Absence of low abundance ions may indicate low signal concentrations and can be a non-exclusionary difference between two spectra. However, the absence of relevant ions is not acceptable.

vi. Spectral averaging and background subtraction can be utilized.

vii. A copy of relevant spectra will be included in the case file.

Procedure or Analysis (the method below may vary slightly depending on the manufacturer of the instrument used)

A. Independent of the injection method
   1. Heat the oven to a temperature that is at least 10°C higher than the highest temperature in the program that will be used. Heat for at least 15 minutes.
   2. Load the GC/MS program that will be used for the type of sample being analyzed (this will cool the oven to the beginning temperature of the program being loaded).
   3. Once the oven is cooled perform an autotune and ensure its acceptability.
   4. Analyze the appropriate system check sample (i.e. SAM for fire debris, 1968 Ford paint for paint analysis) and examine the data for acceptability.

B. Standard GC/MS injection
   1. A blank of any solvent used and/or the syringe will be run prior to each item. The method selected for the blank should be the same one that will be used for casework.
   2. Enter in all relevant case data into "Sample Information" field and select "Start Run".
   3. Use the appropriate syringe for the type of sample being injected (liquid syringe for liquid sample, air tight syringe for headspace sample).
   4. Once the instrument's method is ready, inject the sample in a way that is reproducible. This injection method may vary slightly from analyst to analyst.
   5. Press the start button on the face of the GC portion of the instrument to start the method.

C. Pyrolyzer
   1. The pyrolyzer should be kept in standby mode when not being used.
   2. Go to the pyrolyzer program and change the pyrolyzer method to the SS550_SS method (this will need to be done prior to analyzing the reference sample).
   3. Remove the sample holder and clean.
      a. Suggested cleaning for sample cup:
         i. Scrape residue with probe. Pressurized air can be used to remove any remaining residue.
         ii. The sample holder can be heated with a burner.
         iii. Handle the sample holder with forceps to prevent contamination from hands.
   4. A blank of the pyrolyzer cup should be run prior to each item.
   5. Allow a few minutes for the cup to cool and then load the sample into the cup.

a. Weigh the sample, if possible, prior to placing it into the cup.

6. Place 10 – 75 micrograms of sample into the sample holder.
7. Attach the cup to the sampler and screw the sampler into the pyrolyzer.
8. Allow one minute for the inlet to stabilize and the outside air to purge out of the pyrolyzer.
9. Enter in all relevant case data into the "Sample Information" field and select "Start Run".
10. Once the GC/MS software is ready, press the "Start" button in the pyrolyzer software, wait until the yellow progress bar is finished and a second dialog box with "Start" appears.
11. Release the cup into the oven by pressing down on the sampler and press "Start".
12. At the end of the run, examine the sample cup under a stereomicroscope and remove any remaining charred sample.

Report Wording

See Appendix I.

References

A. ASTM E1610-18 "Standard Guide for Forensic Paint Analysis and Comparison", ASTM International, West Conshohocken, PA, 2018, www.astm.org.
B. ASTM E1618-19 "Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry", ASTM International, West Conshohocken, PA, 2018, www.astm.org.
C. ASTM E2997-16 Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography-Mass Spectrometry, ASTM International, West Conshohocken, PA, 2018, www.astm.org.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

Protocol: Instrumentation

Method: X-Ray Diffraction (XRD)

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

INTRODUCTION

X-ray diffraction is a technique used to identify crystals in a sample. In x-ray diffraction, an x-ray hits the planes/faces of a crystal and are scattered. A portion of the scattered x-rays are detected, and an x-ray diffraction pattern is produced. This pattern is based upon the atoms in the crystal and the shape and size of the crystal. Every crystalline substance gives a pattern and the same substance always gives the same pattern. About 95% of all solids can be described as crystalline and therefore, can be analyzed using this technique. In a mixture of substances, each produces its pattern independently of the others. This produces a spectrum that is unique to the substance. Identification is achieved by comparing the x-ray diffraction pattern with patterns in a reference database.

X-ray diffraction may be used alone or in combination with other analytical techniques to provide identification of a chemical compound or chemical mixture. Identifiable diffraction patterns may be obtained on samples as small as 0.2 milligrams. This is a nondestructive technique.

Related Procedures

A. General Unknowns Common Acids, Bases, and Bleaches Identification (MT-IVA-1)

Terminology

A. Amorphous – the atoms are arranged in a random way, i.e. glass.
B. Angstrom – the basic unit of length commonly employed in X-ray diffraction. It is equal to 0.1 nanometers of 10^{-8} centimeters.
C. Bragg angle – the angle between the direct (incident) beam and the diffracted beam.
D. Bragg's law – a mathematical expression relating X-ray wavelength (λ), diffraction angle (θ), and interplanar spacing (*d*). $n\lambda = d \sin\theta$ Here n = an integer (1, 2, 3,).
E. Crystalline – material that shows repeating interval arrangements of atoms.
F. Crystal lattice – a three-dimensional array of atoms or molecules made up of identical repeat units (unit cells).
G. D-spacing – the spacing between consecutive layers of high atomic density in a crystal lattice.
H. Miller indices – a system for identifying sets of planes within a crystal lattice. Miller indices are determined by taking the reciprocals of intercept length in three orthogonal directions and clearing of fractions.
I. Preferred orientation – the tendency of crystallites, due to their shape, to lay in a certain direction relative to the plane of the analyzed surface of the specimen. The resulting specimen is non-random, and the observed intensities are weighted toward the preferred planes.
J. Takeoff angle – the angle between the plane of the surface of the x-ray tube anode and the emergent x-ray beam.

Safety

A. Periodically check the safety interlocks on the instrument.
B. Do not override the safety interlocks without supervisor's approval.
C. X-rays are produced by this instrument. Operators should wear an x-ray body badge to monitor their exposure to the x-rays.

Preparations

A. Zero background sample holder
B. Spatula
C. Mortar and pestle

Instrumentation

A. X-Ray Diffraction unit with Diffractometer
B. Instrument Collection Parameters
    a. Select scan range to obtain sufficient diffraction peaks for classification or identification.
    b. Select slits and counting parameters with will permit a diffraction pattern from crystalline samples less than one milligram.
    c. Select copper tube with nickel filter with accelerating voltage and amperage ≥ 40KV, 30MA.

Minimum Standards and Controls

The following are requirements to meet the minimum standards and controls for X-Ray Diffraction:

A. Documentation
    1. Digital copies of diffraction patterns must include case number, item number, date, and analyst's initials. Tube target, filter type, accelerating voltage, and amperage must be included in the case file.

B. Function Checks
    1. Performance check for the proper alignment of diffraction lines will be performed annually. The performance check will include uncertainty measurements and ranges of acceptability as listed in the logbook. If checks, even after being repeated, are outside of the acceptability ranges, repair service will be required, and the instrument will be placed "not in service/do not use" until repaired.

C. Comparisons
    1. Pattern comparison

a. The x-ray diffraction pattern from a sample is compared to a reference to determine what phases are present
    i. The position and intensity of the reference should match the data from the sample.
        a) A small amount of mismatch in peak position and intensity is acceptable
    ii. Specimen displacement error will cause a small amount of error in peak positions
        a) This is due to the misalignment of the sample.
        b) Peaks that are close together should be shifted the same direction and by the same amount.
        c) However, the peak shift follows a cosθ behavior, so peak shift might change direction over a large angular range.
    iii. The sample data should contain all major peaks listed in the reference pattern.
        a) If a major reference peak is not observed in the data, then it is not considered to be a good match.
        b) Minor reference peaks could be lost in the background noise, so it may be acceptable if they are not observed.
    iv. Most diffraction data contain K-alpha 1 and K-alpha 2 peak doublets rather than just single peaks.
        a) This is more evident at higher angles 2θ. At low angles, the second peak may not be visible.
        b) The k-alpha 1 peaks are twice the intensity of the k-alpha 2 peaks

Procedure or Analysis

1. The Rigaku MiniFlex 600 has a chiller that must be checked at least every 6 months.
    a. Ensure the water level is correct and clear (change and/or add distilled water if needed). Never use tap or deionized water. The chiller does not need to be turned off to add the water.
    b. Check the filter in the back. Algae can buildup and restrict the water flow. This filter should be changed every 3 months. The chiller and diffractometer must be turned off prior to changing the filter.
    c. If the flow rate is still low after checking water level and filter, check the filter (mesh) in the x-ray tube and clean if needed.
2. Sample preparation
    a. Grind the sample to an appropriate size to avoid preferential orientation.
        i. Very small crystallites. Average diameter < 0.01µ. Lines are so broad that it is not possible to distinguish them from the general background.

ii. Small crystallites average diameter range 0.01µ to 0.2µ. Lines produced are resolved, but slightly broadened.
iii. Crystallites of proper size. Average diameter range 0.5µ to 10µ. This size gives sharp diffraction lines.
iv. Crystallites which are too large. Average diameter > 10µ.
a. Clearly discernible diffraction lines consisting of many spots closely spaced. 10µ to 40µ.
b. Sizes are visible, but a scattering of spots deviating from the true line position. 40µ to 80µ.
c. Lines are no longer visible, many spots randomly scattered. 80µ to 200µ.
d. Few scattered spots. >200µ.

b. If the sample is suspected to by hydroscopic, use heat lamp or incandescent lamp while preparing sample.
c. Place the sample in the center of sample holder. Sample size less than 0.5mg should be arranged in a rectangular shape with long side perpendicular to x-ray tube.
d. Sample height must be level with surface of sample holder, or the expected peak positions will be shifted and cause errors.
e. The depression in the holder should be completely covered with sample unless a zero-background holder is used.
f. Sample surface should be as flat as possible.

3. Instrument hardware
a. Choose the correct divergence slit and ensure it is installed.
i. The 1.2 degree slit allows more x-rays through.
ii. The 0.625 degree slit allows better resolution but lower intensity. If this slit is used, the analysis time should be increased.
b. Ensure the Kβ filter and 8mm scatter slit are inserted in the first clip by the detector

4. Software Procedure (Miniflex Guidance)
a. Setting up the beam
i. Select Control
ii. Select XG (X-ray generator)
iii. Select X-ray ON (20,2 sleep mode)
iv. Select Control
v. Select Aging
vi. Select Execute (this will slowly turn on the beam)
b. To collect a sample
i. Select General Management
ii. Change the name
iii. Choose the method by selecting Condition
iv. Select Run

Report Wording

See Appendix I.

References

A. Sullenger, D.G.; Contrell, J.S.; Beiter, T.A. "X-Ray Powder Diffraction Patterns of Energetic Materials"; *Powder Diffraction, **1994***, 9, 1, 2-14.

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MICRO/TRACE PROCEDURES MANUAL

APPENDIX I: Report Wording

Reviewed by:

_____
Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____
Bureau Chief Jeffrey Buford
Micro/Trace Command Coordinator
Forensic Sciences Command

Fire Debris Analysis Report Wording Guidelines

I. DISCUSSION:

When the chromatogram from an item displays all features which the analyst deems necessary for a particular class identification, the report will state an ignitable liquid of that class was detected; it will also, when appropriate, name examples of the class.

A. Fire Debris:

1. Positive: A chromatogram was produced that matched a chromatogram of a reference flammable liquid or that met the minimum requirements for a class identification of a flammable liquid.	"Light petroleum distillate, examples of which are cigarette lighter fluids, camp fuels and other solvents." OR
Used when the chromatogram is in the LPD region but doesn't match LPD Reference or when chromatogram has additional compounds such as alcohols, acetates or ketones.	"Light petroleum based product, examples of which are lacquer thinner and other commercial solvents." OR "Medium petroleum distillate, examples of which are paint thinners, dry cleaning solvents, and some brands of charcoal starter fluids." OR "Isoparaffinic product, examples of which are some brands of charcoal starter fluids and odorless mineral spirits." OR "Heavy petroleum distillate, examples of which are fuel oils, diesel fuel, and some brands of charcoal starter fluids." OR

	"Gasoline." “Weathered Gasoline.” “Degraded Gasoline.” OR “Aromatic product, examples of which are some paint and varnish removers, fuel additives, and some insecticides vehicles.” OR “Naphthenic-Paraffinic product, examples of which are some charcoal starters, some insecticide vehicles, and some lamp oils.” OR “Normal-Alkane product, examples of which are some solvents, some copier toners, some candle oils, and carbonless copy forms.” OR “Oxygenated solvent, examples of which are some lacquer thinners, fuel additives, and some industrial solvents.” OR "(Specific product name)" when appropriate.
Wording for heavily weathered gasoline (5-peak group missing).	"Petroleum product characteristic of weathered gasoline." OR “Weathered gasoline”

	A high concentration of alpha-pinene	"Terpenes present. It cannot be determined at this time if these terpenes are a naturally occurring product of the wood or due to the addition of a foreign substance such as turpentine. Submission of a comparative sample of this wood may result in a more conclusive laboratory finding." “Terpenes found, these are found in Turpentines as well as naturally occurring in some types of wood”.
2.	Grey area: Pattern not from substrate present but does not match all of the above requirements for class identification.	"Unidentified petroleum product (or distillate)”. “Heavy petroleum product, an example of which is a biodiesel blend of Diesel Fuel and Fatty Acid Methyl Esters.”
3.	Inconclusive: Pattern may be due to substrate.	"Inconclusive. Submission of a comparison sample of _____ may result in a more conclusive laboratory finding."
4.	Negative:	"No ignitable liquids found."

Optional comment for negative:

A negative result means the laboratory did not identify ignitable liquids in the submitted item(s). There are several possible reasons for a negative result including the possibility that no ignitable liquid was used, or one was used and it is no longer present.

Intoxicating Compound Analysis Report Wording Guidelines

CRITERIA	RECOMMENDED REPORT FORMAT
A. Intoxicating Compound Identified	"(Compound Identified)" OR "(Compound Identified) is listed in the Illinois Statutes as an intoxicating compound."
B. Alkyl Nitrite Present	"(Compound Identified)"
C. No Intoxicating Compound Identified	"No intoxicating compound was found."

Tear Gas Analysis Report Wording Guidelines

Criteria	Recommended Report Format
The lachrymatory compound is identified.	_____ found. _____ is an active ingredient in some chemical aerosol deterrents.
Comparison between standard and unknown with same lachrymatory compound(s)	Items _____ and _____ contain the same active ingredient found in some chemical aerosol deterrents.

SEM Primer Gunshot Residue Report Wording Guidelines

The findings and conclusions for PGSR analysis are based upon an in-house validation study completed by the Illinois State Police Forensic Sciences Division on November 20, 1997.

Particle Classification

Tri-Component Particle Classification

A tri-component particle should possess a non-crystalline appearance and may contain nodules adhering to the surface, surface bright spots, layers and various surface textures. While these particles are best identified as spheroids, PGSR particles may have irregular shapes that are a result of impact fracture. The primary elemental composition that defines a particle as being primer gunshot residue is a combination of barium, antimony and lead. At a minimum, two distinct EDS spectral peaks are required to confirm for the presence of each element Ba, Sb, and Pb. Many other elements (Si, Ca, Al, Fe, Sr, Cu, Zn, S, Sn, K, Cl, Na, Mg, Hg and Co), most often at trace levels, may also be present in the particle along with the previously mentioned combinations. The presence of Zn, Ni, and P will indicate non-PGSR particle types unless the Zn is present along with and at a level equal to or less than Cu, and the Ni is present with Cu and Zn. It is also important that at least one of the primary elements be a major component of the particle composition. If elements other than those listed are present in the particle, it is to be classified as non-PGSR.

Consistent Particle Classification

Consistent particles are classified when combinations of the primary elements such as PbBa, PbSb, BaSb, Sb, Ba, Pb are present. The other elements listed in the tri-component classification may also be present. Several other compositions will fit into this classification because of the new Pb free ammunition that is being manufactured. They will primarily be Sr rich or a combination of Ti/Zn and should be spheroidal.

Additional Particle Classification Rules

- Particles with K or Sn at levels higher than primary Sb (L_α) will no longer be classified as tri-component PGSR particles, but as consistent PGSR particles.
- Cu and/or Al may be the primary peak in the spectra of a tri-component PGSR particle, but either Ba, Sb, or Pb should also be a major peak.
- If the secondary Fe (K_β) peak appears the particle is to be classified as non-PGSR.
- Cl may be present at high levels, but it must be less than the highest peak of at least one of the 3 primary elements. Cl may not be the highest peak in the particle spectrum. If either of these two situations occurs, then the particle is to be classified as non-PGSR.
- Mg may be present, but it must be at a trace level for the particle to be classified as PGSR type particle.

Sample Stub Characterization

Tri-component and Consistent

For this category there must be at a minimum of three tri-component particles and additional consistent particles which have been confirmed by the analyst on the stub. The load factors (particles per square millimeter analyzed) can vary. There does not appear to be any relationship between the number of tri-components and consistent particles detected. The numbers and types of particles can be affected by the ammunition type.

Consistent

For this category samples will contain less than three tri-component particles and a variety of consistent particles which have been confirmed/extrapolated by the analyst. The load factor must be 3.75 or greater. The majority of these particles will probably be of the Lead rich type unless the ammunition used is .22 caliber or the new Lead-free type. The Lead-free ammunition will produce either Strontium, Calcium or Titanium/Zinc particles.

Hand Background

Hand backgrounds are a result of encountering materials through the course of daily life. Occupation can play a major role in the types of particles that are deposited on the hands and may affect whether we choose consistent or hand background. For a stub to be classified as hand background it can contain any type of particle including PGSR type particles. However, the load factor of the PGSR type particles must be less than 3.75 and less than three tri-component particles.

Contaminated

If analysis of the items reveals that the kit/room control sample contains a minimum of three confirmed tri-component particles and/or consistent particle load factor of ≥ 3.75 then there is an indication of potential contamination and no conclusion can be made with respect to the item(s) associated with the kit/room control sample.

Adhesive Blanks

These samples can contain a variety of particle types, but the total number of particles must be no more than 50 and there may be no confirmed tri-component particles.

Criteria	Findings
Negative Room/Kit Control	Similar to an adhesive blank.
Positive Room/Kit Control	Contaminated with primer gunshot residue type particles.
Environmental Type Particles	Contains particles characteristic of background samples.
Tri-component and Consistent Particles	Contains a minimum of three (3) tri-component and additional consistent PGSR particles.
Consistent Particles	Contains consistent type PGSR particles.

Conclusions

Negative Room/Kit Control	No statement needed.
Positive Room/Kit Control	Due to contamination of the control, no conclusions may be drawn from Item #.
Environmental Type Particles (subject)	Indicates that the subject may not have discharged a firearm with the right/left hand(s). If the subject did discharge a firearm, then the particles were not deposited, were removed by activity, or were not detected by the procedure.
Tri-component and Consistent Particles (subject)	Indicated the subject discharged a firearm, contacted a PGSR related item or had the right/left hand(s) in the environment of a discharged firearm.
Consistent Particles (subject)	Indicate that the subject may have discharged a firearm, may have been in the environment of a discharged firearm, contacted a PGSR related item with the right/left hand(s), or received the particles from an environmental source.
Environmental Type Particles (surface)	Indicated that the area sampled may not have been in the environment of a discharged firearm or contacted a PGSR related item. If the area sampled was in the environment of a discharged firearm or contacted a PGSR related item, then the particles were not deposited, were removed by activity, or were not detected by the procedure. Indicates that the area sampled was in the environment of a discharged firearm or contacted a PGSR related item.

Tri-component and Consistent Particles (surface) Consistent Particles (surface)	Indicates that the area sampled may have been in the environment of a discharged firearm, may have contacted a PGSR related item, or may have received the particles from an environmental source.

General Unknown Analysis Report Wording Guidelines

I. DISCUSSION:

The laboratory report findings of a general unknown examination may be diverse.

Criteria	Report Format
Identification:	(Name of unknown material(s)) found.
Characterization: • Non-identification with limited characterization of unknown material.	Acidic chemical substance found.
Inclusion: • Results do not show any relevant differences in chemical composition between or among the items.	Elemental and structural composition of Item 1 could not be differentiated from the elemental and structural composition of Items 2 or 3. Therefore, Items 1, 2, and 3 could have come from the same source or separate sources with the same chemical composition.
Exclusion: • Results show relevant differences in data between or among the samples.	Item 1 contains capsaicin and α-chloroacetophenone (CN). This combination of chemicals was not found in Item 2. Therefore, Item 2 can be excluded as the source of Item 1.
Inconclusive: • Results from which no conclusion can be drawn. The reason for the inconclusive result must be clearly stated in the report.	Due to the presence of sodium chloride in the control sample, no conclusion can be drawn as to the presence or absence of sodium hypochlorite (bleach) in Item 1.
Not identified / Negative Determination: • Results of the analytical examination are negative for the target compound(s) in question.	No adulterant or contaminant found. OR No (name of target compound(s)) found.

The limitations of the tests, if any, must be documented and reported. These limitations may include: specificity of the analytical techniques used, reproducibility of the data, the amount of sample required for the procedure to detect the presence of a certain analyte, the uniqueness of the class of material, and whether one product can be distinguished from another product or class of similar products.

The Conclusion or Findings statements will reflect the interpretation of the limitations and may include the following suggested wording:

- Preliminary tests indicate the presence of….
- ….(trace). OR …., a trace amount.
- ….other chemicals, chemical mixtures, or chemical products have been manufactured that would be indistinguishable from Item 1. Therefore, an individual source cannot be stated to the absolute exclusion of all other sources.
- …., a common (salt).
- ….may be present due to environmental sources.
- Some commercial twine products are known to contain a heavy petroleum distillate. Submission of an identical new twine as a comparison sample may result in a more definitive finding.

The analyst may also report the general properties and potential uses of the substance or class of substances found.

Weight (if necessary) of a substance is determined using a calibrated analytical balance and is reported along with an estimation of measurement uncertainty at a specific confidence level.

- If the measurement uncertainty in ounces is greater than or equal to 0.01 ounce: The uncertainty associated with the mass measurement in Item [Z] is [±x.xx] grams ([±x.xx] ounces) at a 95% confidence level.

OR

- If the measurement uncertainty in ounces is less than 0.01 ounce: The uncertainty associated with the mass measurement in Item [Z] is [±x.xx] grams (less than ±0.01 ounce) at a 95% confidence level.

Below is an example of report wording:

ITEM	DESCRIPTION	FINDINGS
1	One (1) envelope containing two (2) vials.	See findings for Items 1A and 1B.
1A	One (1) vial containing clear liquid.	Acidic chemical substance found.
1B	One (1) vial containing clear liquid.	No corrosive substance found.
2	One (1) gray shirt.	Capsaicin.
3	One (1) vial containing white powder.	Sucrose and potassium nitrate.
4	One (1) paper fold containing white powder residue.	Sucrose and potassium nitrate.

CONCLUSIONS

Item1A contains an acidic substance. No acidic substance was found in Item 1B. Therefore, Item 1B can be excluded as the source of Item 1A.

Item 2 contains Capsaicin. Capsaicin is the active ingredient in some personal protective sprays.

Elemental and structural composition of Item 3 could not be differentiated from the elemental and structural composition of Item 4. Therefore, Items 3 and 4 could have come from the same source or separate sources with the same chemical composition.

MICROSCOPY BASED ANALYSIS REPORT
WORDING GUIDELINES

GENERAL:

Reports from microscopy-based disciples should provide a comprehensive and succinct list of evidence, findings, conclusions, and useful comments relevant to examinations. Because these types of analyses encounter a wide variety of evidence and due to the often, descriptive nature of Findings, the examiner may find that short narratives most clearly convey information to the agency.

General Guidelines for Consideration:

I. Laboratory Description of Items

The examiner is encouraged to give a brief description of each item of evidence (generally excluding the item's packaging) along with from where the item was recovered or its ownership, if known. This description may enhance the information provided by the agency upon submission.

II. Findings

Each item requires a Finding, which describes the examiners observations or results of analysis. In some cases, this may detail the condition of the evidence, summarize the materials observed, or identify a substance. The testing performed or the suitability of the item for comparisons may be included. Alternatively, 'Not Analyzed' or 'Used for Comparison' may be sufficient for a Finding. The narrative in Finding should be a brief presentation of the outcomes of examinations and should answer the agency's request for analysis.

III. Conclusions

A further account of results of analyses may be listed in the Conclusions. Conclusions may present comparisons between recovered materials or explain the significance of the Findings. Along with the Finding, a Conclusion should address an agency's request and articulate probative information which may be discovered during examinations.

IV. Remarks

If necessary, Remarks can be added to request additional submissions or to provide general information.

The following are some examples or templates of Finding and Conclusion statements that may be used in reports.

A. Recovery of Trace Materials

1. Findings

Hairs, fibers, botanical material, soil particles, and miscellaneous debris were observed.
Trace materials were recovered.
Trace evidence was collected from the evidence submitted but will not be analyzed at this time.

B. Hair

1. Findings

Apparent XXX root observed.
Hair fragments were observed.
Among the hairs recovered, XXX hairs, hair fragments, and animal hairs were observed.

2.Conclusions

The recovered hairs are not suitable for submission to the Biology/DNA section.
A representative sample consisting of the proximal ends of XXX hairs with apparent XXX roots were recovered and sub-itemized as Item XXX.

C. Fiber

1. Findings

XXX fibers observed.
Comprised of XXX fibers.
Some of the recovered fibers are suitable for comparison.
XXX fibers were not compared because they are commonly found in our environment.

2. Conclusions

XXX fibers recovered from Item XXX are consistent with the fibers which compose the XXX in Item XXX and dissimilar to XXX; therefore, these fibers could have originated from the XXX in Item XXX and did not originate from XXX.

Fibers recovered from Item XXX are dissimilar to the fibers which compose Item XXX; therefore, no association can be made between Items XXX and XXX.

Similarities were observed to XXX fibers which compose Item XXX; however, because XXX fibers are commonly found in our environment and have little or no comparative value, complete comparison is precluded.

D. Fabric Impressions

1. Findings

A suitable fabric impression was observed

Damage to construction was observed on Item XXX.

A standard fabric impression was made.

2. Conclusions

The fabric impression observed on Item XXX was dissimilar to the XXX in Item XXX, therefore, did not originate from the XXX in Item XXX.

Consistent with the class characteristics of the test impression in Item XXX, therefore, this impression could have originated from the XXX in Item XXX.

E. Pressure Sensitive Tape

1. Findings

The ends appear torn/cut.

Item XXX is in a used and soiled condition

Item XXX and its torn end were used for comparison purposes.

2. Conclusions

Physical and microscopic observations and instrumental analyses of the construction and components of XXX reveal that Item XXX is consistent with Item XXX.

The XXX tape in Item XXX cannot be associated to Item XXX.

F. General Microscopy

1. Findings

Because of the poor quality and insufficient quantity of the XXX in Item XXX, no further analysis could be performed.

Unsuitable for comparison.

Not examined at this time.

Consists of XXX.

2. Conclusions

Dissimilar in XXX to Item XXX.

Similar in XXX to Item XXX.

Inconclusive.

No comparisons were performed.

G. Remarks

If it is determined that the analysis of the evidence will significantly aid in the investigation of this case, please contact the Micro/Trace Section.

At such time as appropriate XXX standards are submitted, comparison with the questioned XXX may be conducted.

If determined to be probative, further analysis may be performed upon specific request and submission of required standards.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

APPENDIX II: Minimum Standards & Controls

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

APPENDIX II

MINIMUM STANDARDS AND CONTROLS

Introduction

For identifications, it is required that data and observations that support conclusions are recorded in the case file.

When the comparison of a known sample (standard) to an unknown (questioned) sample is performed, both samples must be treated in the same manner. Adequate standard materials must be submitted for comparison purposes.

If there is a possibility that an item may be consumed in analysis, or that an item may be permanently altered to prevent examination by another analyst, the examiner shall follow EVH-32. Prior to consuming or altering the evidence, images and/or photomicrographs shall be taken.

Verification by a second analyst shall be required for all probative associations that base conclusions on microscopical or visual examinations. Verifications shall be documented by the verifying examiner.

Terminology

A. Blank - an analysis performed on a laboratory-prepared sample, which includes all components of the unknown sample except for the material of interest. All solvents, preparatory steps, and analysis conditions will be identical to that used on the unknown sample.
B. Control - a test performed on a known sample under identical conditions as that used on the unknown sample.
C. Performance check - a test performed on a known sample as a check of an instrument's output.
D. Minimum Standard of Analysis - the minimum required analytical tests to be performed in order to report a conclusive finding.
E. Preliminary Test - an analytical procedure that yields information about a sample, but by itself cannot be used as a basis for a conclusive finding.
F. Confirmatory Test - an analytical procedure that will specifically identify an unknown sample.
G. Reference File - a collection of spectra or chromatograms of known materials to be used as a reference collection for comparison to an unknown sample.
H. In-House Reagent – a chemical solution, chemical mixture, or dilution that laboratory personnel prepared by combining two or more chemical compounds.
I. Stock chemical – an element, chemical compound, or a mixture of chemical compounds purchased from a vendor and which is stable over time. It is usually used as a solvent or as a component for in-house reagents.
J. Normalization - the process of adjusting a set of values to force them to have one or more

common attributes.

K. Normalization factor - an algebraic value used to achieve normalization.
L. Normalization reference material - a material of known properties used to check operational performance of an analytical process or provide numerical normalization factors.
M. Reference Material – material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties (ISO Guide 30:192(E) Amendment 1:20-08, JCGC 200:2008).
N. Internal Reference Material – typically acquired from a "secondary source" or made in the laboratory. Internal reference materials shall be checked against traceable reference material unless they are from a known source, if possible.
O. Reference Collection – groups of data, items, or materials encountered in casework which are maintained for identification, comparison, or interpretation purposes. Examples of reference collections include but are not limited to ignitable liquids, explosives and energetic materials, paints, Munsell color book, fibers, in-house chromatographic and spectral data, and purchased spectral libraries.
P. Stacked profile – the display of a reference profile and a questioned sample profile in a vertical arrangement such that comparative peaks (ions) are readily located and viewed.
Q. Standard – also Known Sample – also Exemplar – A case item taken from a known source to be used as a representative sample for comparison. Examples of a standard would be the victim's head hair, fibers from the vehicle carpet, or paint from the suspect's vehicle.
R. Unknown – also Questioned Sample – a case item or portion of an item that has been submitted for identification and/or comparison.
S. Demonstrative image/photograph – image of an item to document its appearance. This image does not support technical conclusions nor document evidence prior to consumption or alteration.
T. Meaningful difference(s) – a feature or property of a sample that does not fall within the variation exhibited by the comparison sample, considering the limitations of the sample or technique, and therefore indicates the two samples do not share a common origin. The use of this term does not imply the formal application of statistics.

Guidance to Comparison Findings

Several Micro/Trace disciplines are comparison type analyses between a submitted known sample and questioned sample to determine if they could have originated from the same source. Below are guidelines on the possible findings for these types of analyses.

A. Similar/Indistinguishable – no differences in features or properties are observed between compared items that are substantial enough to conclude they originated from different sources. For spectral data comparisons, data are 1) within the variability of spectra from the same source, or 2) can be explained by lack of sample heterogeneity, contamination, damage, etc.

B. Dissimilar/Distinguishable – exclusionary differences in features or properties are observed between compared items that are sufficient to conclude they originated from separate sources. For spectral data comparisons, data are 1) outside the variability of spectra from the same source, and 2) differences cannot be explained by considerations of sample heterogeneity, contamination, damage, etc.

C. Inconclusive - exclusionary differences are observed in features, properties, or spectral data which may be due to damaged, contaminated, or insufficient sample. No definitive conclusion can be drawn.

Documentation

A. All items/containers must be marked with case number, item number, date, and analyst's initials.

B. Worksheets
   1. State all tests performed and their results.
   2. All non-routine tests will be fully detailed.
   3. An accurate description of the evidence will be recorded.
   4. If a result of inconclusive is reported for any item, a detail explanation of the reasoning behind the finding will be documented in the worksheet.

C. If the item may be consumed in analysis, follow procedure in the Command Directives Manual, EVH-32.
   1. An accurate description of how the evidence is repackaged will be noted.

D. Shorthand Notation
   1. In general, there is no need to write everything on laboratory notes in longhand if the abbreviations used are readily comprehensible to a reviewer and clearly documented.

E. Routine Formulations and Standard Tests
   1. There is no need to notate on worksheets how tests are performed, the formulation of reagents used, and instrument parameters so long as they are contained in the Micro/Trace Procedures Manual.

General Instrumentation and Tests

A. Blanks and controls must be run at the same conditions as the samples.

B. All digital copies of case items will be preserved in the case file.

C. All instrument data generated from case items must contain the case number, item number, analyst's initials, and date.

D. Pertinent instrument parameters used for an analysis must be documented in the

procedures manual or digital copy placed in the case file.

E. All performance check data must be documented and contain the analyst and date.

F. Identification or classification of items must include comparison to a reference.

G. Identification and classification made by comparison to any reference must be documented by placing a digital copy of the reference in the case file.

H. Identification or classification made via computer search must be confirmed by visual comparison to the computer's generated reference and documented by placing a digital copy of the reference in the case file.

I. The use of Gas Chromatography/Mass Spectrometry (GC/MS) in the analysis of fire debris has additional requirements for the use and documentation of reference comparisons. These additional requirements supersede 6, 7, and 8 above for the specialty area of fire debris analysis only.

J. Instrument Logs - each instrument maintained by the Micro/Trace Section will have an appropriate maintenance/performance check log.
    1. The maintenance log will note all scheduled preventative maintenance performed and all other repairs, along with the date and analyst performing the maintenance. Following maintenance or repair, and before resuming casework, appropriate instrument functional checks will be performed and documented in the log. (See also: Individual Instrumentation and Tests and Specialty Areas for specific requirements.)
    2. The performance check log shall contain the results of all required performance checks along with the Date, Initials, and Case number.

K. Reference Materials
    1. Reference materials shall, where possible, be traceable to an accredited reference material producer or certifying body. If no such traceability exits, alternative reference materials must be fully documented as to their origin, accuracy, stability, and/or uncertainty factors. Documentation must be maintained in LAM.
        a. Used in casework/performance checks
            - Copper/Aluminum (Cu/Al)
            - Perfluorotributylamine (PFTBA)
            - Polystyrene (PS)
            - NIST SRM 640d silicon powder
            - MultiSpeck or similar fluorescent material
            - Traceable Homium Oxide, Didymium, and Neutral Density (ND) filters (certified every two (2) years)
            - Refractive Index Liquids
            - Traceable stage micrometer (certified every ten (10) years)

- Traceable balance weights (certified every two (2) years)

2. Internal reference material must be checked against traceable reference material unless they are from a known source. Documentation must be maintained in LAM.
    a. Used in casework/performance checks
        i. Primer Gunshot Residue (PGSR) Control
3. The above materials have no expiration dates unless noted.

L. Individual Instrumentation and Tests
1. Thin Layer Chromatography (TLC)
    a. TLC chambers will be properly labeled (solvent system) when in use.
    b. Control samples will be run on the same plate as unknowns.
    c. Worksheets will indicate the solvent system, visualizing procedure, controls run, and results of the analysis.
    d. Bottles containing visualizing reagents will be properly labeled, dated, and initialed by the preparer.
2. Chemical Reactivity Tests - All in-house reagents must be properly labeled with name, initials of preparer and expiration date. Add concentration, hazard warning, and storage labels (if relevant).
    a. In-house reagents will be authenticated by testing with positive controls when prepared and concurrently in casework to ensure reliability and the results recorded either in case notes or logbook.
    b. All in-house reagents expire one (1) year from date prepared. The expired in-house reagents can be re-authenticated by meeting the original defined criteria from the first authentication and given a new expiration date that is one year from the re-authentication.
    c. Stock chemicals must be properly labeled with date received and initials. Manufacturer assigned expiration dates for stock chemicals must be followed. If the manufacturer does not provide an expiration date, stock chemicals will have no expiration date and will be marked/labeled appropriately.
    d. Controls and appropriate blanks, if applicable, will be run each time an in-house reagent is used in casework and the results will be noted in the case notes.
3. Balances
    a. Using traceable weights, balances will be checked every month or, if used infrequently, prior to each use. The balance performance must fall within the defined measures of uncertainty and ranges of acceptability set by the Forensic Sciences Command Quality Manual (QM-11). If checks, even after being repeated, are outside of acceptability ranges, repair service will be required, and the instrument will be placed “not in service/do not use” until repaired. A record of the performance check will be placed in LAM.
    b. Weight will be reported to a hundredth of a gram and a hundredth of an

ounce. Conversion is grams x 0.03527 = ounces.

c. Weight will not be rounded off. If the measured amount of sample is less than 0.01 gram (less than 0.01 ounce), weight will be reported as "less than 0.01 gram (less than 0.01 ounce)."
d. Balances will be calibrated by an ISO accredited vendor on a semiannual basis.
e. Weight sets will be certified by an ISO accredited vendor every six years.
f. The measurement uncertainty must be included on the report.
g. Measurement Uncertainty for Mass Determination
    i. All measurements are associated with a level of uncertainty. When the most significant contributing sources are considered, a reasonable approximation of the true uncertainty can be given. The uncertainty of measurement is a calculated range of values at a given confidence interval that allows us to express how certain we are that the "true" value is within the stated interval.
    ii. The measurement uncertainty associated with mass determination is affected by factors such as the resolution of the balance, repeatability, linearity, and the uncertainty associated with the calibration standards. Each individual measurement has an uncertainty associated with it. When multiple measurements are combined to obtain a total mass, their uncertainties must also combine.
h. Determination of Measurement Uncertainty for a Balance
    i. All balances used in casework are calibrated semi-annually by an ISO accredited vendor. As part of this calibration, the estimated measurement uncertainty associated with that balance is to be calculated by the vendor.
    ii. The measurement uncertainty calculation should consider, at a minimum, the resolution of the balance, the repeatability of the balance, the balance's linearity, and the uncertainty associated with the calibration weight standards. A coverage factor of k = 2, corresponding to a 95% confidence level, will be used.
    iii. The measurement uncertainty estimate given by the ISO accredited vendor is a snapshot of the uncertainty associated with that balance's measurements at the time of calibration. The balances are function checked according to the policy in QM-11. Continued acceptable results of the function check demonstrate that the snapshot is still valid.
i. Application of Measurement of Uncertainty for Mass Determination
    i. The measurement of uncertainty will be determined for every mass that appears on a casework laboratory report.
    ii. Obtain the measurement uncertainty estimate from the most current calibration certificate for the balance used in the mass

determination.

iii. Calculate the measurement uncertainty at a 95% confidence level associated with the final mass using the following equation:

$$\sqrt{N \text{ x } (u)2} = \pm MU$$

where N = number of measurements needed to obtain final mass

u = measurement uncertainty estimate for balance (95% confidence)

MU = total measurement uncertainty at a 95% confidence level for final mass

iv. Note that if only one measurement is required to determine the final mass, then $N = 1$, and the equation reduces to $u = \pm MU$. Thus, if only one mass measurement is made, then the measurement uncertainty at a 95% confidence level associated with the final mass is equal to the measurement uncertainty estimate for the balance.

v. The measurement uncertainty must always be calculated to the same number of decimal places as the balance reading. It must always be rounded up (never down or truncated).

vi. If the conversion of the calculated total measurement uncertainty from grams to ounces results in a value that is less than 0.01 ounce, the total measurement uncertainty value in ounces will be reported as "less than ± 0.01 ounce."

j. Microbalances

i. A performance check, using a certified weight of 200 mg, will be performed on a daily basis, when the microbalance is in use. The performance check must result in a weight that is within ±3% of the certified weight. If the check, even after being repeated, is outside of the acceptability range, repair service will be required, and the instrument will be placed "not in service/do not use" until repaired. A record of the performance check will be placed in LAM.

4. Fourier Transform Infrared Spectrometer (FTIR)
   a. See Fourier Transform Infrared Spectroscopy method (MT-IXA)
5. Mass Spectrometry
   a. See Gas Chromatography/Mass Spectrometry method (MT-IXC)
6. Scanning Electron Microscope with Energy Dispersive X-Ray System (SEM/EDS)
   a. See Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy method (MT-IXB)
7. X-Ray Diffraction (XRD)
   a. See X-Ray Diffraction method (MT-IXD)

8. Gas Chromatography
    a. See Gas Chromatography/Mass Spectrometry Method (MT-IXC)
9. pH Meters
    a. pH meters will be checked on the day of casework using a traceable buffer solution. A record of the check will be placed in the case file.
10. Micrometers/Calipers
    a. The micrometer/caliper will be checked prior to use utilizing the appropriate gauge blocks to an established tolerance of +/-0.003". A record of this check will be maintained in LAM.
11. Attenuated Total Reflection (ATR) Accessory
    a. See Fourier Transform Infrared Spectroscopy Method (MT-IXA)
12. Berek Compensator
    a. A reference check for the proper operation of the Berek Compensator will be made each time it is used. This will be done using fiber(s) from the reference collections. The range of acceptable birefringence values for the fiber will be documented in LAM. If the determined birefringence value is outside the accepted limits, the check should be performed again. If the value remains outside accepted limits, the compensator is to be removed from service until repaired.
    b. Reference birefringence values will be maintained in LAM.
13. Microspectrophotometer
    a. Wavelength performance (holmium oxide and didymium) and photometric performance (ND 0.1, ND 0.5, and ND 1.0) checks will be conducted each day before use. The performance check will include uncertainty measurements and ranges of acceptability. If the data does not include uncertainty measurements, a manual check of the holmium oxide 446.1 nm wavelength will be measured and have a value of 446.1 +/- 3.0 nm and check of the ND 0.5 +/- 0.047. If checks, even after repeated, are outside of acceptability ranges, repair service will be required.
    b. Performance check materials will be certified biannually. The results of these certifications will be stored in LAM.
    c. A background will be run for each sample.
    d. The sample preparation and mode (absorbance/transmission/reflection) must be recorded and included in the case file.
    e. Fluorescence spectrometry performance checks should be performed using a check sample of known fluorescent material each time the analysis is performed. The results are to be checked for reproducibility. The results are to be stored in LAM and the case file. If checks, even after repeated, are dissimilar to previously recorded spectra, repair service will be required.
    f. Precise fluorescence spectral measurements require the use of quartz slides and cover slips.
    g. For fluorescence the filter cubes used must be recorded and included in

the case file.

14. Microscopes
    a. The analyst will assure that the microscopes are in a clean condition prior to use.
    b. It is recommended that the microscopes are covered at the end of each day.
    c. Microscopes shall be thoroughly checked on an annual basis for cleaning and maintenance by a qualified service technician. Results of annual service checks will be stored in LAM.
    d. Ocular scales will be checked using a traceable stage micrometer initially and at a period of at least every ten (10) years. Values for ocular scale divisions (OSD) shall be determined for each objective used. A record of the checks will be stored in LAM.
    e. Fluorescence microscopes will be checked using a check sample of known fluorescent material. A record of the result shall be maintained in an appropriate log and the case file. If checks, even after repeated, are outside of acceptability range, repair service will be required.
15. Pipettes
    a. All pipettes shall be calibrated by a company qualified to provide certification for their accuracy and precision. Calibrations are to be performed annually. Any pipette for which measurements are outside of the manufacturer's specifications is to be removed from casework and submitted for repair and calibration.

M. Specialty Areas
1. Fire Debris
    a. See Fire Debris Protocol (MT-I)
    b. Headspace (Oxygenates)
        i. See Fire Debris Protocol Headspace Procedures (MT-IA-2)
2. Paint
    a. See Paint Analysis Protocol (MT-II)
3. Acids and Bases
    a. Processing for specific acids must be accompanied with appropriate blanks and controls. If an extraction is performed, a blank of the extraction liquid and the substrate must be performed.
    b. Minimum standard of analysis - pH, chemical tests, and elemental analysis.
4. Intoxicating Compounds
    a. Minimum Standard of Analysis - identification of an intoxicating compound requires infrared spectroscopy or mass spectrometry.
5. Metal Identification
    a. Minimum Standard of Analysis - elemental analysis. Common names of alloys will be included.
6. Tear Gas Analysis

a. Minimum Standard of Analysis - infrared spectroscopy or mass spectrometry.
b. Blank - a solvent extract of a clean area of the substrate material will be performed as necessary.

7. Physical Match
    a. Representative images/photomicrographs shall be required of physical matches that result in meaningful associations. A detailed representative drawing may suffice instead of a photographic image.
    b. All class characteristics and individual features not apparent from the photograph or drawing will be listed.
    c. Physical match procedures, report wording, and minimum standards and controls requirements are in the Physical Math Procedures Manual.
8. Gunshot Residue Analysis
    a. See Primer Gunshot Residue Analysis Protocol (MT-IIIA)
9. General Unknowns
    a. Minimum standards of analysis for identification of organic compounds are infrared spectroscopy, mass spectrometry, or x-ray diffraction. Minimum standards of analysis for identification of inorganic compounds are x-ray diffraction or a combination of chemical tests, infrared spectroscopy and elemental analysis.
    b. Identification will be based only upon completion of the minimum standards of analysis. It is recognized that it will not always be possible or necessary to perform a complete analysis on a given piece of evidence because of sample quality, size limitations, or agency request. When this is the case, the analyst will perform as complete an analysis as possible or necessary and his/her report will be based upon that analytical level of characterization.
10. Hair
    a. Identification and evaluation of hairs shall be done by using transmitted light microscopy.
    b. Test and Controls – maintain a reference collection of animal hairs and commercial furs for identification of animal hairs.
    c. Submission of hairs for DNA analysis shall be conducted by evaluation with DNA analysts on a case-by-case basis. The Micro/Trace analysis may prioritize hair submissions using the root growth phase or presence of adhering root material.
11. Fibers
    a. Man-made fibers for comparison shall be identified as to generic class using polarized light microscopy and at least one more identification technique such as solubility tests, refractive index determination, Berek compensator analysis, or FTIR.
    b. Animal fibers for comparison shall be identified by transmitted light microscopy (MT-VIA).
    c. Natural (vegetable and mineral) fibers for comparison shall be identified

by a minimum of Brightfield microscopy. All vegetable fibers, except cotton will be cross sectioned for identification and compared to the fiber reference collection. Distinguishing characteristics must be present for identification.

d. Fiber comparisons shall be conducted using a comparison microscope. The only exception shall be when fiber standards have been submitted and show significant visual or stereoscopic differences and the fibers do not represent a transfer. Microscopic dissimilarities may defer further testing.
e. Fiber comparisons shall include fluorescence microscopy observations.
f. Fiber comparisons shall use microspectrophotometry for fibers that are dyed sufficiently to produce an adequate spectrum.
g. Fiber comparisons shall also be required between standards and the following questioned fibers if a previous association has been established.
    i. Fibers adhering to questioned fabric impressions.
    ii. Questioned yarns for comparison to fabrics.
    iii. Fibers in questioned ropes.
    iv. Fibers adhering to questioned buttons/fasteners.
h. Other techniques such as cross-sections and refractive index determinations may be used for comparison of fibers when there are no significant differences between the question and standard fibers.
i. Fabric impressions shall be examined and compared visually, physically and which magnification. Representative images/photomicrographs shall be required of fabric impression comparisons that result in meaningful associations.
j. Fiber and fabric impression comparisons involving meaningful transfers shall be verified by a second Micro/Trace analyst. Fiber verifications shall be made by microscopic comparison of all standards and pertinent fibers. Fabric impression verifications shall be compared by comparison of all standards or exemplars and pertinent impressions. Verifications shall be documented by the verifying examiner.
k. Test and Controls
    i. Maintain an up-to-date reference collection of fibers for identification of fiber types.
    ii. All solvents and reagents used in solubility testing shall be checked by using known fiber from the reference collection before use on each case. The results of solubility reagent checks will be recorded in the case file.
    iii. Maintain a reference collection of ropes and cordage to aid identification.
    iv. Fabric impression test impressions should be used for comparison in most two-dimensional questioned impression case when the known item cannot readily be eliminated.

v. Fabric impression test impressions should be produced in a manner similar to the question impression(s), if at all possible. Adequate exemplars of fabric must be made while attempting to duplicate any distortion due to force.

12. Particulate Matter
    a. Particulate matter shall be examined stereoscopically and, where applicable, microscopically and instrumentally.
    b. Particulate matter shall be compared to standards or appropriate reference collections before an identification is reported.
    c. Tests and Controls – maintain a reference collection of known substances for identification.
13. Pressure Sensitive Tape
    a. A reference collection for various tapes shall be maintained.
    b. The use of equipment and instrument shall include performance checks. Case notes shall document characteristics and test results which resulted in comparisons. Associations shall show no unexplained differences between probative tape specimens.
    c. Minimum requirements for association are:
        i. Macroscopic observations and physical measurements.
        ii. Fiber comparison of reinforcement material if present.
        iii. FTIR of adhesives and backing.
        iv. Elemental (SEM/EDX or XRD) of adhesives.

Report conclusions are to be based on the tests performed.

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MICRO/TRACE PROCEDURES MANUAL

APPENDIX III: Data Suitability for Comparison

Reviewed by:

Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

APPENDIX III

Before the data of an unknown sample analysis can be compared to a reference standard or a known sample, it must first be evaluated for suitability. Occasionally, this includes an assessment of limitations inherent to any particular analytical technique given the presence of a specific substance. The following suitability assessments shall be considered prior to any comparison between a sample and a reference standard or a known sample and a questioned sample.

ASSESSING THE SUITABILITY FOR COMPARISON OF SAMPLE DATA

I. For Instrumental Techniques (GC-MS, FTIR, SEM/EDX, Microspectrophotometer)

A. Detector Response

1. When using a technique that produces a detector response, the response should be at least three times the standard deviation of the background at the peak position to be considered a peak suitable for comparison. This peak height can be approximately estimated from the noise level, or thickness of the baseline, on either side of the peak of interest.

B. Chromatographic Peak Shape

1. Acceptable peaks are typically Gaussian, but slight asymmetry and tailing is normal with temperature-gradient methods.

2. The peak of a single analyte should produce a single apex to be used for comparison.

3. In the event that a chromatographic peak is not Gaussian-shaped, the analyst shall evaluate the level of fronting or trailing to determine the significance of the irregularity. Even in circumstances of asymmetry, a single apex should be present for a single sample component to be integrated when retention time is referenced during analysis. In the event that a single apex is not discernible when retention time is to be considered, mitigation steps shall be taken to generate acceptable peak shapes for use in comparison to a reference standard.

C. Peak Separation

1. Peak separation shall be sufficient to show distinct analytes in chromatographic techniques. Peak Separation shall also be sufficient to differentiate between IR bands (FTIR) and mass assignments (GC-MS). Analysts must assess the extent to which any shouldering or overlap may

affect identification of a specific analyte to the exclusion of interfering analytes or impurities and note any manipulations of data employed to produce a clear result from a composite of overlapping substances.

D. Concentration Disparities

1. Characteristics that may be affected by concentration disparities may include chromatographic peak shape, retention time, mass spectral ion ratios, etc. Part of ensuring similar experimental conditions between the sample and the reference standard is ensuring substantial similarities between the concentration of a sample analyte and that of a reference standard, to the degree that meaningful comparisons can be made.

II. For Non-Instrumental Techniques (Solubility, Microscopy, Measurements)

A. Presence or Absence of data

1. For those techniques that do not provide detector responses, analysts shall visually assess the results for the presence or absence of positive test indications as defined by Minimum Standards and Controls or in comparison to a reference standard (e.g. solubility reactions, color reactions).

B. Physical Measurements

1. For comparisons of physical attributes, the same measuring device (ruler, caliper, balance, etc.) is to be used for both questioned and known items.

C. Microscopical Comparisons

1. Samples for microscopical comparisons are to be prepared in the same manner. Mounted specimens should use the same type of medium, slides, and coverslips. The optics and illumination for both sides of a comparison microscope unit are to be as closely matched as possible.

MINIMUM STANDARDS AND CONTROLS FOR TESTING TECHNIQUES

Minimum standards and controls for each individual procedure or testing technique can be found in the relevant section of the procedures manual.

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MICRO/TRACE PROCEDURES MANUAL

APPENDIX IV: MICROSCOPE CLEANING & MAINTENANCE

Reviewed by:

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Approved by:

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Bureau Chief Timothy A. Tripp
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Forensic Sciences Command

APPENDIX IV

MICROSCOPE CLEANING & MAINTENANCE

INTRODUCTION

These techniques are provided to assist the Forensic Scientist when cleaning any of the microscopes used within their section and maintaining them in proper working condition. These guidelines are not intended to be inclusive of all microscope types (i.e., stereo microscope, compound microscopes, comparison microscopes, etc.) and manufacturers (Olympus, Nikon, Leica, etc.). Rather, its purpose to clarify and supplement the recommendations provided by the instruments' manufacturer.

SAFETY CONSIDERATIONS

Standard Laboratory Precautions.

PREPARATIONS

Standard Laboratory Practices.

Chemical	NFPA
Acetone	1-3-0
Methyl Alcohol	1-3-0
Xylene	2-3-0
Xylene Substitute	2-3-0
Water, distilled	0-0-0

Microscope Cleaning kit composed of:
- -aspirator with camel hair brush
- -compressed air
- -lens cleaning fluid
- -lens tissue (lint free)
- -swabs

MINIMUM STANDARDS & CONTROLS

A. The analyst will assure that the microscopes are in a clean condition prior to use.
B. It is recommended that the microscopes are covered at the end of each day.

C. Microscopes shall be thoroughly checked on an annual basis for cleaning and maintenance by a qualified service technician. Results of annual service checks will be stored in a logbook.

D. Ocular scales will be checked using a traceable stage micrometer initially and at a period of at least every ten (10) years. Values for ocular scale divisions (OSD) shall be determined for each objective used. A record of the check will be maintained in a file or logbook.

INSTRUMENTATION

Microscope
Traceable stage micrometer as needed.

PROCEDURE

Care needs to be applied anytime a microscope is cleaned as well as operated. The microscope does contain delicate mechanical parts which can be easily misaligned and even damaged. The use of solvents should be limited to the minimal quantities needed in order to remove the adhering debris. Solvents may destroy the plastic parts of a microscope and, when applied to the lenses, may dissolve the cement used in bonding the various portions of the lens system, as well as eroding the coatings on the surfaces of some of the lenses.

Compressed air or an aspirator with camel hair brush may be used to remove the accumulation of dust on oculars (eyepieces), objectives, stage, condensers, etc. Small amounts of a lens cleaner may be applied to lens tissue to remove simple adhering debris, such as fingerprints, from the lenses without damage to the cement or the coating. Further tough adhering debris can be removed with a minimal amount of an appropriate solvent, such as Xylene (Xylene Substitute) or Acetone applied to a swab. A small amount of the solvent can be placed on a piece of lens paper which is then used to wipe off the debris. Avoid prolong and repeated contact of the solvent with any of the lenses. A small amount of alcohol on a swab or lens tissue should then be used to remove any remaining solvent on the lens. Once cleaned by any of the aforementioned methods, the lens should be gently wiped with clean lens paper.

The best way to maintain the microscope in proper working condition is with daily maintenance which should include a cursory inspection of the microscope and examination of the optical and, if possible, light paths (Kohler illumination). At the conclusion of its use, the operator should ensure the microscope is clean and free of any contaminates which may have been deposited during the examination. The microscope(s) should be covered at all times with a lint-free cover when not in use. Disassembling the microscope should be avoided if at all possible, unless by properly trained personnel.

Ocular Scale Performance Check

Ocular scales (sometimes referred to as a reticule), when available in an ocular of a compound microscope, will allow the examiner to rapidly determine measurement of microscopic particles. In order to provide measurements, a performance check of the ocular scale divisions (OSD) must be performed utilizing a traceable stage scale micrometer for *each* objective used.

Insert the stage scale into the microscope and obtain the best focus of the micrometer divisions. Align or superimpose the ocular scale over the stage scale (usually divided into micrometers) so that the first point, or far left measurement, or each scale will coincide. Count the total number of divisions from the first point of the oculars to one of the divisions near the end, far right measurement, of the oculars where it exactly aligns with one of the lines from the stage scale.

Calculate the number of micrometers per each ocular scale division using the *largest* areas of both scales possible.

Divide the stage micrometer by the ocular units to obtain the approximate measurement of one (1) OSD for that objective:

$$\frac{\text{Stage Micrometer}}{\text{Ocular Scale Divisions}} = \text{Approximate measurement of one (1) OSD.}$$

Document this performance check in the microscope Log Book.

Preventive Maintenance

Preventive maintenance will be done by a *qualified* professional on a yearly basis on microscopes regularly used and as needed on those used infrequently. The maintenance performed will include, but is not limited to:

- thoroughly clean and inspect all exterior surfaces,
- thoroughly clean and inspect optics and illumination system,
- thoroughly clean and inspect mechanical parts,
- remove old lubricant and reapply fresh lubricant where needed,
- clean and inspect all accessories,
- establish Kohler alignment of the illumination system,
- check microscope and attachments for proper operation,
  provide documentation and explanations of the maintenance performed and any repairs made to the instrument and record in the microscope Log Book.

REFERENCES

1. McCrone, W.C.; McCrone, L.B.; Delly J.G.; *Polarized Light Microscopy*, Ann Arbor Science: Ann Arbor, MI, 1978; pp. 98-99.

ILLINOIS STATE POLICE

MICRO/TRACE PROCEDURES MANUAL

APPENDIX V: Clean Technique Procedure for Non-DNA Personnel

Reviewed by:

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Forensic Scientist Adrienne Bickel, Chairperson
Micro/Trace Command Advisory Board

Approved by:

_____

Bureau Chief Timothy A. Tripp
Micro/Trace Command Coordinator
Forensic Sciences Command

Appendix V

Clean Technique Procedure for Non-DNA Personnel

Any forensic scientist who will be handling any item of evidence that had the potential to go to the DNA section for analysis must follow the following procedures:

1. The analyst must wear a mask, lab coat with disposable sleeve covers or disposable lab coat and gloves while examining any potential DNA evidence.

   A. The gloves must either be sterile or the gloves must be bleached and then dried with a paper towel after the gloves are put on. Gloves must be changed between exhibits. Gloves must also be changed after handling non-evidence items prior to returning to casework. These non-evidence items may include but are not limited to, refrigerators/freezers, biohazard waste bins, equipment, computers and telephones. Gloves should be changed following common sense and clean technique.

   B. The face mask must be worn over the nose and mouth to prevent the transfer of aerosols from both the nose and mouth of the analyst to the evidence. Whenever the mask is removed from the face it must be disposed of and a new mask used. For example, if an analyst removes the mask to talk on the phone, the mask must be disposed of, not hung around the neck or placed on the counter and reused.

   C. Lab coats must be fully buttoned or snapped.

2. First decontaminate the surface on which samples are to be processed with a 10% bleach solution. Wet the surface (counter top, lab bench, etc.) that will be utilized to examine evidence thoroughly with 10% bleach solution. Spread the 10% bleach across entire surface with a paper towel. Ensure surface is dry before examining evidence. Don't store bleach solutions in open containers. Replace the bleach solution daily with a fresh bleach solution.

3. All instruments which will be used to process forensic samples (e.g., forceps, scissors, scalpel/razor blades, pipetters and metal probes) must be decontaminated by autoclaving or rinsing with a 10% bleach solution. Caution: some surfaces may resist wetting and will require addition of a detergent. In addition, these items may also be placed under an ultraviolet light source for at least 15 minutes. Note: UV light will not destroy DNA on surfaces that are not directly exposed to the light.

4. Place evidence samples in clean containers or on clean surfaces for processing. Large glassine weighing papers are suggested.

5. Use a 10% bleach solution to rinse or wipe instruments between samples. Instruments may be rinsed with distilled water. After rinsing with a 10% bleach solution, use kim wipes to wipe the instrument. Use a new kim wipe each time.

f. Items will be processed one at a time. Put away the previous item before opening the next item. Clean instruments and fresh paper must be used for each item.

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MICRO/TRACE PROCEDURES MANUAL

APPENDIX VI: Abbreviations

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Abbreviations

The following are abbreviations utilized by forensic scientists in the Micro/Trace Section. They can be used as written or with any variation in case type (upper or lower). Instrument names, chemical formulas, generally accepted nomenclature, and routinely used scientific abbreviations may also be used. The below list includes examples but is not a fully comprehensive list.

Abbreviation	Meaning	Discipline
(+), pos	positive, positive result, plus	General
(-), N, Neg	negative, negative result	General
(s)	sealed	General
< , ≤	less than, less than or equal to	General
>, ≥	greater than, greater than or equal to	General
@ cmpd	at compound microscope	General
@ stereo	at stereo microscope	General
α	alpha	General
λ	wavelength	General
µ, µm	micro (10-9), micron	General
µg	micrograms	General
µl	microliter	General
ρ	density	General
Θ	theta, angle	General
≠	not equal to, is not	General
≡ =	equal, equivalent to	General
~	approximately, possibly	General
Ø	no, none, is not	General
arrow symbol	presumptive, associated with	General
check symbol	acknowledgment of information	General
triangle symbol	defendant or difference	General
', "	feet, inches	General
‖	parallel	General
a, A	area	General
Abd, abs	absorption, absorbance	General
ACE	Active charcoal elution	Fire Debris
ACR	Acrylic	Paint
adj	adjacent	General
admin, adm	administered, administration	General
Agg	aggravated	General
ALK IPH	Isophthalic Alkyd	Paint
ALK OPH	Orthophthalic Alkyd	Paint
ALK TER	Terephthalic Alkyd	Paint
ammo	ammunition	General
ana	anagen root	Micro
ANAB	ANSI-ASQ National Accreditation Board	General
anim, anim. h.	animal, animal hair(s)	General
ANSI-ASQ	Quality	General
app., approx., ~, ≈	approximately, apparently, apparent	General
ASA	Assistant States Attorney	General
ASTM	American Society for Testing and Materials	General
ATR	attenuated total reflectance	General
auto	automotive	Paint
avail.	available	General
B, Bi	paper bag, beta, Birefringence	General
Ba	barium	PGSR
batt	battery	General

bc	bar code	General
Berek, Berek comp, BC	Berek compensator analysis	Micro
bet	Blue evidence tape	General
Bio, Biol	Biology	General
bkg	background	PGSR
blk	blank	General
Bot, bot. debr.	Botanical, botanical debris	General
bpb, BPBs	Brown paper bag(s)	General
Br, brn	brown	General
bx	box	General
C	consistent	PGSR
C	Cloth, Carbon, Centigrade	General
C/C	Clear coat	Paint
C/O	cut open	General
cal	caliber	PGSR
canc	Cancellation, cancelled	General
CAR CAC	Calcium Carbonate	Paint
CAR CAC ARA	Calcium Carbonate Aragonite	Paint
CAR CAC CAL	Calcium Carbonate Calcite	Paint
cat	Catagen root	Micro
CC	Carbon coated, carbon copied, Cook County, clear coat	General
CCSAO	Cook County States Attorney Office	General
CCSPD	Cook County Sheriff's Police Department	General
CERTS	Chicago Police Evidence and Recovered Property tracking system label	General
CEV	Charcoal put in extract vial, vial put in can	Fire Debris
Charac, chx	Characteristic(s)	General
CHCl3	chloroform	General
chem	Chemical, chemistry	General
Chgo	Chicago	General
chk, Ck, ck, cks	check(s)	General
CHR KZC	Chromate Potassium Zinc	Paint
CHR SCH	Chromate Strontium	Paint
cir	circular	General
CL, cl	clear	General
cloth	clothing	General
coeff	coefficient	General
coll, col	Collected, collection	General
comp	Compound, comparison, compensator	General
conc	concentrated	General
conf	Confirmatory test	General
Cons, consis	consistent	General
cont, ctg, ctns	Continuous, continued, contains, containing,	General
contr	contrast	General
conv	conversation	General
COPA	Civilian Office of Police Accountability	General
CP	Carpet pad	Fire Debris
CPD	Chicago Police Department	General
CR	Complaint register	General

ETOH, EtOH	ethanol, ethyl alcohol	General
ETS	Evidence tape sealed	General
Evid val	Evidential value	General
Evid	Evidence, evidential	General
Ex	exhibit, example	General
Ext, ext pt, EXT	Extinction, extinction point, exterior, Extracted ion	General
F, fib	fibers	General
F/A, FA	Firearm, firearm section	General
F/C, FC	File copy	General
FAME	Fatty Acid Methyl Ester	Fire Debris
FB, FBU	Forensic Biology, Forensic Biology Unit	General
Fc, fs	Fingernail clippings, fingernail scrapings	General
FD	Fire Department	General
FLM	Fluorescence light microscope	General
Fluor/ FL	fluorescence	General
Frag, fragm	Fragment, fragmented	General
FS	Forensic Scientist	General
FT-IR, FTIR	Fourier Transform Infrared Spectroscopy	General
fvc/vt	Fibers of various colors and types	General
g	Grams, gasoline	General
G.P.P.	Glassine paper packet	General
g/cc	grams/cubic centimeter	General
g/mL	grams/milliliter	General
gal	gallon	General
GC	Gas chromatograph(y), gallon can	General
	Gas Chromatography Mass Spectrometry, Gas Chromatograph	
GC/MS, GCMS	Mass Selective Detector	General
GSR	Gunshot residue	General
GSW	gunshot wound	General
H, h	Head space, hour, hair(s)	General
H/H, hh, Hs	Head hair(s), hairs	Micro
Heav, hvy	heavy	General
HH, HHS	heated headspace	Fire Debris
Hol	Holmium	General
hom	homicide	General
HPD	Heavy petroleum distillate	Fire Debris
Hr(s)	Hour(s)	General
i	initials	General
I, Inc.	Inconclusive, insoluble	General
I.D., ID	Inside diameter, identification identify, initial + date	General
i/s	inside	General
ID'd	identified	General
ind	indeterminate	General
info	information	General
INJ, inj	injected	General
inits	Initals	General
inst	Instruction, instrument	General
int	interior	General
inv	Inventory, investigator, inventory number	General
ES	Exam sheet	General
esp	especially	General
ET in orig package	Evidence tape sealed in original package	General

IPA	Isopropyl alcohol	General
IR	Infrared spectroscopy	General
Irreg	irregular	General
ISO	International Organization for Standardization	General
ISO, Isopar	Isoparaffinic product, Isoparafin, isotropic	Fire Debris
ISP	Illinois State Police	General
ISP SAK, ISPSAECK	Illinois State Police Sexual Assault kit	Micro
ISPBS	Illinois State Police bar code sticker	General
It./ It	item	General
J	jar	General
K	Known, thousands	General
Kev	Kilo electron volt(s)	General
kg	kilograms	General
kv	Kilo volt(s)	General
l	liter	General
L, lg	large	General
L, liq	liquid	General
l.c.v, LCV	Limited comparative value, little comparative value	General
L/H, LF, LH	Left hand	PGSR
L/L	Lower layer	Paint
L/P, LP	Latent Prints, Latent Prints Section	General
L/S	Long sleeve	General
lab	Label, laboratory	General
LB	left back	PGSR
lbs	pounds	General
LM	Light microscope	General
LPD	Light petroleum distillate	Fire Debris
LPP	Light petroleum product	Fire Debris
LR white	Liquid Resin White	General
LS	left shoe	General
lt, LT, L	light, left	General
m	microscopical, minutes	General
M, med	medium	General
M.d., misc	miscellaneous debris	General
MAIS	major accident investigation section	General
man	manilla	General
mat	material	General
max	maximum	General
ME, MEO, M.E.O	Medical Examiner, Medical Examiner's Office	General
MEL	Melamine	Paint
MEOH	methanol, methyl alcohol	General
mess	message	General
Mfr	manufacturer	General
micr., Micro	microscopy, microscopic, microscope	General
min, m	minute(s)	General
misc	miscellaneous	General
misc. debr.	miscellaneous debris	General
ml, mL	milliter	General
mm	millimeter	General

mns	microns	General
mod	moderately, moderate	General
MPD	Medium Petroleum Distillate	Fire Debris
MSP, microspec	Microspectrophometer, microspectrophometry	General
N	Non PGSR	PGSR
N	no, not	General
n	normal, refractive index	General
n	refractive index, perpendicular - parallel	Micro
n alkanes, n-alk, n-alkanes	normal alkanes	Fire Debris
n w/t obs	normal wear and tear observed	General
N, Neg	Negroid, negative, negligible	General
n.d.r.o., NDOARO	no damage of apparent recent origin	General
n.l.	normal length	General
n.o.e.v.	nothing of evidential value	General
n/a, NA	not applicable, no analysis, not available	General
n/e, NE	not examined	General
n/s, NS	not suitable	General
NaRho	Sodium rhodyzonate	General
nat	natural	General
NBS	National Bureau of Standards	General
NC	nitrocellulose	General
NEUCP	no extinction under crossed polarizers	Micro
NIRCL	Northeastern Illinois Regional Crime Laboratory	General
NIST	National Institute of Standards and Technology	General
norm	normal	General
ns, NS	not seen, no seals, closed/no seals	General
Nu, Nv	ultraviolet 365, 405 nm	General
NWT	normal wear and tear	General
O	other(s)	General, PGSR
o.p.	original packet	General
O/L	outer layer	General
o/s	outside	General
obs, OBS	observed	General
OD	outside diameter	General
OEM	Original Equipment Manufacturer	Paint
off	office	General
OP, orig. pkg	original package	General
OPS	office of professional standards	General
orig	Original	General
OSFM	Office of the State Fire Marshal	General
OT	Original Topcoat	Paint
OU	Original Undercoat	Paint
OXI	Oxide	Paint
OXI FEO RED	Iron Oxide (red)	Paint
OXI FEO YEL	Iron Oxide (yellow)	Paint
OXI SIO	Silicon Dioxide	Paint
OXI SIO OPA	Silicon Dioxide Opal	Paint
OXI SIO QUA	Silicon Dioxide Quartz	Paint
OXI TIO	Titanium Dioxide	Paint

OXI TIO ANA	Titanium Dioxide Anatase	Paint
OXI TIO RUT	Titanium Dioxide Rutile	Paint
P	plastic bag, paper	General
P, prev	previously confirmed	PGSR
P.O., PO	police officer	General
P/C	primer coat	Paint
P/L	primer layer	Paint
P2P	peak to peak ratio	General
part	particulate, particle, partial(ly)	General
pass, psngr, pssr	passenger	General
pb	paper bag	General
Pb	Lead	General
PbBa	lead barium	PGSR
PbSb	lead antimony	PGSR
PC	pint can	General
PCE	passive charcoal elution	Fire Debris
PD	police department	General
PDQ	Paint Data Query	Paint
PE	paper envelope	General
perf	performance, perforation	General
Perf Ck	performance check	General
p-ester, poly	polyester	General
PGSR	primer gunshot residue	General
PHO ZNP	Zinc Phosphate	Paint
phone conv.	phone conversation (record)	General
photo	photocopies, images	General
pigm, pgmt, pig	pigment, pigmentation	General
pkg	package	General
pl, plb	plastic, plastic bag	General
pleo	pleochroic	General
PLM	Polarized Light Microscope/ Microscopy	General
pmt, permt, perm	permount	General
poly, polyest	polyester	General
poly, polysty	polystyrene	General
poss	possibly, possible	General
pp F/B	previously processed by Forensic Biology	General
PPE	personal protective equipment	General
pr	pair	General
prev, P	previously, previous	General
prob. val.	probative value	General
prom	prominent	General
prop	property	General
prox	proximal	General
pt	pint can	Fire Debris
PUR	Polyurethane	Paint
PyGC/MS	pyrolysis gas chromatograph/ mass spectrometer	General
pyro	pyrolysis	General
Q, quest	question(ed)	General
QA	quality assurance	General

QC	quart can	General
QR	quality review	General
Qt	quart can	Fire Debris
R	retardation, right, reverse, right	General
R/H, rh	right hand	General
R/S, rs, rep smpl	representative sample	General
RB	right back	PGSR
RC	room control	PGSR
RD, RD#	Record Division, Record Division number	General
re	reason	General
REA	Urea	Paint
rec, rcv'd, rec'd	received, recovered, recent, record	General
REF	reflective	Paint
ref, refl	reflectance, reflective, reflected, reference	General
rep	representative	General
Ret, RET, ret	retardation colors, red evidence tape	General
Rev	revolver, reverse	General
RI	refractive index	General
Rm	room	General
rnd	round	General
rot	rotate(d)	General
Rs	right shoe	General
RT	Refinish Topcoat	Paint
Rt, RT	right, retention time	General
RU	Refinish Undercoat	Paint
RxN	reaction	General
S	sulfer, soil, small, sealed, soluble	General
S&W	Smith & Wesson	General
s.o.p.	see original page	General
s.p.p.	see previous pagee	General
S/S	sample size, short sleeve	General
SA	sexual assault, semi-automatic	General
SAECK	sexual assault evidence collection kit	General
SAK	sexual assault kit	General
SAM	sensitivity/standard accelerant mixture	Fire Debris
SAO	State's Attorney Office	General
Sb	antimony	PGSR
SB, sblk	system blank	General
scat	scattered	General
SDI	sealed, dated, initialed	General
SE	solvent extract(ion)	General
sec	second	General
SEM	Scanning Electron Microscopy/Microscope	General
SEM/EDX	Scanning Electron Microscopy/ Energy Dispersive X-ray	General
semi cont	semi-continuous	Micro
sens	sensitivity	General
sig	significant	General
sign	sign of elongation	Micro
SIL	Silicate	Paint
vis	visible	General

W.P.B.	white paper bag	General
W.S.	worksheet	General
w/	with	General
w/o, w/out	without	General
WET, wet	white evidence tape	General
WP, W.P.	white paper	General
WPP	white paper packet, white paper product	General
X	power, for magnification, etc.	General
x-polars	crossed polars	General
x-sect, XS, xsection	cross sectional, cross section	General
xyl sub/subst	xylene substitute	General
Y	yes	General
yel	yellow	General
YET	yellow evidence tape	General

ILLINOIS STATE POLICE

MICRO/TRACE
PROCEDURES MANUAL

APPENDIX VII: Safety – NFPA Codes

Reviewed by:

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Approved by:

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Safety - NFPA Codes

The NFPA system identifies the hazards of a material in terms of three principal categories in this order: "health," "flammability," and "reactivity." These categories indicates the degree of severity by a numerical rating that ranges from four (4), indicating a severe hazard, to zero (0), indicating no hazard.

Signal	Health	Signal	Flammability	Signal	Reactivity (Stability)
4	Materials that on very short exposure could cause death or major residual injury.	**4**	Materials that will rapidly or completely vaporize at atmospheric pressure and normal ambient temperature, or that are readily dispersed in air and that will burn readily.	**4**	Materials that in themselves are readily capable of detonation or of explosive decomposition or reaction at normal temperatures and pressures.
3	Materials that on short exposure could cause serious temporary or residual injury.	**3**	Liquids and solids that can be ignited under almost all ambient temperature conditions.	**3**	Materials that in themselves are capable of detonation or explosive decomposition or reaction but require a strong initiating source or which must be heated under confinement before initiation or which react explosively with water.
2	Materials that on intense or continued but not chronic exposure could cause temporary incapacitation or possible residual injury.	**2**	Materials that must be moderately heated or exposed to relatively high ambient temperatures before ignition can occur.	**2**	Materials that in readily undergo violent chemical change at elevated temperatures and pressures or which react violently with water or which may form explosive mixtures with water.
1	Materials that on exposure would cause irritation but only minor residual injury.	**1**	Materials that must be preheated before ignition can occur.	**1**	Materials that in themselves are normally stable, but which can become unstable at elevated temperatures and pressures.
0	Materials that on exposure under fire conditions would offer no hazard beyond that of ordinary combustible material.	**0**	Materials that will not burn.	**0**	Materials that in themselves are normally stable, even under fire exposure conditions, and which are not reactive with water.

The examiner should use all class 4 chemicals and class 3 reactivity chemicals in a fume hood. The examiner should use all class 3 and class 2 health and flammability chemicals in a fume or other type of hood.