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01 QUALITY ASSURANCE

TE-01-01 TRACE SERVICES

1 Overview
   A. The Trace Evidence Discipline offers laboratory service for the detection, recovery, analysis and comparison of trace evidence by competent examiners.
   B. Trace Evidence sub-disciplines for which service is provided are:
      1. Trace Evidence Recovery
      2. Physical Comparison
      3. Hair
      4. Fiber
      5. Paint/Polymer
      6. Glass
      7. Imprint/Impression
      8. Gunshot Primer Residue
      9. Filaments
     10. Unknown Substance
     11. Pressure Sensitive Tape
     12. Fire Debris

2 Examiner Approval
   A. An examiner shall demonstrate competency and be authorized by the Laboratory Director before supervised work will be allowed.
   B. The Trace Evidence Training Manual contains modules for each sub-discipline. The training records of each employee documents completion of the required reading materials, training exercises and testing whereby the employee demonstrates competency to perform the sub-discipline.

3 Examiner Assessment
   A. Each examiner shall successfully complete a proficiency test, interlaboratory comparison or intralaboratory comparison every other year in the following sub-disciplines for which they perform casework:
      1. Fiber
      2. Paint
      3. Glass
      4. Physical Match
      5. Hair
      6. Filament
      7. Unknown Substances
B. Additionally, each examiner shall successfully complete a proficiency test, interlaboratory comparison or intralaboratory comparison every year in the following disciplines, as recognized by OSAC and adopted by ANAB, for which they perform casework:

1. Fire Debris
2. Gunshot Primer Residue
3. Impression
4. Trace (Materials)
TE-01-02 CONTROLS, MATERIALS/COLLECTIONS, AND SPECTRAL LIBRARIES

1 Scope
These policies cover the use of controls, reference material and/or collections, and reference spectral libraries.

2 Related Documents
CLS Manual: Standards, Reference Materials/Collections, Databases, and Controls

3 Practices
3.1 General
A. Controls, reference material/collections, and reference spectral libraries will be identified as to their intended purpose.
   1. Instrument performance and method verification;
   2. Identification, comparison or interpretation in casework; or
   3. Training
B. Laboratories shall maintain lists of controls, reference material/collections, and reference spectral libraries indicating their intended purpose, source and unique identification.
C. If a control, reference material/collection, or reference spectral library is used, it shall be documented in the case record and/or an appropriate log.
D. Controls, reference material/collections, and reference spectral libraries are to be used only for their intended purpose.

3.2 Controls
A. A sample of known qualitative value is used to verify the performance of a procedure or instrument before use on evidence samples. The sample can also be used to demonstrate that a procedure or instrument can perform as expected and the results of the analysis are acceptable.
B. The identity of a sample shall be confirmed before it can be used as a control. Documentation of the confirmation of identity, lot number (if applicable), and source will be maintained.
C. The quality control data sheets supplied with some commercially prepared samples will be retained.
D. Controls will be stored according to manufacturer's recommendations.

3.3 Reference Material/Collections
A. Reference material/collections maintained for identification, comparison, or interpretation in casework shall be clearly listed for use in casework, uniquely identified and controlled.
B. Controls and reference material/collections identified for use in casework may also be used for training. They shall be properly controlled, handled and stored to prevent contamination and/or deterioration.
C. Controls and reference material/collections identified for use in training shall be used for training purposes only and documented in a training record and/or log.
4 Reference Spectral Libraries

A. Reference libraries of spectra used in identification of compounds must be fully documented, uniquely identified, and properly controlled.

B. Commercial libraries of mass spectra and infrared spectra in electronic form that were acquired from external sources for use with the laboratory’s analytical instrumentation meet these requirements, as do published reference collections and reputable scientific literature.

C. For reference libraries produced by the laboratory, at least one of the following requirements shall be met for each entry used to confirm the identity of evidentiary substances:

1. The compound used to generate the spectrum shall be traceable. The person that generates the spectrum shall note, either on the reference spectrum itself or in the information that accompanies it, the manufacturer’s or supplier’s company name and lot number, the date the entry was generated, and his or her initials; or

2. The spectral information in the entry shall be matched to information for the same compound that is published in an approved library or literature. The person that performs the comparison shall note, either on the reference spectrum itself or in the information that accompanies it, the date the match was verified, the source of the reference used for the comparison, and his or her initials.

5 Records

Lists of controls and reference materials/collections
TE-01-03 REAGENTS

1 Scope
To establish quality assurance procedures for reagents used in trace evidence examinations.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Practice
A. Refer to the respective chapter for the required grade specification and/or concentration of reagents, if specified.
B. Reagents should be stored as recommended by the manufacturer.
C. Refer to the respective chapter for the laboratory-prepared reagents that shall be quality tested against a known substance on the day of use to demonstrate they react as expected. This shall be documented in the case file.
D. Commercially-prepared reagents are not required to be quality tested (examples include xylene(s), xyless II, glycerol, etc.)
E. Laboratory-prepared reagents that are intended for one day use shall have the preparation date and components used in preparation documented in the case record.
F. A logbook shall be maintained for laboratory-prepared reagents that are intended to be used for more than one day. The logbook will include the following information:
   1. Reagent preparation date
   2. Components used in preparation
   3. Initials of the preparer
   4. Results of quality testing (if applicable)
G. All reagent bottles shall be marked with the name of the solution, preparer's initials and date of preparation. The date of preparation may serve as the lot number correlating to the logbook for reagents that are retained.
H. No reagent will be used if it is not working properly or is contaminated. Additionally, a reagent shall not be used if its stated shelf life has expired, unless a performance verification has been completed supporting the use of reagents beyond their expiration date. The performance verification will be recorded on the Validation/Verification form (LAB-408).
TE-01-04 LABORATORY EQUIPMENT

1 Scope
To establish quality assurance procedures for equipment in the Trace Evidence discipline.

2 Related Documents
CLS Manual: Laboratory Equipment
CLS Manual: Validations and Performance Verifications

3 General Requirements

A. Each piece of significant equipment shall have an associated Equipment Log (LAB-405) containing information related to usage, verification, calibration and maintenance.

B. Operating instructions for significant equipment shall be readily available.

C. The operating conditions, such as number of scans, methods used, temperature programs, for significant equipment used to obtain data shall be documented in the case record, unless otherwise specified.

D. Significant equipment shall be performance checked as required. If the performance check does not pass, perform troubleshooting in an attempt to locate and correct the issue. If the equipment is unable to pass a performance check, advise the supervisor and follow procedures outlined in CLS. Consultation with a service engineer may be needed.

E. If significant equipment fails the performance check after troubleshooting attempts, is observed to be out of calibration, is non-functional, or gives questionable results, it is removed from service and its "out of service" status shall be clearly displayed. The LAB-405 is updated, a LAB-410 is completed, and the supervisor shall be notified.

F. If significant equipment is shut down, it shall be performance checked before use on case work.

G. Any repairs performed by a service engineer are considered to be a major repair.

H. After a major repair of significant equipment, a performance verification will be completed as outlined below before it is returned to service.

I. The process outlined in the Validations and Performance Verifications section of the Crime Laboratory Service Manual will be followed when returning significant equipment to service.

4 Significant Equipment

4.1 Fourier Transform Infrared Spectrometer (FTIR)

A. Performance Check

1. A matte-finish polystyrene film obtained from the manufacturer and verified against published reference spectra is used to verify performance of the detectors being used.

2. The spectrum of the polystyrene film will be obtained each day the FTIR is used. The peaks at 3025, 1600, 1028 and 698 cm⁻¹ shall be marked on the spectrum and be within +/- 4 cm⁻¹ of their expected value in order to pass.
3. Document the date, examiner initials, and pass/fail status of the polystyrene film on the Equipment Log (LAB-405). The spectrum of the polystyrene film will be placed in the case record.

B. Maintenance

2. Performance verification after major repair:
   a) The spectrum of the polystyrene film will be obtained and the peaks at 3025, 1600, 1028 and 698 cm\(^{-1}\) shall be marked on the spectrum and be within +/- 4 cm\(^{-1}\) of their expected value in order to “pass”.
   b) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

4.2 Pyrolysis Gas Chromatograph Mass Spectrometer (PGCMS)

A. Performance Check

1. The mass spectrometer shall be tuned on the day of use for individual samples and evaluated before case samples are run:
   a) The mass selective detector is tuned to ensure that the mass-to-charge ratios (m/z) are assigned correctly and to check for leaks.
   b) The tune values shall be within the range of accepted values as defined during the validation of the instrument or manufacturer recommended values in order to pass.
   c) Document the pass/fail status of the tune on the Equipment Log (LAB-405). The tune is printed and retained in the logbook.
2. Polyethylene is used to verify resolution of the pyrolysis gas chromatograph mass spectrometer. To pass, the pyrogram of the polyethylene shall display a series of at least 10 resolved triplet peaks and be in general agreement with previously accepted polyethylene standards.
   a) The standard run is valid for seven days after the previous run.
   b) Document the date, examiner initials and pass/fail status of the polyethylene sample on the Equipment Log (LAB-405). The pyrogram of the polyethylene will be placed in the case record.
3. A blank shall be run before each sample.

B. Maintenance

2. Performance verification after major repair:
   a) To pass, the pyrogram of the polyethylene shall display a series of at least 10 resolved triplet peaks and be in general agreement with previously accepted polyethylene standards.
   b) The tune values shall be within the range of accepted values as defined during the validation of the instrument or manufacturer recommended values in order to “pass”.
   c) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.
4.3 Microspectrophotometer (MSP)

A. Performance Check

1. Each instrument has a traceable filter set that has documented expected values, generated by the manufacturer and tested against the National Institute of Standards and Technology Standard Reference Material.

2. The visible and/or UV range spectra of the holmium oxide filter and the neutral density filters are obtained each day the instrument is used in order to check performance.
   
   a) All holmium oxide peaks shall be within +/- 3 nm of their expected value in order to pass.
   
   b) Absorbance values for the neutral density filters shall be within the determined acceptable range of the NIST values in order to pass.
   
   c) If the ultraviolet range is used, absorbance values for the neutral density filters for wavelengths greater than 270 nm shall be within the determined acceptable range of the NIST values in order to pass.
   
   d) Document the date, examiner initials and pass/fail status of the verification on the Equipment Log (LAB-405). The spectra and/or data chart will be placed in the case record.

B. Maintenance


2. Performance verification after major repair:
   
   a) All holmium oxide peaks shall be within +/- 3 nm of their expected value in order to “pass”.
   
   b) Absorbance values for the neutral density filters shall be within the determined acceptable range of the NIST values in order to “pass”.
   
   c) If the ultraviolet range is used, absorbance values for the neutral density filters for wavelengths greater than 270 nm shall be within the determined acceptable range of the NIST values in order to “pass”.
   
   d) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

4.4 Scanning Electron Microscope-Energy Dispersive Spectrometer (SEM-EDS)

A. Performance Check

1. A commercially available sample of an acceptable element is used to check the performance of the SEM-EDS. A Gunshot Residue Standard is used to check the performance for Gunshot Residue analysis.

2. Calibration of the detector is required prior to elemental analysis.
   
   a) If the adjustment is outside the detector range, an error message will appear. The instrument will be taken out of service until repairs are made.

   b) Document the date, examiner initials and the calibration on the Equipment Log (LAB-405). The printout of the calibration will be placed in the case file.
3. A Gunshot Residue Standard and an environmental blank are analyzed each time an automated gunshot residue analysis is performed.
   a) Performance check consists of the instrument passing a Quant Optimization and finding the proper number of particles on a GSR QC stub, including a 1um particle.
   b) Detection of particles on the standard shall agree with the expected values in order to pass.
   c) The pass/fail status of the Gunshot Residue Standard is documented on the Equipment Log (LAB-405) and the print-out is placed in the case record.
   d) If gunshot residue particles are detected on the environmental blank, the results of the evidence samples should be considered suspect. The results may be reported with a qualifying statement.

B. Maintenance
   2. Annual service contracts, to include a minimum of one preventive maintenance visit for each instrument, will be renewed with the manufacturer, as funding permits.
   3. Performance verification after major repair:
      a) Performance verification consists of the instrument passing a Quant Optimization and finding the proper number of particles on a GSR QC stub, including a 1um particle.
      b) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

4.5 X-Ray Diffractometer (XRD)

A. Performance Check
   1. A silicon wafer obtained from the manufacturer is used to check performance.
   2. Each day the instrument is used, the silicon wafer is analyzed to demonstrate proper operation.
      a) The lines on the diffraction pattern shall be marked and be located at 28.44° +/- 0.06°, 47.30° +/- 0.06° and 56.12° +/- 0.06° in order to pass.
      b) If the lines are not within this tolerance, adjust the goniometer and repeat the analysis of the silicon wafer. If the lines remain outside tolerance, remove the equipment from service, advise the supervisor and/or the manufacturer representative before performing casework.
      c) Document the date, examiner initials and pass/fail status of the silicon wafer on the Equipment Log (LAB-405). The silicon diffraction pattern will be placed in the case file.
B. Maintenance


2. Performance verification after major repair:
   
   a) The silicon wafer is analyzed and the lines on the diffraction pattern shall be marked and be located at 28.44° +/- 0.06°, 47.30° +/- 0.06° and 56.12° +/- 0.06° in order to “pass”.

   b) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

4.6 Glass Refractive Index Measurement (GRIM) Instrument

A. Performance Check

1. A NIST or Locke Scientific glass standard corresponding to the expected refractive index range of the evidential glass samples will be run each day of use to ensure the instrument is operating correctly.

2. The average $N_D$ of the measured edges shall fall within acceptable limits as defined during the instrument validation in order to pass.

3. The results of the performance check will be placed in the case record and the measured $N_D$ value indicated on the Equipment Log (LAB-405).

B. Maintenance


2. Performance verification after major repair:

   a) A NIST or Locke Scientific glass standards of all three refractive index ranges will be run to verify the instrument is operating correctly. The average $N_D$ of the measured edges shall fall within acceptable limits as defined during the instrument validation in order to pass.

   b) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

4.7 Gas Chromatograph Mass Spectrometer (GCMS)

A. Performance Check

1. The mass spectrometer shall be tuned on the day of use for individual samples or on the initial day of use for a batch of samples and evaluated before case samples are run:

   a) The mass selective detector is tuned to ensure that the mass-to-charge ratios (m/z) are assigned correctly and to check for leaks.

   b) The tune values shall be within the range of accepted values as defined during the validation of the instrument or manufacturer recommended values in order to pass.

   c) Document the pass/fail status of the tune on the Equipment Log (LAB-405). The tune is printed and retained in the logbook.

2. A solvent blank shall be run before each sample.
B. Maintenance


2. Performance verification after major repair:
   a) The tune values shall be within the range of accepted values as defined during the validation of the instrument or manufacturer recommended values in order to pass.
   b) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

4.8 X-Ray Fluorescence (XRF)

A. Performance Check

1. Each day the instrument is used, the aluminum/copper standard is analyzed to demonstrate proper operation and energy calibration. The aluminum/copper standard should be run at all of the time constant values that will be used for sample analysis. The aluminum peak should be at 1.486 keV and the copper peak at 8.04 keV.
   a) Document the pass/fail status of the energy calibration on the Equipment Log (LAB-405). The energy calibration summary is printed and placed in the logbook.

2. If the calibration fails to meet expected values, manual adjustments can be made and a re-calibration performed. If the calibration continues to fail to produce acceptable results, then the equipment is removed from service and consultation with a service engineer may be needed.

B. Maintenance


2. Performance verification after major repair:
   a) The aluminum/copper standard should be run at all-time constant values that will be used for sample analysis. The aluminum peak should be at 1.486 keV and the copper peak at 8.04 keV.
   b) A known set of reference or competency samples will be run and compared to previously generated data to ensure the equipment is functioning as expected.

5 Non-Significant Equipment

Equipment that does not require daily performance checks prior to use or a log of usage includes the following:

5.1 Microscopes

The Trace Evidence Section utilizes stereomicroscopes and compound transmitted light microscopes (including polarized light microscopes, comparison microscopes, and fluorescence microscopes).

A. Microscopes used to measure diameter with an ocular scale will have the scale performance checked for each objective-eyepiece combination.

1. Performance checks are only necessary when a new objective, eyepiece or ocular scale is installed.
B. Comparison microscopes will have the magnification of each objective-eyepiece combination on both stages compared for uniformity upon initial set-up.

C. Normal maintenance which includes keeping the microscopes clean and changing the lamps as needed does not need to be recorded.

5.2 Balances

A. Laboratory balances will be evaluated by a calibration service provider annually.

B. Balances will be evaluated whenever they are moved from one location to another.
   1. Perform at least two different weight measurements using NIST traceable reference standards.
   2. The observed measurements shall be within the tolerance of the electronic balance as stated on the calibration certificate.
   3. The evaluation will be retained in a locally maintained logbook.

C. Routine maintenance is not required to be recorded.

5.3 Calipers/Micrometers

Calipers/micrometers are evaluated on day of use.
   1. Perform at least two different size measurements using NIST traceable gauge blocks or other equivalent reference standard.
   2. The observed measurements shall be within +/-0.02 mm of the specified gauge block.
   3. The results of the evaluation will be recorded in the case record.

5.4 General Equipment

General equipment with settings that cannot significantly affect the test or result (such as stirrers, hot plates, centrifuges, cameras, flashlights, alternative light source, ohmmeter, etc.) are maintained by visual examination only. General equipment does not require the use of an Equipment Log (LAB-405).

6 Records

Equipment Log (LAB-405)
Validation/Verification Form (LAB-408)
Equipment Out of Service Incident Form (LAB-410)
1. **Case Reviews**

All Trace Evidence cases will be subjected to technical and administrative review prior to the report being released, as outlined in *Review of Laboratory Records* (CLS Manual).

2. **Related Chapters/Documents**

Fracture Physical Match Comparison  
Human Hair Comparison  
Thin Layer Chromatography of Fibers  
Comparison of Impression Evidence  

CLS Manual: Review of Laboratory Records, sections on  
- Technical Review  
- Administrative Review  
- Examination Verification  
- Review Resolution Process

3. **Practices**

3.1 **Verification**

In addition to the laboratory system requirements outlined in the *Review of Laboratory Records* chapter of the CLS Manual, the following conditions apply:

A. The verification process is outlined in the chapters of the Trace Evidence Manual where such a process is required:
   1. Fracture Physical Match Comparison,  
   2. Human Hair Comparison,  
   3. Thin Layer Chromatography of Fibers, and  
   4. Comparison of Impression Evidence.

B. No other sub-disciplines require verification.

C. Verification is a separate process from technical review.

3.2 **Technical Review**

A. A technical review is a thorough examination of the test record and includes report(s), notes, worksheets, spectra, photographs, etc. which form the basis for a scientific conclusion.

B. In addition to the laboratory system requirements outlined in the *Technical Review* section of the CLS Manual, the following conditions apply:

   1. All cases undergo a technical review by an individual, other than the examiner, who has expertise gained through training and experience in the sub-discipline being reviewed and is authorized to perform technical review.

   2. The technical reviewer shall have sufficient knowledge of the sub-discipline to verify compliance with the relevant technical procedures and that the conclusions
stated in the laboratory report are supported with the examiner's documentation, using the correct terminology.

3. If the technical reviewer and the examiner do not concur with the discrepancies or recommended changes, the issue(s) will be resolved as outlined in the Review Resolution Process section of the CLS Manual.

4. Multiple reports within a case will undergo separate technical reviews.

5. Technical Review is documented in LIMS.

3.3 Administrative Review

A. An administrative review is a thorough examination of the case record for required laboratory report elements, logic and completeness, factual and consistent information, and correct spelling and grammar.

B. Administrative Review is documented in LIMS.
TE-01-06 GUIDELINES FOR COUNTING TRACE EXAMINATIONS

1 Scope

The count of Trace Evidence examinations is maintained in order to evaluate the work performed per case, compare workload trends, and identify areas where a shift of resources could result in casework being completed in a more efficient manner. The count of trace evidence examinations will include the following:

A. “Number of Items Screened” is the number of items processed to recover trace evidence.
B. “Screening Examinations” is the number of examinations performed to locate or identify the presence of trace evidence on an item. Screening exams (tape lifts, scraping, etc.) are a prelude to the analysis of specific items such as fiber, paint, hair, etc.
C. “Specific Tests” are the analysis tests performed in order to reach a conclusion.
D. “Positive Results” occur when a determination can be made which associates the questioned item to a known source that demonstrates probative value. Examples include a physical match placing a suspect vehicle at the scene, finding hair like the victim’s hair in the suspect’s vehicle, determining that a lamp filament was on when damaged, detecting gunshot primer residue on the suspect’s hand, etc.

2 Practices

2.1 Number of Items Screened

A. An item labeled, initialed, and processed for items of evidentiary value should be counted as one item.
B. If only tape lifts or debris containers are submitted, the number of items screened is equivalent to the number of items from which the trace evidence was recovered.
C. Do not count what is observed on or removed from the screened item.
D. Do not count containers.
E. Examples:
   1. A bloody shirt from which fibers and paint have been removed is counted as one item
   2. A bumper, a windshield, or a headlight is each counted as one item
   3. A vehicle search is counted as one item
   4. A Gunshot Residue kit that is not analyzed counts as one item
   5. Three strips of tape used to recover trace evidence from a shirt counts as one item

2.2 Number of Screening Examinations

A. The number of screening examinations includes the number of tests or examinations performed to locate or isolate the trace evidence for analysis.
B. Recovery techniques (scraping, taping, or vacuuming an item) are not counted as examinations.
C. Examples:
   1. Each tape lift, Petri dish or other container examined by stereoscope for relevant trace evidence is counted as an examination. For example, isolating six fibers from...
three of the four tape lifts of a shirt is counted as four screening examinations, one for each tape lift.

2. A thorough visual inspection is counted as one exam per item. It is necessary to document the relevant properties of the item such as color, texture, size, and stains in order to count the visual inspection as an exam.

3. Count presumptive tests if any are performed on a stain. Controls for presumptive tests are not counted.

4. Screening one item with an alternate light source is counted as one exam, regardless of the number of wavelengths used.

3 Number of Specific Tests Performed

This section applies to the number of analytical tests performed on the recovered trace evidence that provides the basis for a conclusion. In general, do not count quality control samples or blanks.

3.1 Filaments

The examination of filaments from a headlight or taillight is counted here. Count the number of tests actually performed on each filament. The tests that are commonly performed for filament examination includes: stereoscopic examination and continuity testing.

3.2 Fibers

Count the number of tests actually performed on each questioned fiber and on the known sample. Each known sample counts as one fiber. Count one test for each of the following: polarized light microscopic examination, comparison microscopy, cross-section, infrared analysis, thin layer chromatography, and microspectrometer analysis.

3.3 Impressions

Count the number of tests actually performed on each shoe print, tire track, shoe, photograph, cast and cloth impression. Count one test for each of the following: visual comparison, macroscopic comparison, overlay comparison, enhancement techniques (such as amido black, DAB, or luminol) and electrostatic lift.

3.4 Glass

Count the number of tests actually performed on each glass fragment compared. Count one test for each of the following: visual comparison, thickness measurement, microscopic examination, fluorescence, and refractive index determination.

3.5 Gun Shot Primer Residue

Count the number of kits examined. When stubs from inanimate objects are examined, the count number should be the number of stubs examined divided by two.

3.6 Hairs

A. Count the number of hairs (animal or human) compared macroscopically. A conservative estimation is adequate when the number of hairs is large.

1. A known sample of head or pubic hair used for a macroscopic comparison should be counted as one sample.

2. If a mass of questioned hair is compared macroscopically, count it as one sample.
3. If one hair from the mass is selected and compared macroscopically, count it as another sample.

B. If a microscopic examination is conducted to identify somatic or racial origin and no comparison is made, the examination count is the same as the number of individual hairs mounted.

C. If a microscopic examination is conducted to compare questioned hair to a known sample, the examination count is the same as the number of individual hairs mounted.
   1. Each known hair mounted and used counts as one sample.
   2. Count one microscopic test for each mounted hair regardless of the number of magnifications used.
   3. A scale cast counts as one examination.

3.7 Physical Match and Physical Comparison

Count the number of items used to determine a physical match and/or a physical comparison. For example, comparison of a left shoe to a right shoe to see if they could be mates would be counted as two tests (one for each shoe).

3.8 Paint

A. Count the number of tests actually performed on each paint chip. Count one test for each of the following: stereoscopic examination, microscopic comparison of layer sequence, solubility of each layer by chemical, infrared analysis of each layer and the pyrolysis of each sample.
   1. Multiple pyrolysis runs of one sample are counted individually.
   2. Do not count blanks or controls.

B. Database searches are counted as one test per layer of paint.

3.9 Other

A. Count the total number of analytical tests performed on each item examined but not counted elsewhere. For example: stains of a textile garment analyzed for foreign substances, such as petroleum jelly, are usually compared to an unstained portion of the garment. A test of the stained and unstained portion of the garment would each constitute a separate test.

B. Automobile, motorcycle and bicycle parts are counted here if an examination beyond documentation is done on those items. Tests performed on these parts include a physical match and identifying manufacturer logos, part numbers, etc.

4 Opinion Rendered

A. Checking the “Opinion Rendered” box provides a method of determining how many cases have resulted in a significant probative result. If the test results in a conclusion that is of probative value, the “Opinion Rendered” box is checked.

B. If the conclusion is indeterminate, inconclusive or no association of probative value can be drawn, the “Opinion Rendered” box should not be checked. For example, the “Opinion Rendered” box is checked when:
   1. A filament is determined to have been on,
   2. A physical comparison and/or match is made,
3. Samples from an individual are positive for primer residue,
4. Questioned paint is matched to the suspect paint, or
5. The suspect's shoe has the same class and/or unique characteristics as the questioned impression, etc.

5 Examples of Reporting Trace Evidence Examinations

5.1 Headlight
A dual filament headlight is examined.

A. The number of automobile parts screened is one.
B. The number of screening examinations is one for visual inspection
C. The number of tests is four – one for the continuity test of each filament and one for the stereoscopic examination of each filament
D. If a conclusion is made that a filament was on when damaged, check the “Positive Result” box.

5.2 Hair
A victim's shirt is taped to recover hair. The three pieces of tape used to lift the hair are examined with a stereoscope and four hairs are selected and removed from the tapes for a microscopic comparison. Ten hairs from the suspect's known hair samples are mounted for comparison. The four questioned hairs are determined to match the suspect's hair sample. A written verification of the microscopic match is obtained from a second examiner.

A. The number of items screened is counted as one for the shirt
B. The number of screening exams is four, one for the shirt and three for the tape lifts
C. The number of hair tests is fourteen - four for the questioned hair and ten for the known hair sample
D. The “Positive Result” box is checked

5.3 Hit and Run
A victim's pants and shirt are scraped and the debris is placed into two Petri dishes. The dishes are examined with a stereoscope and three paint chips are recovered. Each of the paint chips and the known paint from the suspect's car is examined with a stereoscope to determine color and layer sequence. Chloroform solubility and reaction of each layer with Diphenylamine reagent are noted on one questioned paint chip and on one known paint chip. Infrared analysis is performed on all three layers of both the questioned and known paint, as well as a QC sample to demonstrate the FTIR is operating properly. Pyrolysis of the questioned paint chip, the known paint chip and a QC sample is also performed.

A. The number of items screened is two, one each for the pants and shirt
B. The number of screening exams is four: one for examining the pants and one for examining the shirt, and one each for the examination of the debris in the two Petri dishes to retrieve the paint chips
C. The number of paint samples examined is four, three questioned paint chips and one known paint chip
D. The number of paint tests is twenty-six - four for the initial stereoscopic comparison of the colors of the four paint chips, two for comparison microscopy of the layer sequence, twelve for solubility testing, six for infrared analysis and two for pyrolysis.

E. The “Positive Result” box is checked if analysis results in an association.

5.4 Automobile Search

A hit and run suspect car is searched. Two tape lifts are taken from the fender. Both tape lifts are examined with a stereoscope and five fibers are removed and mounted on slides. The five questioned fibers and fibers from the victim’s pants are examined with a polarized light microscope and with a comparison microscope. One questioned fiber, one known fiber and a QC sample undergo infrared analysis. The color of one questioned fiber and the known fiber sample is compared using the microspectrometer. The questioned fiber was determined to be different than fibers of the victim’s pants.

A. The number of items screened is two, one for the automobile and one for pants

B. The number of screening examinations is four, one for the automobile search, two for the tape lifts examined stereoscopically and one for examination of the victim’s pants

C. The number of fibers examined is six (five questioned and one known sample)

D. The number of fiber tests is sixteen - six for polarized light microscopy comparison, six for comparison microscopy, two for infrared analysis and two for color comparison by microspectrometer

E. The “Positive Result” box is not checked
# TE-01-07 Standard Abbreviations List

## 1 Scope

This list of abbreviations commonly used by the TE discipline has been generated to assist in the interpretation of case record notes and is not a standardized list of required abbreviations. While as comprehensive as possible, the list may not be complete. Note that some abbreviations may be combined, such as KHHV to represent “known head hair of victim”.

## 2 Abbreviations

This is a listing of abbreviations commonly used by trace evidence examiners.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>∴</td>
<td>therefore</td>
</tr>
<tr>
<td>( N_{II} )</td>
<td>refractive index parallel to the long axis</td>
</tr>
<tr>
<td>( N_{\perp} )</td>
<td>refractive index perpendicular to the long axis</td>
</tr>
<tr>
<td>AA</td>
<td>atomic absorption</td>
</tr>
<tr>
<td>AH</td>
<td>animal hair</td>
</tr>
<tr>
<td>Amt</td>
<td>amount</td>
</tr>
<tr>
<td>AN</td>
<td>acrylonitrile</td>
</tr>
<tr>
<td>Ang</td>
<td>angular</td>
</tr>
<tr>
<td>App</td>
<td>apparent</td>
</tr>
<tr>
<td>Assn</td>
<td>association</td>
</tr>
<tr>
<td>Ass’y</td>
<td>assembly</td>
</tr>
<tr>
<td>BH</td>
<td>body hair</td>
</tr>
<tr>
<td>Biref</td>
<td>birefringence</td>
</tr>
<tr>
<td>Bld</td>
<td>blonde</td>
</tr>
<tr>
<td>Blk</td>
<td>black</td>
</tr>
<tr>
<td>Br or brn</td>
<td>brown</td>
</tr>
<tr>
<td>C or Cauc</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Calc</td>
<td>calcareous</td>
</tr>
<tr>
<td>Cg</td>
<td>coarse grained</td>
</tr>
<tr>
<td>Char(s)</td>
<td>characteristic(s)</td>
</tr>
<tr>
<td>Cls</td>
<td>colorless</td>
</tr>
<tr>
<td>Cont</td>
<td>continuous</td>
</tr>
<tr>
<td>Config</td>
<td>configuration</td>
</tr>
<tr>
<td>Cort fusi, cor fusi</td>
<td>Cortical fusi</td>
</tr>
<tr>
<td>Del or Delust</td>
<td>delusterant</td>
</tr>
<tr>
<td>Demark</td>
<td>demarcation line</td>
</tr>
<tr>
<td>Disc or discont</td>
<td>discontinuous</td>
</tr>
<tr>
<td>Dissim</td>
<td>dissimilar</td>
</tr>
<tr>
<td>Dist</td>
<td>distribution</td>
</tr>
<tr>
<td>Dk</td>
<td>dark</td>
</tr>
<tr>
<td>DPA</td>
<td>Diphenylamine</td>
</tr>
<tr>
<td>Evid</td>
<td>evidence</td>
</tr>
<tr>
<td>Ext or Extinct</td>
<td>extinction</td>
</tr>
<tr>
<td>Fg</td>
<td>fine grained</td>
</tr>
<tr>
<td>Foll</td>
<td>follicular</td>
</tr>
<tr>
<td>Frag</td>
<td>fragment or fragmented</td>
</tr>
<tr>
<td>Gran</td>
<td>granules, grains</td>
</tr>
<tr>
<td>Grn</td>
<td>green</td>
</tr>
<tr>
<td>GSR</td>
<td>Gunshot Residue</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>HC</td>
<td>head combing</td>
</tr>
<tr>
<td>HH</td>
<td>head hair</td>
</tr>
<tr>
<td>HHS</td>
<td>head hair standard</td>
</tr>
<tr>
<td>HO</td>
<td>Holmium Oxide</td>
</tr>
<tr>
<td>Hv</td>
<td>heavy</td>
</tr>
<tr>
<td>ISId</td>
<td>Instant Shooter Identification Kit</td>
</tr>
<tr>
<td>Lt</td>
<td>light</td>
</tr>
<tr>
<td>M or Mong</td>
<td>Mongoloid</td>
</tr>
<tr>
<td>M or Med</td>
<td>medium</td>
</tr>
<tr>
<td>MA</td>
<td>Methyl acrylate</td>
</tr>
<tr>
<td>Man</td>
<td>manila colored</td>
</tr>
<tr>
<td>N or Neg or Negr</td>
<td>Negroid</td>
</tr>
<tr>
<td>NA, na</td>
<td>not applicable</td>
</tr>
<tr>
<td>NWP</td>
<td>no work performed</td>
</tr>
<tr>
<td>NR</td>
<td>no reaction</td>
</tr>
<tr>
<td>Ob, OB, ov bodies</td>
<td>Ovoid bodies</td>
</tr>
<tr>
<td>P</td>
<td>Permount</td>
</tr>
<tr>
<td>Pass</td>
<td>passenger</td>
</tr>
<tr>
<td>PC</td>
<td>pubic combing</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>PET</td>
<td>polyethylene terephthalate</td>
</tr>
<tr>
<td>PH</td>
<td>pubic hair</td>
</tr>
<tr>
<td>PHS</td>
<td>pubic hair standard</td>
</tr>
<tr>
<td>Pig or pigm</td>
<td>pigment or pigmentation</td>
</tr>
<tr>
<td>Pl</td>
<td>plastic</td>
</tr>
<tr>
<td>Poss</td>
<td>possible</td>
</tr>
<tr>
<td>Precip</td>
<td>precipitate</td>
</tr>
<tr>
<td>Prelim</td>
<td>preliminary</td>
</tr>
<tr>
<td>Pres</td>
<td>present</td>
</tr>
<tr>
<td>Prox</td>
<td>proximal</td>
</tr>
<tr>
<td>Qtz</td>
<td>quartz</td>
</tr>
<tr>
<td>RAC, RACs</td>
<td>Randomly Acquired Characteristic(s)</td>
</tr>
<tr>
<td>Rec'd, Recv'd</td>
<td>received</td>
</tr>
<tr>
<td>RN</td>
<td>registration number</td>
</tr>
<tr>
<td>Rnd</td>
<td>round</td>
</tr>
<tr>
<td>RT</td>
<td>retention time</td>
</tr>
<tr>
<td>S</td>
<td>soluble or suspect</td>
</tr>
<tr>
<td>SAK or SA Kit</td>
<td>sexual assault kit</td>
</tr>
<tr>
<td>SE</td>
<td>sign of elongation</td>
</tr>
<tr>
<td>Sim</td>
<td>similar</td>
</tr>
<tr>
<td>Sl or Slt</td>
<td>slight</td>
</tr>
<tr>
<td>Trans, transp</td>
<td>transparent</td>
</tr>
<tr>
<td>USFC</td>
<td>unsuitable for comparison</td>
</tr>
<tr>
<td>USFI</td>
<td>unsuitable for identification</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet wavelength range (250-400 nm)</td>
</tr>
<tr>
<td>V</td>
<td>victim</td>
</tr>
<tr>
<td>VA</td>
<td>Vinyl acetate</td>
</tr>
<tr>
<td>VC</td>
<td>Vinyl chloride</td>
</tr>
<tr>
<td>VDC</td>
<td>Vinilidene chloride</td>
</tr>
<tr>
<td>Vfg</td>
<td>very fine grained</td>
</tr>
</tbody>
</table>
Vis = visible or visible wavelength range (400-800 nm)
Wh, wht = white
WPL = Wool Products Label number
Xln = crystalline
X-sec, X-section, XS = cross section
Yell = yellow

### 3 Fire Debris Abbreviations/Definitions

This is a listing of abbreviations commonly used by fire debris examiners within the Trace Evidence discipline.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>Activated Charcoal Strip (C-strip)</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>BEN, Ben, ben</td>
<td>Benzene</td>
</tr>
<tr>
<td>BIO</td>
<td>Biohazard material</td>
</tr>
<tr>
<td>Btl</td>
<td>Bottle</td>
</tr>
<tr>
<td>BPB</td>
<td>Brown paper bag</td>
</tr>
<tr>
<td>BS</td>
<td>Broken Seal</td>
</tr>
<tr>
<td>BWP</td>
<td>Burned Wood Present</td>
</tr>
<tr>
<td>C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>C-strip</td>
<td>activated charcoal strip (ACS)</td>
</tr>
<tr>
<td>CS2</td>
<td>Carbon Disulfide</td>
</tr>
<tr>
<td>DND</td>
<td>Do Not Destroy</td>
</tr>
<tr>
<td>EB</td>
<td>Ethylbenzene</td>
</tr>
<tr>
<td>EIC</td>
<td>Extracted Ion Chromatogram</td>
</tr>
<tr>
<td>etoh</td>
<td>Ethanol</td>
</tr>
<tr>
<td>F</td>
<td>Degrees Fahrenheit</td>
</tr>
<tr>
<td>FD</td>
<td>Fire debris</td>
</tr>
<tr>
<td>FSC</td>
<td>Full Scale Chromatogram</td>
</tr>
<tr>
<td>Gal</td>
<td>Gallon can</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatograph</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Gas Chromatograph/Mass Spectrometer</td>
</tr>
<tr>
<td>GC/FID</td>
<td>Gas Chromatograph/Flame Ionization Detector</td>
</tr>
<tr>
<td>HPD</td>
<td>Heavy Petroleum Distillate</td>
</tr>
<tr>
<td>Hrs</td>
<td>Hours</td>
</tr>
<tr>
<td>HS</td>
<td>Hinge Seal</td>
</tr>
<tr>
<td>i.d.</td>
<td>Identified (or id, ID)</td>
</tr>
<tr>
<td>Injs</td>
<td>Injections</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl Alcohol</td>
</tr>
<tr>
<td>IS</td>
<td>Improper Seal</td>
</tr>
<tr>
<td>Isopar</td>
<td>Isoparaffinic Product</td>
</tr>
<tr>
<td>LPD</td>
<td>Light Petroleum Distillate</td>
</tr>
<tr>
<td>MEK</td>
<td>Methyl-ethyl Ketone</td>
</tr>
<tr>
<td>MIBK</td>
<td>MIBK Methyl Isobutyl Ketone</td>
</tr>
<tr>
<td>MPD</td>
<td>Medium Petroleum Distillate</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectra or Mass Spectrometer</td>
</tr>
<tr>
<td>N</td>
<td>No or Not</td>
</tr>
<tr>
<td>N-P</td>
<td>(or naphth-para) Naphthenic-Paraffinic product</td>
</tr>
<tr>
<td>NA</td>
<td>Not Applicable (also, n/a or N/A can be used)</td>
</tr>
<tr>
<td>Neg</td>
<td>Negative</td>
</tr>
<tr>
<td>OHC</td>
<td>Other hydrocarbons detected</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>P</td>
<td>Pentane</td>
</tr>
<tr>
<td>PB</td>
<td>Paper bag</td>
</tr>
<tr>
<td>PB</td>
<td>Partially Burned</td>
</tr>
<tr>
<td>PBW</td>
<td>Partially Burned Wood</td>
</tr>
<tr>
<td>PLB</td>
<td>Plastic bag</td>
</tr>
<tr>
<td>Pos</td>
<td>positive</td>
</tr>
<tr>
<td>Pt</td>
<td>Pint can</td>
</tr>
<tr>
<td>Qt</td>
<td>Quart can</td>
</tr>
<tr>
<td>RMT</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>RT</td>
<td>Retention Time</td>
</tr>
<tr>
<td>SAM</td>
<td>Standard Accelerant Mix</td>
</tr>
<tr>
<td>sig</td>
<td>Significant</td>
</tr>
<tr>
<td>SIM</td>
<td>Standard Ignitable Mix</td>
</tr>
<tr>
<td>solv</td>
<td>Solvent</td>
</tr>
<tr>
<td>spec</td>
<td>Spectra</td>
</tr>
<tr>
<td>std</td>
<td>Standard</td>
</tr>
<tr>
<td>Sty</td>
<td>Styrene</td>
</tr>
<tr>
<td>Sub</td>
<td>Substituted</td>
</tr>
<tr>
<td>Subst</td>
<td>Substituted</td>
</tr>
<tr>
<td>TIC</td>
<td>Total Ion Chromatograph</td>
</tr>
<tr>
<td>Tol</td>
<td>Toluene</td>
</tr>
<tr>
<td>TP</td>
<td>Tape Sealed</td>
</tr>
<tr>
<td>UB</td>
<td>Unburned</td>
</tr>
<tr>
<td>UBW</td>
<td>Unburned Wood Present</td>
</tr>
<tr>
<td>UL</td>
<td>Unseated Lids</td>
</tr>
<tr>
<td>UPS</td>
<td>Uninterrupted Power Supply</td>
</tr>
<tr>
<td>Y</td>
<td>Yes, affirmative</td>
</tr>
<tr>
<td>/</td>
<td>(handwritten slash on worksheet) affirmative/confirmed</td>
</tr>
</tbody>
</table>

### 4 Abbreviations Used by Investigators on Fire Debris Laboratory Submission Forms

This is a listing of abbreviations commonly used by investigators when submitting evidence for fire debris analysis. This list is to be used only for interpretation of investigators’ notes. **The abbreviations listed below are not approved for use by fire debris examiners in their case file notes unless the meaning is defined in the record.**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABDN</td>
<td>Abandoned</td>
</tr>
<tr>
<td>AC</td>
<td>Air Conditioner</td>
</tr>
<tr>
<td>ADJ</td>
<td>Adjacent</td>
</tr>
<tr>
<td>APPROX</td>
<td>Approximately</td>
</tr>
<tr>
<td>APT</td>
<td>Apartment</td>
</tr>
<tr>
<td>BLDG</td>
<td>Building</td>
</tr>
<tr>
<td>Blvd</td>
<td>Boulevard</td>
</tr>
<tr>
<td>BPB</td>
<td>Back Passenger Floorboard</td>
</tr>
<tr>
<td>BR or BD</td>
<td>Bedroom</td>
</tr>
<tr>
<td>BS</td>
<td>Back Seat</td>
</tr>
<tr>
<td>BSMT</td>
<td>Basement</td>
</tr>
<tr>
<td>BTWN</td>
<td>Between</td>
</tr>
<tr>
<td>CAR</td>
<td>Carpet</td>
</tr>
<tr>
<td>CL</td>
<td>Closet</td>
</tr>
<tr>
<td>DEC</td>
<td>Decedent/deceased</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>DG</td>
<td>Detached Garage</td>
</tr>
<tr>
<td>DR</td>
<td>Driver/Drive (content)</td>
</tr>
<tr>
<td>Dr. or Dr</td>
<td>Drive</td>
</tr>
<tr>
<td>DW</td>
<td>Dishwasher</td>
</tr>
<tr>
<td>DWLG</td>
<td>Dwelling</td>
</tr>
<tr>
<td>E</td>
<td>East</td>
</tr>
<tr>
<td>ENG</td>
<td>Engine</td>
</tr>
<tr>
<td>ENT</td>
<td>Entry</td>
</tr>
<tr>
<td>EXT</td>
<td>Exterior</td>
</tr>
<tr>
<td>FDB</td>
<td>Front Driver Floorboard</td>
</tr>
<tr>
<td>FDR</td>
<td>Front door</td>
</tr>
<tr>
<td>FDS</td>
<td>Front Driver Seat</td>
</tr>
<tr>
<td>FLR</td>
<td>Floor</td>
</tr>
<tr>
<td>FPB</td>
<td>Front Passenger Floorboard</td>
</tr>
<tr>
<td>FPS</td>
<td>Front Passenger Seat</td>
</tr>
<tr>
<td>GAR or G</td>
<td>Garage</td>
</tr>
<tr>
<td>HB</td>
<td>Half Bath</td>
</tr>
<tr>
<td>HWY</td>
<td>Highway</td>
</tr>
<tr>
<td>INT</td>
<td>Interior</td>
</tr>
<tr>
<td>K or KIT</td>
<td>Kitchen</td>
</tr>
<tr>
<td>L/Lft</td>
<td>Left</td>
</tr>
<tr>
<td>LN or Ln</td>
<td>Lane</td>
</tr>
<tr>
<td>LNU FNU</td>
<td>Last Name Unknown, First Name Unknown</td>
</tr>
<tr>
<td>LR</td>
<td>Living Room</td>
</tr>
<tr>
<td>M BDR</td>
<td>Master Bedroom (or M BR)</td>
</tr>
<tr>
<td>ME</td>
<td>Medical Examiner</td>
</tr>
<tr>
<td>N</td>
<td>North</td>
</tr>
<tr>
<td>NE</td>
<td>Northeast</td>
</tr>
<tr>
<td>NW</td>
<td>Northwest</td>
</tr>
<tr>
<td>OFF/OFC</td>
<td>Office</td>
</tr>
<tr>
<td>PSG/PSGR</td>
<td>Passenger</td>
</tr>
<tr>
<td>R/Rt</td>
<td>Right</td>
</tr>
<tr>
<td>REFG</td>
<td>Refrigerator</td>
</tr>
<tr>
<td>RESD</td>
<td>Residential</td>
</tr>
<tr>
<td>S</td>
<td>South</td>
</tr>
<tr>
<td>SDR</td>
<td>Side door</td>
</tr>
<tr>
<td>SE</td>
<td>Southeast</td>
</tr>
<tr>
<td>St</td>
<td>Street (or St.)</td>
</tr>
<tr>
<td>Sus</td>
<td>Suspect</td>
</tr>
<tr>
<td>SW</td>
<td>Southwest</td>
</tr>
<tr>
<td>TRK</td>
<td>Truck</td>
</tr>
<tr>
<td>UNK</td>
<td>Unknown</td>
</tr>
<tr>
<td>UT or UTIL</td>
<td>Utility Room</td>
</tr>
<tr>
<td>VAC</td>
<td>Vacant</td>
</tr>
<tr>
<td>VEH</td>
<td>Vehicle</td>
</tr>
<tr>
<td>VIC/V</td>
<td>Victim</td>
</tr>
<tr>
<td>W</td>
<td>West</td>
</tr>
<tr>
<td>WRHSE</td>
<td>Warehouse</td>
</tr>
<tr>
<td>YD</td>
<td>Yard</td>
</tr>
</tbody>
</table>
1 Scope

The laboratory report is the summation of work in a case and shall clearly and accurately reflect the work done as well as any conclusions and/or opinions made by the analyst.

In the sub-disciplines of Trace Evidence, a statistical assessment of significance is not possible. The addition of value statements (categories of association) will help customers and the general public in better understanding reported conclusions.

Given the wide variety of analyses possible in trace evidence and the varying nature of the evidence examined, it is not feasible to create a complete list of pre-determined reporting statements. A recommended format is provided that will require certain information be present, but still allow the analyst to have flexibility in the arrangement or wording as the case demands.

2 Related Chapters

Appendix: Categories of Association

3 General Reporting

3.1 Requirements

A. Indicate what items were examined, results, opinions (if rendered), categories of association and disclaimers (when applicable).

B. Indicate in report all trace evidence items not examined.

C. Indicate disposition of evidentiary items including items forwarded to other sections and/or laboratories, or retained by the laboratory.

3.2 Reporting Statements

Below is the recommended format for reporting statements. While not every possibility is represented, what is listed below can be adapted to all disciplines and evidence types. Areas in [bold] are variable depending on the case.

A. Screening statements

1. [Trace or type of evidence] evidence was recovered from [item(s)]. If further analysis is needed, please contact this examiner [or request standards].

2. The following items were processed for [trace or type of evidence] evidence: [item(s)]. If further analysis is needed, please contact this examiner [or request standards].

3. Due to the circumstances of this case [or other reason], no analysis was performed on this evidence.

B. Comparison statements

1. [Evidence compared] from [item(s)] are similar in [characteristics] to [item(s)].

2. [Evidence compared] from [item(s)] are dissimilar in [characteristics] to [item(s)].
C. Determination/identification statements

1. **[Number]** characteristic and **[number]** indicative gunshot primer residue particles were confirmed on the GSR kit from **[item(s)]**.

2. **[item]** is a **[condition][dual/single]** filament bulb. The **[type of lamp/bulb/filament]** filament is **[list characteristics observed/tested]**, and exhibits characteristics consistent with being **[on/off]** at the time of damage.

3. The **[characteristics]** data from the **[paint chip description]** was used to search the Paint Data Query (PDQ) database. The data from this recovered paint chip is most similar to a **[year, make/model]**. A search for a suspect vehicle should include but not be limited to these vehicles.

4. The **[item description]** is consistent with **[identification]**. **[Identification]** is commonly found in **[products that contain {identification}]**.

5. The **[shoe/tire]** impression from **[item]** is consistent in tread design to a **[make/model] [shoe/tire]**. A search should begin with, but not be limited to, **[make/model] [shoes/tires]**.

6. A physical match was made between **[item]** and **[item]**.

7. The hair recovered from **[item]** exhibits **[Caucasoid/Negroid/Mongoloid]** racial characteristics.

4 Pre-determined Statements and Wording

4.1 Requirements

A. “Similar” and “dissimilar/different” shall be used in comparison statements where applicable. This does not apply to determination/identification statements.

B. When a comparison results in an opinion, include the analyst’s opinion statement. The format is:

   It is my/our opinion that the **[evidence]** from **[item(s)]** could/could not have originated from **[item(s)]**.

   Please note this format does not apply to inconclusive statements. The format for those types of statements is at the analyst’s discretion.

C. The use of disclaimers is established in the SOPs. Please refer to specific SOPs for wording and directions when required.

D. The Categories of Association appendix will be sent with the final report to provide further explanation to the recipient.

4.2 Categories of Association Scale

Categories of association shall be used when an opinion is reported regarding a comparison between a known and questioned sample. Not all types of analysis will require a category of association. Please note that the statements included are for guidance only.

A. Category 1 (Source Identity/Source Attribution)

   Examples could include physical fracture matches, unique accidental associations, or other types of analysis where a source is confirmed or identified.

   “The compared samples exhibit characteristics demonstrating that the items were once part of the same object. Alternatively, the sample is positively identified.”
B. Category 2A (Associations with distinct characteristics)

Examples could include after market repaints, custom made products with limited distribution, certain manufacturing defects for tire and shoe molds, and other evidence of that nature.

“These items share distinct characteristic(s) that would not be expected to be encountered in the general population.”

C. Category 2B (Association with conventional characteristics)

Examples could include OEM paints, most fibers, shoes, and other mass produced items.

“These items share characteristics that have been manufactured or occur in nature and would be indistinguishable from the submitted evidence. Other items which share these properties may be available in the general population.”

D. Category 2C (Association with limited characteristics/examinations)

Examples could include nearly featureless hairs or fibers, paint samples with a partial layer sequence, partial impressions with little detail and other evidence of that nature or evidence types that are prevalent in society (ex blue denim).

“These items share characteristics that are common amongst these kinds of natural or manufactured materials and would be indistinguishable from the submitted evidence. Limitations to this association could include variations due to condition of the evidence or limited characteristics present.”

E. Category 3 (Inconclusive)

“The observed, measured and/or chemical properties do not provide enough information to associate or eliminate the compared samples.”

F. Category 4 (Dissimilar but not complete exclusion)

Examples could include cases where the source for comparison may not be representative.

“Items are dissimilar in observed, measured, and/or chemical properties indicating they did not originate from the same source. However, the compared items share enough general characteristics that, due to reasonable variations in the source, the source itself cannot be associated or eliminated based on the specimen received.”

G. Category 5 (Elimination/Exclusion)

“These items are dissimilar in observed, measured, and/or chemical properties demonstrating they did not originate from the same source.”

4.3 Example of a Reporting Statement

The red paint chips recovered from the victim’s shirt are similar in color, layer sequence, paint type and composition to the known red paint from the suspect’s vehicle. It is my/our opinion that the paint from the victim’s shirt could have originated from the suspect’s vehicle. (Category 2B)
02 EXAMINATION AND RECOVERY TECHNIQUES

TE-02-01 PHYSICAL EVIDENCE EXAMINATION

1 Scope

The primary purpose of these procedures is to establish unifying documentation and collection procedures that will be utilized by the laboratory.

The initial examiner of an item is primarily responsible for the collection and preservation of evidentiary materials that may be on that item.

Persistence and transfer studies demonstrates that debris on clothing surfaces can be lost if garments are handled, shaken, etc. Items being submitted for trace evidence examination shall be handled as little as possible to minimize loss of the trace evidence and to limit exposure of the items to contaminants until the trace evidence has been collected and preserved.

The procedures presented are intended to assist the examiner in the inspection of physical evidence. They are to be used in conjunction with all applicable laboratory policies, good laboratory practice, and proper scientific methodology. The examiner will be given flexibility to determine an appropriate course of action in regard to the collection, preservation, documentation, and analysis of evidence to attain the ultimate goal of quality and efficiency.

2 Related Documents

CLS Manual:
- Receipt and Review of Laboratory Requests for Service
- Submission and Receipt of Evidence
- Evidence and Database Sample Integrity
- Evidence Processing
- Return of Evidence
- Externally Provided Products and Services

3 Safety

A. All firearms should be treated as if loaded. All firearms shall be rendered safe prior to handling, marking, and/or packaging. The position of the safety should be noted. Rendering a firearm safe does not necessarily mean that it must be unloaded. It means that it shall be placed in such a condition that it cannot be fired if it is dropped or the trigger accidentally pulled.

B. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

1. Wear a lab coat and disposable gloves to protect your own clothing from contaminating the evidence with trace materials and vice versa.

2. When handling possible biohazard materials, wear a lab coat, disposable gloves, approved eye protective devices, and a mask.
4 Equipment and Materials
Varies with the type of technique used to collect the trace evidence
- Envelopes, tape, examination paper
- Etching pencil, scribing tool, or marker
- Tweezers, scalpel, scissors, probe and other implements as necessary
- Plastic bags, paper envelopes, or appropriate evidence containers
- Stereoscope
- Camera

5 Standards, Controls, and Calibration
None

6 Procedure
A. All items considered evidence by the laboratory will be handled according to the Crime Laboratory Service (CLS) Manual.

B. Where possible, this procedure shall be applied to questioned items of evidence before being applied to items of evidence submitted as a known.
   1. Retrieve evidence from evidence storage, evidence custodian, or another examiner.
   2. Verify that the “Laboratory Submission Form” is properly completed and a chain of custody is maintained.
      a) Administrative changes or additions to the form shall be initialed.
      b) Identify the pertinent forensic question(s).
      c) Plan the approach to the case.
      d) Evaluate the potential value of trace evidence relative to the items of evidence submitted for examination.
   3. Describe the individual items of evidence (a sketch and/or photograph may also be included). Note whether other items were packaged together with the selected item. Visually examine the evidence and record as available:
      a) Condition of the evidence and/or possible contamination or preservation issues
      b) Physical description such as color, morphology, texture, size, holes and tears, broken parts, missing parts, or other modifications that make the item appear unusual.
      c) Measurements can be determined with calipers or micrometers (+/- 0.01 millimeters).
      d) Manufacturer’s identification, serial numbers, or other marks
      e) Method used to collect trace evidence
      f) Nature, location and collection of relevant stain(s)
      g) Presence of fractured, torn or cut portion(s) of an item
      h) Presence of patterned marks or impressions on an item
4. As needed for comparison, record, collect, and uniquely label known substrate sample(s) to represent all variations of color, weave, fabric layers, wear, and/or treatment.

5. Collected samples shall be protected from loss or contamination and may be sorted and preserved for future or immediate analysis.

6. Any collected trace evidence may be packaged separately or with the original item, as long as it is uniquely labeled, sealed, initialed and dated. All original exhibits will be re-packaged in the original container, if possible. The evidence is re-sealed in a manner that would detect tampering.

7. Transfer evidence to the evidence storage area, evidence custodian or appropriate examiner.

8. The following are supplemental documentation forms that are available for use, but not required:
   a) *Paint Examination (LAB-TE-03)*
   b) *Glass Examination (LAB-TE-04)*
   c) *Filament Examination (LAB-TE-05)*
   d) *Hair Examination (LAB-TE-06)*
   e) *Fiber Examination (LAB-TE-07)*
   f) *Trace Evidence Instrument Worksheet (LAB-TE-09)*
      i. if the Instrument Worksheet is not used or the operating parameters are not included on the Instrument Worksheet, the operating parameters shall be included elsewhere in the case record.

7 Interpretation

Evaluation of the case synopsis, scene, and evidence will be conducted on a case-by-case basis with the determination of the appropriate method of recovery and documentation made by the forensic examiner at the time of observation.

8 Precautions

A. If environmental conditions are observed which would jeopardize the quality of the testing, the testing will be immediately halted. The Supervisor or Quality Manager shall be immediately notified of the situation and the initiation of a quality incident report may be considered.

B. Contamination
   1. Clean work areas, paper, and equipment before and between the examination of each evidence item.
   2. The screening of evidentiary items for questioned trace evidence shall occur first and that evidence will be properly collected and retained to prevent loss or contamination.
   3. At all times during the examination, items from different sources will be kept separate from other items. For example, items from suspect(s) and items from associated victim(s) are examined in different locations and/or times.
   4. Always keep known materials (glass, paint, etc.) separated from the materials to be searched.
5. Personal protective apparel shall be changed as necessary to ensure safety and to avoid contamination or transfer between evidentiary items.

6. No articles of evidence shall be stored in an unpackaged state, when possible.

7. No more than one article of clothing in an unpackaged state is allowed on an examination table at one time unless the items were submitted in the same package.

8. Note instances where packaging or handling of the evidence creates a potential for contamination. These instances should be brought to the attention of the supervisor, other involved examiners, and the investigator. Such instances may preclude the examination of the evidence.

9. Evidence will be examined in dedicated examination areas. The analyst performing the examinations shall be cognizant of environmental conditions that could affect the quality of the tests being performed. Such environmental conditions may include dust and particulate matter in the air or on surfaces, and other examinations taking place in the area.

10. Access to the examination area should be controlled to limit the possibility for contamination and loss. Access to these areas should be limited by locating the examination areas away from the normal traffic flow or by closing off the examination areas.

11. If necessary, examinations can be coordinated with other laboratory sections in order to preserve evidence and protect it from contamination.

12. Should an examination area become contaminated or need to be cleaned prior to performing examinations, the following may be used to remove residual DNA and/or decontaminate surfaces: 10% bleach solution, a commercial surface cleaner, and/or manual dusting/sweeping tools.

C. Insect activity

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

2. If the insects are still living, examine the object as quickly as possible, take samples of evidentiary material and re-seal the object.

3. If necessary, the evidence may be placed in the freezer to kill the insects to prevent possible degradation of the evidence.

D. Wet or Damp Evidence

1. If the analyst discovers an item is received in a wet condition, the problem shall be corrected before any further damage may occur.
   a) The container should be opened and the object allowed to air dry. This may entail spreading the object out on a flat surface and exposing it to the air for a period of time.
   b) The wet items should not be heated, nor exposed to direct sunlight, nor should a fan be used in an attempt to accelerate drying. Care should be exercised not to lose evidential materials when attempting to dry the object.
   c) The submitting officer should be advised to ensure that all objects are dried before submission.
2. If the analyst subsequently discovers that interim storage of undried objects has occurred, steps shall be taken to minimize the damage caused by the dampness.

   a) The object shall be air dried as soon as possible. Delayed air-drying causes a problem if the object has become foul smelling due to decomposition.

   b) Such items shall be placed in the hood with the exhaust fan running to remove odors and moisture. Caution should be exercised to prevent the loss of evidential materials that might result from the movement of air currents inside the hood.

   c) Communication with the submitting agency in order to explain the problem of objects being submitted wet and to prevent a recurrence is appropriate.
1 **Scope**

Trace Evidence employs several techniques for the recovery of evidence. These techniques include picking, taping, scraping, vacuuming, and combing. The specific recovery technique employed in an examination depends on the type of evidence and/or the case circumstances.

2 **Safety**

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Follow biohazard precautions, if applicable

3 **Equipment and Materials**

- Varies with the technique used to recover the trace evidence
- Stereomicroscope
- Forceps, probe, scalpel and other implements as needed
- Adhesive tape
- Plastic sheeting
- Spatula, long blade knife, or similar implement
- Vacuum fitted with filter trap
- Filters
- Comb
- Cotton balls

4 **Standards, Controls, and Calibration**

None

5 **Procedure**

5.1 **Picking**

A. Picking involves the use of forceps or other implements to pick trace evidence, such as hairs and fibers, from an item. Picking may supplement other collection techniques.

B. Picking is particularly useful for small items and offers the advantages of:

1. Recovery of all the trace evidence on the item;
2. The location of the trace evidence on the item is known; and
3. A limited chance for contamination.

C. Steps

1. Prepare the working surface to prevent contamination and loss of trace evidence.
2. Examine the item using normal light assisted by other forms of illumination, as necessary. Use of the stereomicroscope may enhance the detection of some forms of trace evidence. Document the presence of stains, damage, size, color and other pertinent information.
3. If possible trace evidence is observed, its location is documented and it is separated from the item by using clean forceps or other implement.
4. The recovered evidence is immediately packaged or stored to prevent its loss or contamination.

5. The evidence containers are marked with case number, item number, examiner initials and date.

5.2 Taping

A. Taping or “lifting” involves the use of adhesive tape to remove trace evidence, such as hairs and fibers, from an item.

B. Taping offers the advantages of:
   1. Being relatively quick and efficient;
   2. The potential for contamination and loss is limited; and
   3. The secured trace evidence can be easily viewed.

C. Steps
   1. Prepare the working surface to prevent contamination and loss of trace evidence.
   2. Examine the item using normal light assisted by other forms of illumination, as necessary. Document the presence of stains, damage, size, color and other pertinent information.
   3. A length of adhesive tape is pressed or rolled onto the surface of the item, causing loosely adhering trace evidence to stick to the tape.
   4. When the tape loses its ability to remove trace evidence or becomes loaded with recovered trace evidence, use additional pieces of tape to complete processing the item.
   5. The tape strips are placed onto clear plastic sheeting and are marked with case number, item number, examiner initials and date.
   6. The plastic sheeting are placed in appropriately sized envelopes and stored pending examination.

5.3 Scraping

A. Scraping involves the use of a spatula or similar implement to dislodge trace evidence, such as paint and glass, from an item.

B. Scraping offers the advantages of:
   1. Being relatively quick, and
   2. Easy handling of the collected debris.

C. Steps
   1. Prepare the working surface to prevent contamination and loss of trace evidence.
   2. Examine the item on an appropriate sized sheet of clean paper, using normal light assisted by other forms of illumination, as necessary. Document the presence of stains, damage, size, color and other pertinent information.
   3. The item may be suspended from a rack or clothesline, or manually held above the clean paper.
   4. A clean spatula or similar tool is vigorously brushed against the item, causing loosely adhering trace evidence to dislodge and fall onto the clean paper.
5. The debris on the paper is poured into a container, such as a petri dish.
6. The containers are marked with case number, item number, examiner initials and date.

5.4 **Vacuuming**

A. Vacuuming involves the use of a vacuum fitted with a filter to trap trace evidence vacuumed from an item.

B. Vacuuming offers the advantages of:
   1. Being relatively efficient, and
   2. Being the method of choice in certain situations where large areas must be screened for trace evidence.

C. Steps
   1. Prepare the working surface to prevent contamination and loss of trace evidence.
   2. Examine the item on a sheet of clean paper, if possible, using normal light assisted by other forms of illumination, as necessary. Document the presence of stains, damage, size, color and other pertinent information.
   3. The trap of the vacuum is thoroughly cleaned and a new filter is inserted.
   4. The item or area of interest is vacuumed to collect trace evidence.
   5. As the filter becomes loaded with debris or upon completion of the vacuuming, the filter is changed and immediately packaged to prevent loss or contamination.
   6. The filter containers are marked with case number, item number, examiner initials and date.

5.5 **Combing**

A. Combing involves the use of a comb to trap and collect trace evidence, such as hairs, fibers, and glass, from the hair of an individual.

B. Combing offers the advantages of:
   1. Being relatively efficient, and
   2. Being the method of choice to recover trace evidence from the hair of an individual.

C. Steps
   1. Prepare the working surface to prevent contamination and loss of trace evidence.
   2. Place clean paper on the surface.
   3. Prepare the comb by placing cotton in the teeth of a new, unused comb to enhance the ability of the comb to retain trace evidence.
   4. While an individual is leaning over the clean paper, the prepared comb is used to comb the hair thoroughly.
   5. The comb with the collected debris is placed in the center of the paper.
   6. The paper with the comb is folded and sealed to prevent loss or contamination.
   7. The paper is marked with case number, item number, examiner initials and date.
6 Literature References and Supporting Documentation


TE-02-03 PHYSICAL COMPARISON

1 Scope

An examiner may be requested to conduct a physical comparison of two or more items. This examination involves the comparison of the overall characteristics of the items submitted, including such features as color, style, texture, and dimension.

Examples of this type of examination may include a questioned button submitted for comparison to the buttons remaining on a shirt to determine if the questioned button could have originated from the shirt as represented by the known buttons or a left shoe recovered from a crime scene submitted for comparison to a right shoe recovered from the suspect's house to determine if the shoes could be mates.

2 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Appropriate personal protection against sharp edges, flying debris and biohazards

3 Equipment and Materials

- Calipers, micrometers and other measuring devices
- Stereomicroscope

4 Standards, Controls, and Calibration

None

5 Procedure

1. Perform a separate visual examination (macroscopic/microscopic) of each item. If any trace materials are observed, the item should be processed for the trace material prior to any physical comparison of that item.

2. Document the overall characteristics of the items such as color, style, texture, curvature, and dimensions. Documentation shall be in the form of written notes and diagrams, photocopies and/or photographs.

3. If the physical comparison involves more than visual characteristics, the material shall be examined by personnel authorized to perform work in the appropriate sub-discipline.

6 Interpretation

A. If the overall characteristics exhibited by the items compared are similar, then the conclusion is that the items could belong to a set, be mates, or otherwise could be associated.

B. If the overall characteristics exhibited by the items compared are different, then, depending upon the type of items being compared:

1. The items cannot be associated, or

2. A conclusion cannot be made that the items could otherwise be associated.
7 Literature References and Supporting Documentation


TE-02-04  FRACTURE PHYSICAL MATCH COMPARISON

1 Scope

Physical match comparisons, also known as jigsaw fit comparisons, are performed in an attempt to uniquely associate a piece of material with an item from which it is thought to have originated. This comparison is applicable to situations in which an item has been broken, torn, cut, or otherwise separated into two or more pieces. If the separation involves random processes, the particular separation would not be expected to occur again in exactly the same way. It is therefore possible that the edges of the separate pieces could be fit together in a unique fashion, much like the pieces of a jigsaw puzzle, to demonstrate that the separate pieces were, at one time, joined together.

2 Related Chapters

Case Reviews

3 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Appropriate personal protection against sharp edges, flying debris and biohazards

4 Equipment and Materials

• Calipers, micrometers and other measuring devices

• Stereomicroscope

5 Standards, Controls, and Calibration

None

6 Procedure

1. Perform a separate visual examination (macroscopic/microscopic) of the items. If any trace material is observed the item should be processed for the trace material prior to any physical comparison of that item.

2. Document the overall characteristics of the items such as color, style, texture, curvature, and dimensions. Documentation shall be in the form of written notes, diagrams, photocopies, and/or photographs.

3. Examine the items for possible common fracture edges.

4. Once the examiner has found similar shaped edges, the pieces are tested by carefully holding them close together. If a physical match is found, the pieces will “mesh” or “jigsaw” together.

5. Once the examiner has fit two items together, an examination for scratches, stains, or defects that traverse the broken, cut, or torn edge serves to reinforce the physical match conclusion. Oblique lighting is helpful in this step.

6. Reported physical matches shall be verified by a second authorized examiner.

   a) Document any reported physical match via photograph/photocopy. Verifications can be performed using the photograph/photocopy as long as the documentation is an accurate representation and depiction of the physical match.
b) If unable to photograph/photocopy, then the physical match shall be verified in person by a second authorized examiner. Where possible, documentation photographs will be taken of all non-reported physical matches. Verifications shall be documented in the case record.

7 Interpretation

A. If a physical match is determined, then the items have a common origin and at one time were connected.

B. If a physical match is not determined, but the items have similar overall characteristics, it is possible that:
   1. They did not originate from a common source,
   2. They originated from a common source, but connecting pieces of material are missing, or
   3. They originated from a common source, but lack sufficient characteristics for a conclusive physical match.

C. In order for the examiner to make any determinations regarding the possibility of a common origin in these circumstances, the examiner shall rely on other types of examinations specifically designed for the type of material to be compared.

8 Literature References and Supporting Documentation


TE-02-05  SAMPLING CLOTHING AND OTHER INANIMATE SURFACES FOR GUNSHOT PRIMER RESIDUE (GSR) ANALYSIS

1  Scope

In some circumstances, it may be useful to analyze clothing or other objects for the presence of gunshot primer residue. GSR samples should be collected before tape lifts for hairs and fibers are collected, and before the item is processed for friction ridge and DNA evidence.

2  Related Chapters

Physical Evidence Examination

3  Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4  Equipment and Materials

Scanning Electron Microscopy (SEM) stubs covered with carbon adhesive tabs.

5  Standards, Controls, and Calibration

Negative control SEM stub

6  Procedure

A. Clothing and other items small enough to process inside the laboratory
   1. Screening area shall be suitably isolated from areas used to process ballistics/firearms/GSR distance determination evidence.
   2. Prepare the work surface. Brown recycled paper should not be used. Non-recycled white paper is recommended.
   3. Sample the paper with a new SEM stub as a negative control.
      a) One SEM stub should be sufficient for this; sample multiple locations on the paper.
      b) In some instances, it may not be apparent upon submission that an item is requested for GSR analysis. If an item is opened for examination prior to collection of a paper blank, notation should be made in the case record.
   4. Sample the item by dabbing SEM stubs on the item.
      a) Typically only areas that would be associated with direct GSR deposition from a weapon discharge, such as sleeves and clothing front, and areas associated with carrying a weapon, such as pockets, and waistband, should be sampled.
      b) Use as many SEM stubs as appropriate to collect samples from each individual area.
   5. Place the SEM stub in its plastic vial, and label the vial with the case number, item number, area sampled, examiner initials, and date as appropriate.
   6. Seal the vials in an appropriate container pending GSR analysis by SEM.
B. Vehicles and other objects too large to process inside the laboratory

1. When possible, process the object in a location not known to present a contamination issue (contamination may be an issue near a firing range).

2. Using as many stubs as appropriate, sample the object by dabbing SEM stubs on each surface of interest.

3. Place the SEM stub in its plastic vial and label the vial with the case number, item number, area sampled, examiner initials, and date as appropriate.

4. Seal the vials in an appropriate container pending GSR Analysis by SEM.

7 Limitations

GSR analysis by SEM is not used to create a particle map for distance determination. Therefore, multiple dabs are taken with each individual stub and it should not be attempted to cover every square unit of a surface by dabbing each stub only one time.

8 Literature References and Supporting Documentation


**TE-02-06 DETERMINATION OF CUTS/TEARS**

1 **Scope**

Items are occasionally submitted to the laboratory for examination to determine if the item has been damaged as a result of a tear or cut.

2 **Safety**

   A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
   
   B. Biohazard precautions, if applicable

3 **Equipment and Materials**

   • Stereomicroscope
   
   • Scalpel, knife or other cutting instrument

4 **Standards, Controls, and Calibration**

   None

5 **Procedure**

   A. Examine the damaged edge of the item under the stereomicroscope.
   
   B. Photograph and/or document the observed characteristics of the edges.
   
   C. Whenever possible, make a “test cut” and a “test tear” on an area of the item having the same characteristics as the damaged area. A suspected weapon may be used to make the “test cut” only after it has been completely analyzed for other evidence.
   
   D. Compare the characteristics of the “test cut” and the “test tear” to the damaged item.

6 **Interpretation**

   A. A determination that the damage is characteristic of a cut is made when the edges are uniform in their available characteristics.
   
   B. A determination that the damage is characteristic of a tear is made when the edges display a large amount of variation in their available characteristics.
   
   C. Some items may show characteristics of both a cut and a tear along the length of the damage.
   
   D. Damage with few clear characteristics may be difficult to interpret.

7 **Literature References and Supporting Documentation**


03 PAINT

TE-03-01  PAINT AND POLYMER INITIAL EXAMINATION AND OVERVIEW

1 Scope
Forensic paint and polymer (plastic, rubber, etc.) examination relates to the recognition, identification, and comparison of a questioned sample to its suspected source. Paint and polymer evidence requires basic observations prior to instrumental analyses.

Limited sample size and sample preservation requirements dictate that the comparative tests shall be selected and applied in a reasonable sequence in order to maximize the discriminating power of the test results, without undue consumption of sample. Sample size, condition of evidence, type of sample (e.g. smears and transfer patterns), multi-layered or mixed samples are factors that may hinder comparisons.

2 Related Chapters
Fracture Physical Match Comparison
Paint Chemical Reactivity Tests
Fourier Transform Infrared Microspectroscopy
Pyrolysis Gas Chromatography Mass Spectrometry

3 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. The preparation or collection of known reference samples and/or questioned samples may require scraping, prying, or spraying that provides the opportunity for injury or exposure. Therefore, appropriate safety equipment should be used to prevent injury.
C. Safety equipment includes the appropriate use of safety glasses, gloves, dust masks and clothing as necessary. In some cases, use of a fume hood or good ventilation is appropriate.
D. Biohazard precautions, if applicable

4 Equipment and Materials
- Stereomicroscope
- Comparison microscope
- Scalpel
- Forceps
- Probe
- Spot plate
- Munsell Color Coordinate System books (Matte and Glossy Collection)

5 Standards, Controls, and Calibration
None
6 Procedure

1. Examine known reference samples and questioned evidence separately.

2. Isolate, sort, and/or separate paint and polymer evidence from debris or substrate.

3. Macroscopically and/or microscopically examine representative layers, if present, and record descriptive information:
   a) Approximate number of questioned particles recovered
   b) Physical characteristics (such as soft, hard, brittle, pliable, etc.)
   c) Surface or texture features (such as dull, glossy, rough, pitted, etc.)
   d) Number of layers
   e) Relative color, sequence and thickness of layers. Color may be determined with the Munsell books.
   f) If the sample is of sufficient size and layer structure, prepare cross-sections in order to detect all layers. The number, sequence and appearance of layers are observed and documented.

4. If necessary, prepare a known reference standard from liquid or spray paint:
   a) Thoroughly mix the liquid or spray.
   b) Apply a coat to a clean microscope slide.
   c) Allow it to dry completely.

5. Compare the known and questioned samples macroscopically and/or microscopically. If meaningful differences are observed, it is not necessary to continue with analysis.

6. Perform Fracture Physical Match Comparison. If a fracture physical match is determined between known and questioned samples, it is not necessary to continue with paint analysis.

7. Paint Chemical Reactivity Tests may be performed if there is adequate sample for destructive testing.


9. Document if the sample is inadequate for destructive testing. If there is adequate sample, perform Pyrolysis-Gas Chromatography Mass Spectrometry.

7 Interpretation

A. Automotive paint is typically found as smears or multi-layer fragments, and can usually be recognized by a characteristic sequence of finish coats and primers.

B. Architectural paint fragments may be recognized by their particulate texture and/or rough surface, and may have wood or masonry material as a substrate.

C. Rubber samples can usually be classified by observing the elastomeric characteristics of the sample.

D. If meaningful differences are observed in the color, layer sequence, or polymer type between the questioned and known samples, then the examiner would conclude the questioned sample is not consistent with the known sample and, therefore, did not originate from the area represented by the known sample.
8 Literature References and Supporting Documentation


TE-03-02 PAINT CHEMICAL REACTIVITY TESTS

1 Scope

Paint solubility and chemical reactivity tests allow the examiner to broadly classify some binder types. The most common solvent and chemical reactivity tests are:

A. Chloroform or acetone solubility is used to distinguish between lacquer paint and enamel paint.

B. Xylene solubility distinguishes between dispersion lacquer and solution lacquer paints.

C. The diphenylamine reagent test is used to detect nitrocellulose in paints.

2 Related Chapters

Diphenylamine Test

3 Safety

A. The diphenylamine reagent consists of strong acids.

B. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials

- Chloroform
- Acetone
- Xylene
- Diphenylamine reagent
- Stereomicroscope
- Compound microscope
- Spot plate
- Glass slides
- Coverslips

5 Standards, Controls, and Calibration

A. A paint film known to contain nitrocellulose (most fingernail polishes) or an inorganic nitrate will be used to verify the diphenylamine reagent is working properly. The expected reaction is the development of an intense dark blue color within 5 seconds of applying the diphenylamine reagent.

B. Sodium Chloride or a non-nitrocellulose paint will be used as a negative control.

C. If the reagent performs as expected, document the results in the case record.

6 Procedure

1. Place a small portion of each specimen into a separate well of a white porcelain spot plate (a black spot plate may be used for light color paints) or on a glass slide under a coverslip. It may be necessary to separate multi-layered specimens in order to differentiate reactions on each layer.

2. Apply 1-2 drops of the appropriate solvent or reagent to the specimen and observe any reaction.

3. Document the results of reactivity of the solvents or reagents on each layer of paint.
7 Interpretation

A. Lacquer paint is soluble in chloroform and acetone.
B. Enamel paint is insoluble in chloroform and acetone.
C. Dispersion lacquer paint is soluble in chloroform, acetone, and xylene.
D. Solution lacquer paint is soluble in chloroform and acetone, and is insoluble in xylene.
E. A nitrocellulose based or modified paint will produce an intense dark blue color within 5 seconds of application of the diphenylamine reagent.
F. The following terms may be used to describe the effect of the reagent with the paint:
   1. Soluble- determined to be soluble if the paint completely dissolves within 5 minutes.
   2. Pigment-Leach- determined to have undergone pigment leach if the paint remained substantially intact after 5 minutes, but with pigment extracted by the reagent.
   3. Swell- determined to swell if there is a significant and uniform expansion of the paint with no noticeable solubility or pigment leach.
   4. Curl- determined to curl if there was twisting or bending of particle.
   5. Discolor- determined if there is any noticeable change in color.
   6. Soften- determined to soften if, after exposure to the solvent, the paint chip can be penetrated with a needle but was otherwise not visibly affected.
   7. Breaks Apart- determined to break apart when sample is not soluble but breaks into tiny particles.
   8. No or negative reaction- no noticeable change to the specimen.

8 Literature References and Supporting Documentation


1 Scope

It is usually possible to differentiate motor vehicle repaint from the original equipment manufacturer (OEM) paint. For OEM paint, the color and chemistry of the topcoat and undercoat layers will be useful in identifying manufacturer, model, and year through a search of an automotive paint database. In most cases, a range of possible automotive make, model, and year will be generated by the search.

A Paint Data Query (PDQ) database search may allow determination of a possible manufacturer, plant location, and year range for the questioned paint sample. By narrowing down this information, a possible model can then be determined, even if that particular sample has not been previously entered into PDQ.

The examiner can use the PDQ database not only to suggest possible vehicles as the source of paint collected from a victim or scene, but also to assess an automotive population or how common the chemistry of an evidence paint chip may be.

This procedure uses the Paint Data Query database maintained by the Royal Canadian Mounted Police and software for infrared spectral database search.

2 Safety

None

3 Equipment and Materials

- Munsell Color Coordinate System books
- PDQ Program and Spectral Libraries
- Spectral search software such as Bio-Rad SearchMaster or Know-it-All
- Refinish Color Collections (e.g., DuPont, PPG, BASF)

4 Standards, Controls, and Calibration

None

5 Procedure

A. The information contained in a questioned paint search query may include: location of the specific layer within the paint chip, primer Munsell color values (color/hue, value, and chroma), and binder and extender pigments of each layer.

1. The layer sequence describes the type of paint (O = original, R = repaint), whether it is an Undercoat (U) or a Topcoat (T) and its location in the paint system. The location is determined with reference to the interface of the original topcoats and undercoats.

2. Each primer layer may be individually compared to the Munsell Color Coordinate System to determine its color/hue, value and chroma. Typically, it is not necessary to determine Munsell values for topcoat layers.

3. Infrared spectroscopy data from each layer of paint is used to identify the binder and extender pigments. Topcoat extenders are not coded in the PDQ database and therefore should not be used in the searching criteria.
B. Query the PDQ database. The criteria and results for each search conducted shall be documented, which may include a hit list.
   1. Layer System Query (LSQ).
   2. Spectral libraries search. The name coding that has been applied to the known FTIR spectra in PDQ provides information as to the manufacturer, plant and year of the sample and may be used to help categorize the similar spectra.
   3. Fill-in-the-Blank (FITB). The FITB search allows a search of the database for specific plant, manufacturer, year, etc. information that is available. This search may also be performed based on the results of the LSQ(s).

C. Refinish color collections can be referenced once a possible manufacturer and year range have been established to ensure that the color of the paint sample in question was actually used by the automotive manufacturer in that year range. Observations shall be documented.

D. Determine if search results of the possible vehicle makes, models and production years are an accurate representation using PDQ references and/or FITB searches. Observations shall be documented.

6 Interpretation
A. Each vehicle with data that is consistent with the data of the questioned paint indicates that particular make, model, and year vehicle is a possible source of the questioned paint. Other vehicles made in that same plant and year range are also included as possible sources of the questioned paint.
B. 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 protocols.

7 Limitations
A. Data obtained from a PDQ database provides an investigative lead, not an identification of the vehicle.
B. The PDQ database does not contain all vehicles produced, all paint systems/topcoat colors, trial run paints, or batch differences.

8 Literature References and Supporting Documentation


04  GLASS

TE-04-01  GLASS INITIAL EXAMINATION AND OVERVIEW

1  Scope

Material submitted to the laboratory for glass identification or comparison will require basic observations and tests before more extensive analyses are performed. The initial examination provides information which will assist the examiner in the formulation of a conclusion related to the glass comparison. In addition, information may be derived from this examination to influence the direction of the investigation.

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

2  Related Chapters

Fracture Physical Match Comparison
Glass Refractive Index Determination
Glass Analysis by X-Ray Fluorescence

3  Safety

A. Safety equipment includes the appropriate use of safety glasses, gloves, dust masks and clothing as necessary.

B. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

C. Biohazard precautions, if applicable

4  Equipment and Materials

- Stereomicroscope, polarized light microscope, ultraviolet light
- Scalpel, forceps, probe, spot plate, microscope slides, micrometer, calipers
- Water
- Methanol
- Nitric Acid

5  Standards, Controls, and Calibrations

None

6  Procedure

1. Examine known reference samples and questioned glass evidence separately.

2. Isolate, sort, and/or separate glass evidence from debris or substrate. It may be necessary to clean the glass with water, methanol, or concentrated nitric acid.

3. Macroscopically and/or microscopically examine evidence and record descriptive information which can include:

   a) Approximate the number of recovered questioned glass particles.
b) Determination of glass-like properties:
   i. **Solubility** – glass should not be soluble in either water or methanol
   ii. **Fracture** – glass has irregular shaped broken edges (conchoidal fracture)
   iii. **Hardness** – glass should not become indented when depressed with a metal probe
   iv. **Amorphous** – glass should exhibit isotropic properties when viewed under crossed polars with a polarizing light microscope

4. If it is determined that the evidence is glass-like, document the physical characteristics of the evidence which can include:
   a) Color, type (bottle, window, headlight, tempered, laminated, mirror, reinforced, electric light), shape (curved, flat), surface texture (smooth, orange–peel like, pitted), inclusions, and surface markings.
   b) Fluorescence at various wavelengths.
   c) If two parallel manufactured glass surfaces are available, the thickness can be determined with calipers or micrometers (+/- 0.01 millimeters). Typically, same source glass should be within 0.02 mm.
      i. Calipers/micrometers will need to be checked day of use.
      ii. Perform at least two different size measurements using NIST traceable gauge blocks or other equivalent reference standard.
      iii. If the observed measurements are not within +/-0.02 mm of the specified gauge block, the calipers/micrometers shall be visually inspected for damage or debris. The gauge blocks shall then be re-measured.
      iv. If the observed measurement still falls outside of the acceptable range, the calipers/micrometers shall not be used until the issue is resolved.
      v. The results of the evaluation will be recorded in the case record.

5. If direction of force is to be determined with the glass samples, determine if the edge being examined is from a radial or concentric crack. If a sufficient portion of the glass is not present, this determination may not be possible.
   a) The conchoidal fractures on the edge of the glass will meet the surface at right angles on the side opposite the force when examining a radial fracture.
   b) The conchoidal fractures on the edge of the glass will meet the surface at right angles on the side of the force when examining a concentric fracture.

6. If sequence of cracks is to be determined, examine the cracks that are present in the sample. Fractures will not cross over existing fractures. Document photographically or pictorially the fracture pattern.

7. Perform **Fracture Physical Match Comparison**, as applicable. If a fracture physical match exists between known and questioned glass, it is not necessary to continue with glass analysis.

8. Perform **Glass Refractive Index Determination**, as applicable.

9. Perform **Glass Analysis by X-Ray Fluorescence**, as applicable.
7 Interpretation

A. If no meaningful differences in the physical properties of the questioned glass and the known glass are detected, further testing is warranted if sample size permits.

B. If meaningful differences in the physical properties of the questioned glass and the known glass are detected, then the conclusion is made that the questioned glass is not consistent with the known sample and therefore, did not originate from the source of the known sample.

8 Literature References and Supporting Documentation


TE-04-02  GLASS REFRACTIVE INDEX DETERMINATION

1 Scope

The refractive index determination is a commonly measured physical property of glass because the required sample size is small. The refractive index can be determined using calibrated silicone oils on the GRIM instruments. The wavelength of light is fixed at 589.3 nm, the Sodium D line, while the temperature of the silicone oil is varied until the refractive index of the oil and the glass are the same. This procedure is described by the American Society for Testing and Materials as a standard test method (ASTM E1967-98).

If the sample size is sufficient, elemental analysis of the questioned and known glass samples should also be performed to increase discrimination between samples.

2 Safety

A. Appropriate personal protection from flying glass particles

B. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials

- GRIM instrument
- NIST glass standard 1822 - covers the refractive index range 1.51-1.52
- NIST glass standard 1820 - covers the refractive index range 1.48-1.49
- Locke Scientific reference glass standards - cover the refractive index range 1.46-1.55
- Silicone oils:
  - Oil A - Use for some old window glasses, container glasses
  - Oil B - Use for majority of glasses, particularly window glasses, tableware and some container glasses, which fall in the range 1.510-1.530
  - Oil C - Use for borosilicate glasses such as ovenware and headlamps

4 Standards, Controls, and Calibration

A. A NIST or Locke Scientific glass standard corresponding to the expected refractive index range of the evidential glass samples will be run each day of use to verify the instrument is operating correctly.

1. Five different edges should be measured on the glass standard selected.

2. The average \( N_D \) of the measured edges shall fall within the acceptable limits as defined during the instrument validation in order to pass. Refer to the local policy for acceptable limits.

   a) If the measured values of the standard are not within acceptable limits, perform re-calibration to correct the problem.

   b) If re-calibration fails to produce an acceptable result, advise the supervisor and remove the instrument from service as per CLS policy. Consultation with a service engineer may be needed.

3. The results of the standard will be placed in the case file and the measured \( N_D \) value indicated in the Equipment Log (LAB-405).

B. The GRIM instrument shall be re-calibrated using appropriate glass standards and silicon oils after any major system repairs or instrument relocations.
5 Procedure

5.1 GRIM Instrument and Sample Preparation

1. The GRIM instrument should be allowed to warm up before use.
2. Mount samples on a slide with a cover slip. Add the selected silicone oil to the slide. Samples can be crushed to facilitate analysis.

5.2 Sample Analysis

1. At least ten different edges should be measured on each glass sample, sample permitting.
2. Print the collected data and the calculated $N_D$ values and include in the case record.
3. Repeat sample analysis for each glass sample to be analyzed.
4. Compare the calculated $N_D$ value of the known and questioned samples.

6 Interpretation

A. The acceptable refractive index range for the known sample is the average of the $N_D$ measurements +/- 0.0002.
B. Two glass particles are said to have the same refractive index if their values are +/- 0.0002.
C. Questioned glass having an average refractive index within the acceptable range of the known glass indicates the questioned glass and known glass could share a common source.

7 Literature References and Supporting Documentation


Heideman, Dale, "Glass Comparison Utilizing a Computerized Refractive Indes Data Base", JFS, 1974.


TE-04-03 GLASS ANALYSIS BY X-RAY FLUORESCENCE

1 Scope
The elemental composition of a glass sample can be determined through the use of X-ray fluorescence analysis. Additionally, the elemental composition between glass samples can be compared semi-quantitatively based upon spectral comparisons and elemental peak intensity ratio comparisons.

Elemental analysis of glass samples is routinely performed when physical characteristics and refractive index determinations of each sample are consistent and sample size is sufficient.

Additional information may be found in X-Ray Fluorescence (XRF).

2 Related Chapters
X-Ray Fluorescence (XRF)

3 Safety
A. Protective eyewear shall be worn when filling the detector with liquid nitrogen.

B. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials
- X-ray fluorescence instrument
- Liquid Nitrogen
- Aluminum/copper disk (Al2024) and NIST Standard Reference Material (SRM) 1831 glass
- Sample holders/low element adhesive/sample support film

5 Standards, Controls, and Calibration
A. Each day the instrument is used, the aluminum/copper standard is analyzed to demonstrate proper operation and energy calibration.

1. The aluminum/copper standard should be run at all of the time constant values that will be used for sample analysis. The aluminum peak should be at 1.486 keV and the copper peak at 8.04 keV.

2. If an automated run is performed, the energy calibration will only need to be completed on the day the automated run is started.

3. If the calibration fails to meet expected values, manual adjustments can be made and a re-calibration performed. If the calibration continues to fail to produce acceptable results, then the instrument is removed from service per CLS policy. Consultation with a service engineer may be needed.

4. The Equipment Log (LAB-405) will be marked “pass” or “fail” and the energy calibration summary placed in the instrument log.

B. The NIST Standard Reference Material (SRM) 1831 glass standard will be run day of use. If an automated run is performed on more than 10 samples, a performance check should be completed at the beginning and end of the automated run.

1. The collected spectrum will be compared to historical data. The peak intensity data for the following element ratios will be evaluated: Ca/Fe, Ca/Mg, Ca/K, Sr/Zr, Fe/Zr, Fe/Sr, Fe/Zn.
2. The performance check is considered passing when the average of the elemental intensity ratios fall within the acceptable ranges that were developed during the validation process.
   a) Refer to the local policy for acceptable limits.
   b) Record results of check on the LAB-405.

6 Procedure

6.1 Sample Preparation

1. Fragments for elemental analysis should be approximately 100 µm thick or greater.
2. Analyze the non-float side for full thickness samples.
3. Mount fragments with a relatively flat surface at the sampling point perpendicular to the beam.
4. The questioned fragment and known samples should be of similar size, shape, and thickness.
5. Elevate thinner samples above the stage surface using a sample holder and/or sample support film and adhesive.

6.2 Sample Analysis

A. Recommended parameters:
1. Voltage: 50 keV
2. Set current to achieve < 50% deadtime
3. 1200-1600 Lsec
4. Run samples under vacuum to improve detection of low atomic number elements
5. At least 3 collection points from 3 different pieces of known glass (5 pieces if known sample is container glass) should be run, sample permitting
6. At least 3 collection points per questioned piece of glass should be run, sample permitting

B. Instructions
1. Collect spectrum
2. Perform spectral comparisons:
   a) Examine the spectrum for peaks with characteristic energies and label the peaks
   b) Compare spectral shapes and relative peak heights between your questioned and known samples
3. Perform statistical evaluation of spectra:
   a) Compare the elemental peak intensity ratios between certain elements in the questioned and known glass samples. Possible ratios for evaluation include Ca/Fe, Ca/Mg, Ca/Ti, Ca/K, Sr/Zr, Fe/Zr, and Fe/Sr.
      i. Ratios should be chosen based on the elements present in the samples.
      ii. Ratio pairs should consist of elements with characteristic energies near each other that are sufficiently resolved.
b) The elemental ratios from the known sample should be averaged together and the standard deviation calculated. The acceptable range for questioned samples is the average of the known sample +/- 3 standard deviations.

c) The elemental ratios from the questioned fragment will be averaged together. The average of the questioned fragment elemental ratios will be compared to the range of the known sample.

4. Mark the spectrum with case number, initials, date, and sufficient information to identify the particular sample.

7 Interpretation

A. An exclusion can be made when significant differences are detected in the elemental spectra (different elements and/or observable differences in relative peak heights) or in the calculated elemental peak intensity ratios. This indicates that the questioned and known glass do not share a common source.

B. Caution should be used when trying to eliminate samples based on the absence of an element in one sample that is at or near the limit of detection in the other sample. Additional criteria should be used.

C. An inclusion can be made when no significant differences are detected in the elemental spectra or in the calculated elemental peak intensity ratios. This indicates that the questioned and known glass could share a common source.

8 Literature References and Supporting Documentation


05  LAMP FILAMENT

TE-05-01  LAMP FILAMENT EXAMINATION

1  Scope
Lamps, such as headlights and taillights from vehicles, are often submitted for laboratory examination to determine if the lamps were incandescent at the time of an accident. An evaluation of the filament(s) within the lamp is made to determine if the lamp was on or off at the time of the accident. The laboratory is unable to examine high intensity discharge (HID) lamps or light emitting diode (LED) lamps.

2  Safety
A. Protective eyewear and gloves shall be worn while opening sealed lamps.
B. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3  Equipment and Materials
- Ohmmeter
- Stereomicroscope
- Heating device
- Water
- Hacksaw or other cutting device
- Vise
- Diamond scribe
- Insulated gloves
- Protective eyewear

4  Standards, Controls, and Calibration
A. The ohmmeter operation is checked before use:
   1. Turn the ohmmeter on.
   2. Set the ohmmeter to read resistance (ohms).
   3. The ohmmeter is operating properly if the resistance reads zero when the leads are in contact and if the resistance reads infinity (one) when the leads are not in contact.

5  Procedure
1. Record the lamp manufacturer’s identifying information.
2. Label all lamps to allow identification of their origin (item number or other information identifying vehicle source and location).
3. Document the lamp with photographs that accurately represent its condition.
4. If the filament appears to be continuous, test for continuity with the ohmmeter and document the condition of each filament.
5. Examine each filament visually and with a stereomicroscope.
6. If necessary for complete examination, the glass envelope can be broken to expose the filament(s) for examination. Take care not to damage the filament(s):
   a) Glass headlamps: score the back of the headlamp, apply heat along the score and quickly direct a stream of water onto the score, causing the headlamp to crack. Continue to alternately apply heat and water until the back of the headlamp can be removed from the body.
   b) Plastic lamps: cut the lamp until the filaments are exposed.
   c) Small lamps: brake, signal and other small lamps can be placed inside a plastic bag and carefully squeezed in a vise to break the glass envelope and expose the filaments.

7. Record the condition and characteristics of each filament in the case record.

8. Upon completion of the examination, repackage the lamps in a manner to prevent any further damage to the filament.

6 Interpretation

A. Interpretation will be dependent upon the abnormalities observed (see Table 05-01-A).

B. Based on the observed characteristics, possible conclusions may include:
   1. Lamp/bulb/filament determined to be incandescent (on) at the time of damage.
   2. Lamp/bulb/filament determined to be cold (off) at the time of damage.
   3. Lamp/bulb/filament determined to be burned out.
   4. Lamp/bulb/filament found to be indeterminate (weak, questionable or confusing indications should lead to an indeterminate conclusion).

C. No analysis can be performed on the lamp (HID and LED lamps). When lamps are determined to be either incandescent (ON) or cold (OFF) at the time the filament was damaged, the following disclaimer will be used in the laboratory report: “We/I are/am unable to determine if the lamp/bulb/filament was damaged as a result of this particular incident/the incident in question.”

D. When intact peanut style lamps are determined to exhibit distortion only, the following disclaimer will be used in the laboratory report: “Due to the style of this bulb/lamp, we/I are/am unable to determine if the bulb/lamp was on or off.”
### Table 05-01-A: Lamp Examination After Collision


<table>
<thead>
<tr>
<th>Observed Abnormalities</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filament incandescent</td>
</tr>
<tr>
<td>Glass broken</td>
<td>+</td>
</tr>
<tr>
<td>Glass broken or unbroken</td>
<td>+</td>
</tr>
<tr>
<td>Glass broken or unbroken</td>
<td>+</td>
</tr>
<tr>
<td>Glass broken or unbroken</td>
<td>+</td>
</tr>
<tr>
<td>Glass broken or unbroken</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Definite indication of condition  
P = Possible indication of condition

* It should be noted that in lamps with broken glass, filament distortion can be caused by mechanical means.

**Note:** “Incandescent” generally means that the filament was lighted and “Cold” means not lighted. However, determination of whether light was “on” or “off” may require consideration of other circumstances or indications of abnormalities.
7 **Limitations**
This examination cannot determine when the lamp was damaged.

8 **Records**
Filament Examination worksheet

9 **Literature References and Supporting Documentation**


06 HAIR

TE-06-01 RACIAL CHARACTERISTICS AND SOMATIC ORIGIN DETERMINATION OF HUMAN HAIR

1 Scope
The racial characteristics and somatic origin of a questioned human hair can be determined by an examination of the gross appearance and/or microscopic characteristics of the hair. This is most often done as an investigative lead or to eliminate certain hair from further examination.

2 Related Chapters
Macroscopic Examination of Hair

3 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials
- Stereomicroscope with magnification of approximately 10X – 50X
- Brightfield comparison microscope with objectives and eyepieces to permit observations in the range of approximately 20X - 400X
- A high intensity tungsten light source suitable for photomicrography and equipped with a daylight correction filter
- Microscope slides, cover slips
- Permount mounting medium (or appropriate medium with a known refractive index)

5 Standards, Controls, and Calibration
A. In-house standards and/or reference literature outlining the racial and somatic origin characteristics
B. The lighting should be adjusted to provide balanced Koehler illumination

6 Procedure
1. Isolate, sort, and/or separate hair evidence from debris or substrate.
2. Survey the macroscopic general features of the hair evidence as in Macroscopic Examination of Hair.
3. Foreign hairs, which may have potential evidentiary value as determined by the examiner, are mounted for microscopic assessment and classification. If there is blood and/or debris having no apparent evidentiary value on the hair, then it may be cleaned by using water before mounting.
4. Label hair mounts with case number, item number, examiner initials, and date.
5. Record the observed microscopic characteristics indicative of the racial and somatic origin of the hair, as well as:
   a) Color
   b) Approximate length
   c) The condition of the proximal end of the hair
7 Interpretation

A. Racial characteristics and somatic origin may be determined through comparison of questioned hairs to human hair keys and in-house human hair standard collections.

B. Racial characteristics are determined from the gross appearance and microscopic characteristics of the hair with particular attention given to shaft diameter variation, pigment granule distribution, and cross-sectioned shape.

C. Somatic origin is determined from the gross appearance and/or microscopic characteristics of the hair with particular attention given to shaft diameter, shaft fluctuations, configuration, tip shape and texture.

D. Unless the characteristics are distinctly typical of a certain racial and/or somatic origin, the examiner should not attempt to define the racial characteristics and/or somatic origin of the hair.

E. When racial characteristics of hair are reported out, the following disclaimer will be used: “Please note the observed racial characteristics of the hair do not necessarily reflect the ethnicity of the individual.”

8 Literature References and Supporting Documentation


Hicks, John W., Microscopy of Hair, Federal Bureau of Investigation, 1977.
TE-06-02 MICROSCOPIC EXAMINATION AND COMPARISON OF HAIR

1 Scope

Typically, only hairs from the scalp and pubic regions of the body are involved in microscopic comparisons made by the Crime Laboratory Service. There is more variability in the characteristics of scalp or pubic hairs among different individuals than in the hairs from other body regions, resulting in stronger associations.

A range of values exists for any particular characteristic among the hairs from a single body region of any one person. However, this range is only an extremely small part of the spectrum of values exhibited by the entire population.

2 Related Chapters

Case Reviews
Macroscopic Examination of Hair

3 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials

- Stereomicroscope with magnification of approximately 10X – 50X
- Brightfield comparison microscope with objectives and eyepieces to permit observations in the range of approximately 20X - 400X
- A high intensity tungsten light source, suitable for photomicrography and equipped with a daylight correction filter
- Microscope slides, cover slips
- Permount mounting medium (or appropriate medium with a known refractive index)

5 Standards, Controls, and Calibration

A. Hair standards shall be submitted before any hair comparisons can be performed. Known samples should be requested from all persons who may reasonably be expected to have been a source of questioned hair. If such samples are obtained, the significance of any ensuing associations is increased.

B. From a submitted known sample, a number of hairs will be selected to represent the variation of characteristics within the known sample.

   1. Known Hair Standard - a known representative hair standard is obtained by pulling and combing hairs from different areas of the head and/or pubic region.
      a) The number of hairs collected should consist of at least 25 hairs, with roots, that represent the variation of all the hairs in the region.
      b) It is strongly recommended that greater than 25 hairs be collected as collecting more hairs (up to 100 for head hairs; 50 for pubic hairs) will ensure all variation is represented. An insufficient hair standard may adversely affect the ability to perform a meaningful hair comparison.

   2. At times, a secondary known sample of hair (such as a victim’s brush) may be substituted for comparison purposes during an investigation.
6 Procedure

6.1 Overview

A. Microscopic characteristics exhibited within a human hair can be grouped into two categories: general and distinguishing.

1. Characteristics in the general category include:
   a) Color – hue, pigmentation, variation
   b) Structure – form, diameter, cross-sectional shape, cortex, medullation, shaft aberration
   c) Cuticular traits – scales, thickness, margins, sequence, weathering
   d) Acquired characters – proximal ends (roots), distal ends (tips)
   e) Length

2. Characteristics in the distinguishing category include:
   a) Artificial treatment
   b) Abnormalities
   c) Uncommon structural conditions
   d) An unusual value for a particular general characteristic
   e) Artifacts

B. General characteristics are those commonly found in all human hairs and are the main characteristics examined during hair comparisons. General characteristics can be similar between individuals and therefore are not indicative of a specific person.

C. Distinguishing characteristics may not be commonly found in all human hairs and their presence may add strength to an association. However, distinguishing characteristics can exist in more than one individual.

6.2 Analysis

1. Microscopic comparisons will only be performed on head and pubic hairs since body hairs do not contain a sufficient number of characteristics for comparison.

2. The Macroscopic Examination of Hair chapter is used to determine which questioned hair(s) require(s) microscopic comparison. Mount the questioned hair(s) for comparison and label with case number, item number, examiner initials, and date.

3. Select and mount a representative sample of the appropriate known hair standard and label with case number, item number, examiners initials, and date.

4. The comparison shall be thorough and careful and may involve the use of different magnifications to compare as many of the microscopic characteristics as possible. It is important that characteristics between two hairs be compared at equivalent positions along the hair shaft.
5. With sufficient detail and accuracy, record the general and individual microscopic characteristics used to formulate the conclusions between questioned and standard hairs. The documentation may include:

a) Color
b) Approximate length
c) Racial characteristics
d) Somatic origin
e) Condition of the proximal end
f) Individual characteristics

6. Hairs with roots may be suitable for nuclear DNA analysis regardless of the root growth stage.

a) Hairs should be documented prior to forwarding to the DNA section for further testing. Once a hair is sent to DNA, a portion of that hair will be destroyed. The trace examiner shall determine the appropriate level of documentation based upon the supplied case information.

b) This documentation should include at minimum, a macroscopic examination.

c) A complete microscopic examination, including somatic and racial origin, is optional and should be completed if it is deemed necessary by the examiner.

d) A photograph of the root portion of the hair is optional.

7 Interpretation

A. All microscopic comparisons will be verified by a second authorized examiner.

B. The analyst shall provide the verifier with the actual physical evidence along with all relevant documentation.

C. Associations:

1. No two hairs are exactly the same in minute detail. Therefore, it is important to use a representative known hair standard for comparison. A representative known hair standard captures the range of characteristics present in the person’s head or pubic hair region.

2. In order to conclude that a questioned hair is similar in macroscopic and/or microscopic characteristics with a known sample from a particular person, it shall first be determined that:

   a) The characteristics exhibited by the questioned hair fit within the range of characteristics present in the known sample, and

   b) There are no meaningful differences in these characteristics or their arrangement.

D. Elimination:

If the questioned hair is found to have some major characteristics (race, color, etc.) different from the hairs in the standard sample, or if the characteristics of the questioned hair do not fit within the range of characteristics exhibited by the standard, then the examiner would conclude the questioned hair is dissimilar to the known sample and, therefore, did not originate from the source of the known sample.
E. Inconclusive:
1. It may be that the questioned hair and the known hair exhibit both similarities and unaccountable differences in their physical characteristics.
2. The examiner may be of the opinion that the differences are not sufficient to eliminate the source represented by the known sample as being a possible source of the questioned hair and no conclusion could be reached as to whether or not the questioned hair could have originated from the source of the known sample.

F. Other conclusions and investigative leads:
1. The presence of an anagen root indicates that the hair was forcibly removed and should be reported when it is deemed necessary by the examiner.
2. The presence of individual characteristics should be reported when they may be important to the investigation.
3. Any non-fragmented hair contains a root and may be suitable for nuclear and/or mitochondrial DNA analysis. Any fragmented hair may be suitable for mitochondrial DNA analysis.

8 Limitations
A. If the microscopic characteristics of the questioned hair fall within the range of microscopic characteristics exhibited by the submitted known standard, then the questioned hair could have come from the known standard or any other known standard with similar characteristics.
B. Any hairs with less than ½” of comparable characteristics may not be suitable for comparison.
C. Some hair will not exhibit sufficient microscopic characteristics upon which to base an association. These hairs would be categorized as unsuitable for comparison.
D. A disclaimer accurately describing the limits of a particular conclusion will be reported whenever a positive association is determined. A statement equivalent to the following is adequate:
   1. “It is pointed out that hairs do not possess a sufficient number of unique individual microscopic characteristics to be positively identified as having originated from a particular person to the exclusion of all others.”
   2. “Please note that a conclusion that a hair could have come from an individual cannot be made until a microscopic hair comparison has been performed.”
E. When the time between the questioned hair(s) and the collection of known hair standards is five (5) years or more apart, any comparisons shall include the following disclaimer in the examiner’s report:
   “Due to the time span of five years or more between the shedding of the questioned hairs and the collection of the hair standards, the value of the hair comparison involving these hairs is limited to screening for DNA analysis and/or investigative leads.”

9 Literature References and Supporting Documentation


TE-06-03 SPECIES DETERMINATION OF HAIR

1 Scope
An animal hair may be identified as to genus, and sometimes species, by the physical and microscopic examination of its length and diameter measurements, scale patterns, medulla type, root shape, pigment location, shape, color, and color band pattern.

2 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials
- Stereomicroscope with magnification of approximately 10X – 50X
- Brightfield comparison microscope with objectives and eyepieces to permit observations in the range of approximately 20X - 400X
- A high intensity tungsten light source suitable for photomicrography and equipped with a daylight correction filter
- Microscope slides, cover slips
- Permount mounting medium (or appropriate medium with a known refractive index)
- Suitable casting media (e.g., Permount, nail polish, Norland adhesive, etc.)
- Acetone

4 Standards, Controls, and Calibration
An in-house animal hair standard collection, known samples submitted with the case, and reference literature

5 Procedure
1. Isolate, sort, and/or separate hair evidence from debris or substrate.
2. Survey the general features of the hair evidence. In some instances, depending upon the characteristics present, a preliminary classification of hairs (animal, human) may be made.
3. Questioned hairs of possible animal origin, which may have potential evidentiary value as determined by the examiner, are selected for examination.
4. As necessary, prepare casts of scales to identify the scale patterns of the hair using the scale casting method:
   a) On a suitable substrate place a thin layer of suitable casting media.
   b) Place the hair on the substrate ensuring that the hair is embedded in the casting material.
   c) Allow the medium to dry.
   d) Gently peel the hair from the substrate. An impression of the scales will remain in the casting medium.
   e) Document the substrate and casting medium used.
5. Microscopically observe the scale impressions and compare to standards as necessary.
6. Mount the selected hairs for microscopic assessment and classification. Hair mounts shall be labeled with case number, item number, examiner initials and date.

7. Observe and record characteristics such as length, diameter, scale pattern, medulla type, root shape, pigment location, shape, color and color band pattern and compare to standards as necessary.

6 Interpretation

A. A combination of animal hair keys and examination of the characteristics of the questioned hair in comparison with a known animal hair standard is the best way to identify the genus or species of a questioned animal hair. There are a few common animal hairs (for example, hair from the deer family, dogs, cats and rabbits) that can be identified without the use of keys.

B. Unless the characteristics are distinctly typical of a certain genus or species, the examiner should not attempt to define the genus or species of the hair.

7 Literature References and Supporting Documentation


TE-06-04  MACROSCOPIC EXAMINATION OF HAIR

1 Scope
A visual or macroscopic examination of questioned and known hairs should be performed to determine the need to conduct a microscopic comparison.

2 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials
- Stereomicroscope with magnification of approximately 10X – 50X,
- A high intensity tungsten light source
- Ruler

4 Standards, Controls, and Calibration
None

5 Procedure
1. Separate the hair evidence from any debris or substrate.
2. Survey the features of the questioned hair. If a macroscopic comparison is performed, record the general characteristics including, but not limited to, visual color, configuration, root, and approximate length. A stereomicroscope may be used to observe fine detail.
3. Examine the known hair standard. If a macroscopic comparison is performed, record the general characteristics including, but not limited to, visual color, configuration, root, and approximate length. Keep the questioned hair from being mixed with the known hair.
4. Compare the general characteristics of the questioned hair to the known hair standards.

6 Interpretation
A. Questioned hairs recovered from an individual’s body, clothing or environment and determined to be macroscopically similar to the hair standards from that individual are not generally examined further.
   1. When a visually similar hair is reported out, the following disclaimer will be used: “Please note that this is a limited comparison and a conclusion that hair could have come from an individual cannot be made until a microscopic hair comparison has been performed.”

B. Questioned hairs recovered from an individual’s body, clothing or environment and determined to be macroscopically dissimilar to the known hair standards from that individual may be subject to a microscopic comparison by an authorized hair examiner

C. The decision to perform a microscopic comparison rests on the potential evidentiary value of the hair and the case circumstances
7 Literature References and Supporting Documentation


07 FIBER

TE-07-01 FIBER INITIAL EXAMINATION AND OVERVIEW

1 Scope
Forensic fiber examination relates to the recognition, comparison and/or identification of a questioned fiber sample to its suspected source. Limited sample size and sample preservation requirements dictate that the comparative tests shall be selected and applied in a reasonable sequence to maximize the discriminating power of the test results without undue consumption of sample.

2 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Safety equipment includes the appropriate use of safety glasses, gloves, dust masks and clothing as necessary. In some cases, use of a fume hood or good ventilation is appropriate.
C. Biohazard precautions, if applicable

3 Equipment and Materials
- Stereomicroscope
- Scalpel, forceps, probe

4 Standards, Controls, and Calibration
None

5 Procedure
1. Isolate, sort, and/or separate fiber evidence from any debris or substrate.
2. Examine known textile sources and document physical characteristics, such as fiber type(s), color, and construction, as needed for comparisons. Known samples should be taken from all appropriate areas.
3. Perform Fracture Physical Match Comparison and/or Determination of Cut/Tear as necessary. If a fracture physical match is determined between known and questioned samples, it is not necessary to continue with fiber analysis.
4. Mount questioned fibers selected for further examination for comparison and/or identification.
5. Perform applicable comparisons, as needed. If meaningful differences are observed at any point during fiber comparison, it is not necessary to continue with analysis.
   a) Microscopic Examination of Fibers
      The generic class of manufactured fibers used to form a reported conclusion shall be confirmed by FTIR.
   b) Comparison Microscopy
      In addition to the comparison microscope, conclusive results from MSP or TLC shall be acquired in order to draw a positive color association.
   c) Microspectrophotometry of Fibers

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d) Fourier Transform Infrared Spectroscopy

e) Solubility Testing of Di-Acetate and Tri-Acetate Fibers

f) Fluorescence Microscopy

g) Cross-sectioning of Fibers

h) Thin Layer Chromatography of Fibers, which may include

i. Thin Layer Chromatography of Cotton Fibers

ii. Thin Layer Chromatography of Wool Fibers

6 Interpretation

A. If no significant differences between the questioned fiber and the known fibers are detected, then the conclusion is made that the fiber could have originated from the source of the known sample.

B. If significant differences of the questioned fiber and the known fibers are detected, then the conclusion is made that the questioned fiber is not consistent with the known sample and, therefore, did not originate from the source of the known sample.

C. Due to the commonality and discriminating value of colorless cotton and blue denim cotton fibers, this type of evidence may have limited significance.

7 Literature References and Supporting Documentation


TE-07-02 MICROSCOPIC EXAMINATION OF FIBERS

1 Scope

Forensic fiber examination relates to the recognition, comparison and/or identification of a questioned fiber sample to its suspected source. Fiber evidence requires initial observations that will lead the examiner to the proper course of analysis.

2 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials

- Polarized light microscope with objectives and eyepieces to permit observations at approximately 100X – 400X
- Full wave and/or quarter wave compensator
- Microscope slides, cover slips
- Permount mounting medium (or appropriate medium with a known refractive index)
- Michel-Levy chart

4 Standards, Controls, and Calibration

Before use, adjust the microscope to obtain Köhler illumination

5 Procedure

5.1 Fiber Screening

1. Mount fibers and examine under polarized light while rotating the stage 360°.
2. Document the type of extinction observed.
   a) If the fiber does not exhibit extinction and appears as a flat, twisting ribbon, this is sufficient to identify the fiber as cotton. Otherwise, a fiber exhibiting no extinction may be a vegetable fiber. Perform Optical Characteristics of Vegetable Fibers, if necessary.
   b) If the fiber exhibits extinction at all angles, it may be a glass fiber. Perform Glass Initial Examination, if necessary.
   c) If the fiber exhibits some extinction and appears to have scales or a medullary structure, it may be a hair. Perform Species Determination of Animal Hair, if necessary.
   d) If the fiber exhibits extinction (or almost complete extinction) at 0° and 90°, it may be a man-made fiber or a silk fiber. Perform the Microscopic Determination of Optical Properties below.

5.2 Microscopic Determination of Optical Properties

Birefringence, sign of elongation, and relative refractive indices are usually sufficient to identify the generic class of most common man-made fibers and silk fibers.

A. Birefringence

1. Rotate the mounted fiber until it is at a 45° angle toward the upper right of the field of view and examine under crossed polars.
2. Observe the interference color (retardation). Thick fibers and deeply dyed fibers can obscure the interference colors.

3. Use the Michel-Levy chart to estimate the order of the observed interference color.

4. Document the birefringence as low for first order colors, medium for second order colors, high for third order colors, and very high for fourth order colors and above.

B. Sign of Elongation

1. Rotate the mounted fiber until it is at a 45° angle toward the upper right of the field of view and examine under crossed polars.

2. Observe the interference color (retardation). Thick fibers and deeply dyed fibers can obscure the interference colors.

3. Insert either a full wave or a quarter wave compensator into the light path. A full wave compensator will introduce a fixed retardation between 530-550 nm. A quarter wave compensator will introduce a fixed retardation between 125-150 nm.

4. Observe the interference color (retardation) displayed at the same point on the fiber as in Birefringence.

5. If the resultant retardation is of a higher order, then the fiber has a positive (+) sign of elongation.

6. If the resultant retardation is of a lower order, then the fiber has a negative (-) sign of elongation.

C. Determination of Relative Refractive Indices

1. Orient the mounted fiber to be parallel to the polarizer (the analyzer is removed).

2. Increase the distance between the objective and the fiber and observe the Becke line (the halo of light surrounding the fiber). Closing the condenser aperture and/or lowering the light intensity may help the Becke line be more visible.

   a) If the Becke line moves into the fiber, then the fiber has a higher refractive index than the mounting media.

   b) If the Becke line moves away from the fiber, then the fiber has a lower refractive index than the mounting media.

3. Record the approximate refractive index in the parallel direction of the fiber ($N_{parallel}$), relative to the refractive index of the mounting medium, based upon the degree of contrast observed between the fiber and the mounting media.

   a) If the contrast is slightly different, then the fiber has a greater (or lower) refractive index than the medium.

   b) If the contrast is great, then the fiber has a much greater (or much lower) refractive index than the medium.

   c) If the edges of the fiber essentially disappear, then the fiber has the same refractive index as the medium.

4. Orient the fiber to be perpendicular to the polarizer.

5. Repeat steps 2 and 3 to estimate the approximate refractive index in the perpendicular direction ($N_{perpendicular}$) of the fiber, relative to the refractive index of the mounting medium.
6 Interpretation

A. The combination of birefringence, sign of elongation and refractive indices are usually sufficient to allow tentative identification of the generic class of most man-made fibers.

B. Table 07-02-A is useful as a guide to interpret the generic class of most common man-made fibers (and silk) from optical properties.

C. If no meaningful differences in the optical properties of the questioned fiber and the known fiber are detected, further testing may be warranted.

D. If meaningful differences in the optical properties of the questioned fiber and the known fiber are detected, then the conclusion is made that the questioned fiber is not consistent with the known sample and, therefore, did not originate from the source of the known sample.

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Property</th>
<th>Index</th>
<th>Birefringence</th>
<th>Sign of elongation</th>
<th>Best characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>(n_{\text{perpendicular}})</td>
<td>1.473</td>
<td>0.004 (low, gray interference color)</td>
<td>Positive</td>
<td>Low birefringence and positive sign of elongation</td>
</tr>
<tr>
<td></td>
<td>(n_{\text{parallel}})</td>
<td>1.477</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylic</td>
<td>(n_{\text{perpendicular}})</td>
<td>1.515</td>
<td>0.002-0.05 (low, gray interference color)</td>
<td>Negative</td>
<td>Low birefringence and negative sign of elongation</td>
</tr>
<tr>
<td></td>
<td>(n_{\text{parallel}})</td>
<td>1.512</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nylon</td>
<td>(n_{\text{perpendicular}})</td>
<td>1.520-1.526</td>
<td>0.049-0.060 (high, 2\textsuperscript{nd} order interference colors for fine fibers)</td>
<td>Positive</td>
<td>(n_{\text{perpendicular}}) very close to Permount in addition to higher order interference colors</td>
</tr>
<tr>
<td></td>
<td>(n_{\text{parallel}})</td>
<td>1.575-1.580</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olefin</td>
<td>(n_{\text{perpendicular}})</td>
<td>1.492-1.496</td>
<td>0.025-0.035 (usually 2\textsuperscript{nd} to 4\textsuperscript{th} order interference colors in center, with 1\textsuperscript{st} order colors at edges)</td>
<td>Positive</td>
<td>Only fiber type with (n_{\text{perpendicular}}) somewhat below Permount in addition to interference colors above 2\textsuperscript{nd} order</td>
</tr>
<tr>
<td></td>
<td>(n_{\text{parallel}})</td>
<td>1.520-1.530</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyester</td>
<td>(n_{\text{perpendicular}})</td>
<td>1.53-1.548</td>
<td>0.150-0.200 (very high, usually only pale reds and pale greens interference colors)</td>
<td>Positive</td>
<td>Considerable contrast between (n_{\text{parallel}}) and (n_{\text{perpendicular}})</td>
</tr>
<tr>
<td></td>
<td>(n_{\text{parallel}})</td>
<td>1.695-1.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rayon</td>
<td>(n_{\text{perpendicular}})</td>
<td>1.520-1.525</td>
<td>0.020-0.022 (usually only 1\textsuperscript{st} order interference colors)</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 07-02-A : Optical Properties of Six Common Man-Made Fiber Types (Approximate Indices)**

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Property</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silk*</td>
<td>$N_{\text{parallel}}$</td>
<td>1.591</td>
</tr>
<tr>
<td>Silk*</td>
<td>$N_{\text{perpendicular}}$</td>
<td>1.538</td>
</tr>
<tr>
<td>Best characteristic</td>
<td>Always a little above Permount, $N_{\text{parallel}}$ always either very slightly below or the same as Permount</td>
<td></td>
</tr>
<tr>
<td>Birefringence</td>
<td>0.053 (high first order to low second order interference colors)</td>
<td></td>
</tr>
<tr>
<td>Sign of elongation</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Best characteristic</td>
<td>Smooth glassy fiber, may exhibit transverse or oblique depressions at intervals, has a rounded triangular cross-section</td>
<td></td>
</tr>
</tbody>
</table>

*Although silk is an animal fiber, its optical properties are similar to those of man-made fibers*

7 **Literature References and Supporting Documentation**


TE-07-03 COMPARISON MICROSCOPY

1 Scope

The comparison microscope is used to compare the color and shade, delusterant size, shape, and distribution; cross-sectional shape, and overall appearance of questioned and known fibers.

2 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials

Comparison microscope with objectives and eyepieces to permit observations at approximately 100X – 400X

4 Standards, Controls, and Calibration

Adjust the microscope to obtain balanced Köhler illumination before use.

5 Procedure

1. Use the fiber mounts prepared in Microscopic Examination of Fibers and Cross-Sectioning of Fibers.
2. Place the mount of known fibers on one stage of the comparison microscope and the mount of questioned fibers on the other stage.
3. Using the same magnification on both stages, adjust the microscopes to obtain, as near as possible, balanced Köhler illumination.
4. Compare the questioned fiber to the known fiber, documenting:
   a) Visual color and shade,
   b) Delusterant characteristics,
   c) Cross-sectional shapes, and
   d) Overall appearance

6 Interpretation

A. If no meaningful differences in the characteristics of the questioned fiber and the known fiber are detected, further testing is warranted before a conclusion can be determined.

B. If meaningful differences in the characteristics of the questioned fiber and the known fiber are detected, then the conclusion is made that the questioned fiber is not consistent with the known sample and, therefore, did not originate from the source of the known sample.

7 Limitations

Color comparison using comparison microscopy has been shown to be less discriminating than microspectrophotometry and thin layer chromatography.

8 Literature References and Supporting Documentation

Robertson, James, editor, Forensic Examination of Fibers, Ellis Horwood Limited, 1992.


**TE-07-04 MICROSCOPESPECTROPHOTOMETRY OF FIBERS**

1 **Scope**

Microspectrophotometry (MSP) provides a nondestructive, objective evaluation of color from very small areas of a fiber. The color of questioned and known fibers can be compared and the possibility of a metameric pair can be eliminated. A metameric pair is when two colors appear visually similar under a certain set of conditions and are actually different.

Since the color of an object is due to the particular dyes, pigments and other colorants in it, the overall shape of the spectral curve should be distinctive and unique to that particular dye combination.

If possible, spectra in the ultraviolet range should be obtained when the visible range is featureless.

2 **Safety**

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 **Equipment and Materials**

- Microspectrophotometer able to scan at least 400-800 nm
- Holmium oxide glass reference
- Didymium glass reference
- Neutral density filters
- Quartz slide and coverslip
- Glycerol

4 **Standards, Controls, and Calibration**

A. Each instrument has a traceable filter set that has documented expected values, generated by the manufacturer, and tested against the National Institute of Standards and Testing Standard Reference Material.

B. The visible and/or UV range spectra of the holmium oxide filter, didymium filter, and the neutral density filters are obtained each day the instrument is used in order to check performance.

1. All holmium oxide and didymium peaks shall be within +/- 3 nm of their expected value in order to pass.

2. Absorbance values for the neutral density filters shall be within the determined acceptable range of the NIST values in order to pass.

3. If the ultraviolet range is used, all holmium oxide and didymium peaks shall be within +/- 3 nm of their expected value in order to pass. Absorbance values for the neutral density filters for wavelengths greater than 270 nm shall be within the determined acceptable range of the NIST values in order to pass.

4. Document the date, examiner initials, and pass/fail status of the check in the Equipment Log (LAB-405). The spectra and/or data chart will be placed in the case record.
5 Procedure

A. Mounted fibers previously examined for optical properties (Microscopic Examination of Fibers) are used in this procedure.

B. Fibers to be scanned in the ultraviolet range shall be mounted in a non UV absorbing medium such as glycerol on quartz slides.
   1. Analyze at least five fibers from a manufactured fiber set or ten fibers from a natural fiber set to represent the color variation of a known item.
   2. Obtain several spectra from each questioned fiber, when possible, since dye intensity can vary along a fiber’s length (particularly with natural fibers) and because of the effects of irregular cross sections. The exact number of runs required shall depend on the homogeneity of the questioned fiber and of the known sample.
   3. Label spectra with case number, sample identification, date, and examiner’s initials.
   4. Print the spectra and place in the case record.
   5. Compare spectra from questioned and known samples.

6 Interpretation

A. Each questioned fiber spectrum shall be compared to the known fiber spectra to determine if a positive association is found. The slope and position of peak maxima (nm), peak width, and peak intensity should be evaluated.

B. A positive association is noted when the questioned spectrum has the same peak positions, falls within the range of intensity, and has the same general shape as the known spectrum.

C. A negative association (exclusion) is made when
   1. The questioned spectrum is different to that of the known fiber set (i.e. spectral shape, peak intensities), or;
   2. The questioned spectrum falls outside the range of the known spectra.

D. An inconclusive result is when there are no significant points of comparison in the spectra (e.g., spectra from microscopically black or from very pale fibers that are outside the dynamic range of the instrument).

7 Literature References and Supporting Documentation


CROSS-SECTIONING OF FIBERS

1 Scope
The cross-section of fibers can give the examiner information for identifying natural fibers, provide a point of discrimination, and help determine end use information for manufactured fibers.

2 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials
- Stereomicroscope with magnification of approximately 10X – 50X
- Brightfield comparison microscope with objectives and eyepieces to permit observations in the range of approximately 40X – 400X
- A high intensity tungsten light source suitable for photomicrography and equipped with a daylight correction filter
- Hot plate
- Alcohol lamp
- Single-edge razor blade or scalpel blade
- Polyethylene film, low density
- Jolliff plates, needle threader, filler yarn (white, black)
- Microscope slides, cover slips
- Permount
- Suitable embedding medium (ex. superglue)

4 Standards, Controls, and Calibration
None

5 Procedure
Fibers may be cross-sectioned using the following methods.

5.1 Polyethylene method
1. Place the fiber between pieces of polyethylene film on a glass slide.
2. Cover the preparation with a second glass slide and place it on a hot plate set at approximately 120 °C or above an alcohol lamp.
3. As the polyethylene starts to clear, apply slight pressure to the covering slide.
4. When the polyethylene is clear and the fiber is covered, move the preparation to a cool surface.
5. When cool to the touch, pull the slides apart.
6. Cut a series of cross-sections perpendicular to the fiber axis.
7. Mount the sections on a microscope slide and label with case number, item number, examiner initials, and date.
8. Observe the characteristics of the cross-section.
   a) For manufactured fibers: the cross-section shape; the size and distribution of
delustrant across the fiber; and the degree of dye penetration. For pigmented
fibers, the size and distribution of pigment are also observed.

9. For natural fibers: the cross-section shape, lumen size, and cell wall thickness.

10. Compare the characteristics of a questioned fiber cross-section to the known fiber
cross-section.

5.2 Alternate to Polyethylene Method
A. The procedure for using these alternate mediums is similar to polyethylene in that the fiber
is embedded in the medium.

B. Other mediums, such as superglue, can be used to embed the fiber(s) in a solidifying
matrix that will not significantly alter the shape of the fiber.
   1. Embed the fiber in the medium and allow to harden (if applicable).
   2. Once the fiber is embedded, proceed as directed under the Polyethylene Method,
beginning at step F.

5.3 Jolliff Method
1. Cut a 2-3 inch length of filler yarn. The color of the filler yarn should be a
contrasting color to the evidence fibers.
2. Insert the threader through a hole from the back side of the Jolliff plate.
3. Pass about half the length of filler yarn through the eye of the threader.
4. Lay the evidence fiber between the free ends of the filler yarn.
5. Pull the threader, drawing the filler yarn and evidence fiber into the hole of the
Jolliff plate.
6. Slice the filler yarn and evidence fiber from both surfaces of the Jolliff plate.
7. Remove the section of the Jolliff plate with the cross-section and cover with a
cover slip on a microscope slide.
8. Document the characteristics of the cross-section.
9. Compare the characteristics of the questioned sample cross-section to the known
sample cross-section.

5.4 Documentation
Photomicrographs of the cross-sections may be taken.

6 Interpretation
A. A positive association is noted when the questioned fiber cross-section exhibits the same
cross-section shape and characteristics as the known fiber cross-section.

B. A negative association (exclusion) is noted when the questioned fiber cross-section
exhibits differences from the known fiber cross-section.

C. A combination of natural fiber keys and examination of the characteristics of the
questioned natural fiber with the in-house natural fiber standard collection or other
references is the best way to identify the genus or species of a questioned natural fiber.
D. Unless the characteristics are distinctly typical of a certain genus or species, the examiner should not attempt to define the genus or species of a natural fiber.

7 Literature References and Supporting Documentation


TE-07-06 THIN LAYER CHROMATOGRAPHY OF FIBERS

1 Scope

Thin layer chromatography is an inexpensive, simple, well-documented technique that, under certain conditions, can be used to complement visible spectroscopy in comparisons of fiber dyes. The principle of the method is that the dye components are separated by their differential migration caused by a mobile phase flowing through a porous, adsorptive medium.

While a straightforward extraction using a 4:3 ratio of pyridine:water is capable of removing the dye from a majority of fiber types, including from cotton and wool, it is known that reactive, sulfur, vat, diazo, ingrain, and pigmented dyes will not extract. For these dyes an alternative extractant will need to be employed. Further, reactive dyes are not easily extracted from cotton and wool, which may necessitate the use of an alternative procedure employing an enzyme to destroy the fiber, releasing the dyes for analysis.

2 Related Chapters

Sodium Hydroxide Solutions
Acetic Acid Solution
Cellulase Solution
Citric Acid Solution

3 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials

- Hot plate
- Alcohol lamp
- Oven
- Torch
- Ultraviolet light (long-wave)
- Fine capillary glass tubes (internal diameter of about 1.5 mm).
- Fine syringe or glass pipette.
- Aluminum backed silica gel 60 F<sub>254</sub> plates (or equivalent)
- Development chamber
- Pyridine:water (4:3)
- 3 M Sodium Hydroxide
- 0.5 M Acetic Acid
- 0.3 M Citric Acid in Methanol
- Cellulase Solution
- N-Butanol
- Ethanol
- Ammonia
- Pyridine
- Water
• Methanol
• Propan-1-ol
• Other extraction solutions (See Table 07-06-A)
• Other eluent solvents (See Table 07-06-D)

5 Standards, Controls, and Calibration

A. The ease of dye extraction and the particular extractant required will depend on the generic class of the fiber and the type of dye present. The generic class of the known and questioned fibers shall be determined prior to TLC analysis.

B. When performing the alternate cotton fiber extraction, dye from the known sample should be extracted and the eluent systems evaluated to determine which system achieves optimum separation of the extract. Dye extracted from equivalent amounts of known and questioned fibers is compared using the selected eluent.

C. Sample preparation shall be identical for all known and questioned fibers being compared on one TLC plate.

6 Procedure

6.1 Dye Extraction for All Fiber Types

1. Place an amount of the known fiber(s) in a short length (about 25 mm) of fine capillary glass tube that has been heat sealed at one end (with alcohol lamp or torch). A fine wire can be useful in pushing the fiber(s) down the tube.

2. Place an equal amount of the questioned fiber(s) to be compared in a separate capillary tube.

3. Add about 10 µL of pyridine:water (4:3) to each tube, ensuring that the fiber(s) are submerged, and heat-seal the tubes.

4. Incubate the tubes in an 80-100°C oven. Periodic checks for dye extraction should be made every 15 minutes for up to 1 hour.

Note: Extractant may still be colorless after the hour incubation.

6.2 Elution for All Fiber Types

1. Apply the known and questioned extracted dyes to the same TLC plate about 1 cm from the lower edge using a syringe or other suitable device.

2. Dry spots using a warm hot plate, repeating applications until the spot is strongly colored. The spot size should be uniform and not exceed approximately 2 mm in size.

   a) It is preferable to have at least two known spots on the plate, on both sides of the questioned sample. A note shall be made of the sample order on the plate, using pencil, so that pen ink does not interfere with the separation.

   b) If the extractant was colorless and did NOT produce a colored spot when applied to the TLC plate, refer to Table 07-06-A to determine another extractant to use.
### Table 07-06-A: Alternative extractants and dye class

<table>
<thead>
<tr>
<th>FIBER TYPE</th>
<th>EXTRACTION SOLUTION</th>
<th>DYE CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypropylene</td>
<td>Methyl acetate/water/acetic acid (5:5:1)</td>
<td>Disperse</td>
</tr>
<tr>
<td>Nylon</td>
<td>Chlorobenzene</td>
<td>Acid</td>
</tr>
<tr>
<td>Polyester</td>
<td>Chlorobenzene/acetic acid (1:1)</td>
<td>Basic</td>
</tr>
<tr>
<td>Polyester</td>
<td>Chlorobenzene</td>
<td>Disperse</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Formic acid/water (1:1)</td>
<td>Basic</td>
</tr>
<tr>
<td>Wool</td>
<td>2% aq. oxalic acid, followed by pyridine/water (4:3)</td>
<td>Metallized</td>
</tr>
<tr>
<td>Cotton or Rayon</td>
<td>Glacial acetic acid</td>
<td>Azoic</td>
</tr>
</tbody>
</table>

c) If fiber type is cotton or wool and does not form a colored spot following extraction with an alternative extractant, proceed to Dye Extraction for Cotton or Dye Extraction for Wool, respectively.

### Table 07-06-B: Dye class for pyridine/water (4:3) extraction

<table>
<thead>
<tr>
<th>FIBER TYPE</th>
<th>DYE CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>Basic or Disperse</td>
</tr>
<tr>
<td>Nylon</td>
<td>Acid or Basic</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Acid or Disperse</td>
</tr>
<tr>
<td>Wool</td>
<td>Acid</td>
</tr>
<tr>
<td>Cotton</td>
<td>Direct</td>
</tr>
<tr>
<td>Rayon</td>
<td>Direct</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>Acid(^i)</td>
</tr>
<tr>
<td>Acetate/Triacetate</td>
<td>Disperse</td>
</tr>
</tbody>
</table>

\(^i\)Dyes rarely extract from Polypropylene, but if they do, they are most likely to be Acidic.
4. Select and prepare an appropriate eluent based on the dye class (refer to Tables 07-06-C and 07-06-D). It is recommended to evaluate at least two eluents.

<table>
<thead>
<tr>
<th>DYE CLASS</th>
<th>ELUENT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>1, 4, 3</td>
</tr>
<tr>
<td>Basic</td>
<td>11, 12, 1</td>
</tr>
<tr>
<td>Disperse</td>
<td>6, 7, 8, 5</td>
</tr>
<tr>
<td>Acid (nylon)</td>
<td>9, 10</td>
</tr>
<tr>
<td>Acid or Metallized (wool)</td>
<td>1, 2</td>
</tr>
<tr>
<td>Azoic</td>
<td>5</td>
</tr>
</tbody>
</table>

*Table 07-06-C: Eluents recommended for certain dye classes*

<table>
<thead>
<tr>
<th>ELUENT</th>
<th>SOLVENTS</th>
<th>PROPORTIONS (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Butanol, acetone, water, ammonia</td>
<td>5:5:1:2</td>
</tr>
<tr>
<td>2</td>
<td>Pyridine, amyl alcohol, 10% ammonia</td>
<td>4:3:3</td>
</tr>
<tr>
<td>3</td>
<td>n-Butanol, ethanol, ammonia, pyridine, water</td>
<td>8:3:4:4:3</td>
</tr>
<tr>
<td>4</td>
<td>Methanol, amyl alcohol, water</td>
<td>5:5:2</td>
</tr>
<tr>
<td>5</td>
<td>Toluene, pyridine</td>
<td>4:1</td>
</tr>
<tr>
<td>6</td>
<td>Chloroform, ethyl acetate, ethanol</td>
<td>7:2:1</td>
</tr>
<tr>
<td>7</td>
<td>n-Hexane, ethyl acetate, acetone</td>
<td>5:4:1</td>
</tr>
<tr>
<td>8</td>
<td>Toluene, methanol, acetone</td>
<td>20:2:1</td>
</tr>
<tr>
<td>9²</td>
<td>n-Butanol, acetic acid, water</td>
<td>2:1:5</td>
</tr>
<tr>
<td>10</td>
<td>n-Butanol, ethanol, ammonia, pyridine</td>
<td>4:1:3:2</td>
</tr>
<tr>
<td>11</td>
<td>Chloroform, butanone, acetic acid, formic acid</td>
<td>8:6:1:1</td>
</tr>
<tr>
<td>12²</td>
<td>n-Butanol, acetic acid, water</td>
<td>4:1:5</td>
</tr>
</tbody>
</table>

*Table 07-06-D: Preparation of selected eluent*

²These eluents form an upper and a lower phase. Use the upper phase as the eluent. NOTE: The ethanol used is 99%; the ammonia 0.880 Specific Gravity, unless otherwise stated.
5. Place the plate vertically into the development chamber containing the selected eluent. The level of the eluent should be at least 0.5 cm below the origin/application spots on the plate. Document the eluent used.

6. Allow the eluent front to rise until good resolution of the dye is achieved (normally 2 cm from the origin), but not so far as to allow the spots to become diffuse.

7. Remove the plate and dry it on a warm hot plate.

8. Proceed to Plate Examination.

6.3 Dye Extraction for Cotton

1. Place a single fiber (or thread) in a short length (about 25 mm) of fine capillary glass tube that has been heat sealed at one end. A fine wire can be useful in pushing the fiber down the tube.

2. Suspend the fiber sample in 5 µL 3 M Sodium Hydroxide (or the thread sample in 50 µL) and keep at 0°C for 4 hours.

3. Remove the liquid and re-suspend the fiber in 5 µl 0.5 M Acetic Acid (or the thread sample in 50 µl) for approximately 20 seconds.

4. Remove the liquid and re-suspend the fiber in 60 µl Cellulase Solution (or the thread sample in 150 µl) for approximately 1 minute.

5. Remove the Cellulase Solution and repeat steps 3 and 4 once.

6. Remove the Cellulase wash and re-suspend the fiber in 3 µl Cellulase Solution (or the thread sample in 150 µl) at 45°C for 20 hours.

7. Add methanol to the solution as follows:
   a) Single fiber samples - add 3 µl Methanol to the samples, mix, then centrifuge for 5 minutes.
   b) Thread samples – centrifuge for 5 minutes. Remove 30 µl dye solution and mix with 30 µl methanol.

6.4 Elution for Cotton

1. Apply both known and questioned dyes to be compared to the same TLC plate about 1 cm from the lower edge using a syringe or other suitable device.

2. Dry spots using a warm hot plate, repeating applications made until the spot is strongly colored. The spot size should be uniform and not exceed about 2 mm in size.

3. It is preferable to have at least two known spots on the plate, on both sides of the questioned sample. A note shall be made of the sample order on the plate, using pencil so that pen ink does not interfere with the separation.

4. Place the plate vertically into the development chamber containing the selected eluent. The level of the eluent should be at least 0.5 cm below the origin/application spots on the plate. The eluent systems are:

5. Allow the eluent front to rise until good resolution of the dye is achieved (normally 2 cm from the origin), but not so far as to allow the spots to become diffuse.
6. Remove the plate and dry it on a warm hot plate.
7. Proceed to **Plate Examination**.

### 6.5 Dye Extraction for Wool

1. A single fiber (or thread) is placed in a short length (about 25 mm) of fine capillary glass tube that has been heat-sealed at one end. A fine wire can be useful in pushing the fiber down the tube.
2. Suspend the fiber sample in 3 µL 0.75 M Sodium Hydroxide (or the thread sample in 100 µL), seal the tube and incubate at 40°C for 24 hours.
3. Add 2 µL 0.3 M Citric Acid in Methanol (or 66 µL to the thread sample).
   **Note**: more Sodium Hydroxide may be used in Step 2 to cover the fiber, but the ratio of Sodium Hydroxide to Citric Acid shall remain 3:2.
4. Mix, then centrifuge for 5 minutes.

### 6.6 Elution for Wool

1. Apply both known and questioned dyes to be compared to the same TLC plate about 1 cm from the lower edge using a syringe or other suitable device.
2. Dry spots using a warm hot plate, repeating applications made until the spot is strongly colored. The spot size should be uniform and not exceed about 2 mm in size.
3. It is preferable to have at least two known spots on the plate, on both sides of the questioned sample. A note shall be made of the sample order on the plate, using pencil so that pen ink does not interfere with the separation.
4. Pre-run the plate by placing it in the development chamber containing methanol:ammonia (13:7). The level of the methanol:ammonia mixture should be at least 0.5 cm below the origin/application spots on the TLC plate.
   a) **Allow the methanol:ammonia mixture to flow past the origin about 4 mm to concentrate the extracted dyes.**
   b) **Remove and dry the plate.**
5. Place the plate vertically into the development chamber containing the eluent. The level of the eluent should be at least 0.5 cm below the origin/application spots on the plate.
   a) **The eluent system is: propan-1-ol:methanol:water:ammonia (6:3:1:4)**
6. Allow the eluent front to rise until good resolution of the dye is achieved (normally 2 cm from the origin), but not so far as to allow the spots to become diffuse.
7. Remove the plate and dry it on a warm hot plate.
8. Proceed to **Plate Examination**.

### 6.7 Plate Examination

1. When dry, immediately examine plates in visible and in long-wave ultraviolet light.
2. Document the observed bands with a color diagram and/or color photograph.
3. Compare the colors/fluorescence, sequence and position of the bands obtained from the dye of the questioned fiber to those from the corresponding known fibers.
4. If the examiner chooses to retain the thin layer plate, mark the plate with case number, item numbers, date, and examiner initials. Seal the plate in a ziplock bag and retain with the evidence or separately.

7 Interpretation
   A. A positive association occurs when band colors/fluorescence, sequence and positions are consistent between questioned and known fibers.
   B. A negative (exclusion) association is noted when either the questioned and known patterns show no similarities or where there are a number of coincident bands but one or more bands are missing from the questioned or known.
   C. An inconclusive result is noted when there are no bands on the TLC plate.
   D. The verification of all TLC results will be documented by a second authorized examiner.

8 Literature References and Supporting Documentation
   Robertson, James, editor, Forensic Examination of Fibers, Ellis Horwood Limited, 1992.
TE-07-07  OPTICAL CHARACTERISTICS OF VEGETABLE FIBERS

1 Scope

The determination of physical and optical properties is sufficient to identify vegetable fibers (cotton, flax, jute, manila, etc.). Many vegetable fibers require processing of the technical fiber and examination of the fiber ultimates for identification.

2 Related Chapters

Phloroglucinol Solution

3 Cross-sectioning of Fibers Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Glacial acetic acid:hydrogen peroxide solution may be explosive. Do not allow to evaporate to dryness.

4 Equipment and Materials

- Polarized light microscope with objectives and eyepieces to permit observations at approximately 100X – 400X
- Microscope slides, cover slips
- Permount mounting medium (or appropriate medium with a known refractive index)
- Heated water bath
- Phloroglucinol solution
- Glycerin/Alcohol solution (60:40) [prepared fresh]
- Acetic Acid
- Hydrogen peroxide (30%) [commercially-prepared reagent]
- Permount

5 Standards, Controls, and Calibration

A. It is best to identify vegetable fibers by their scientific name to avoid confusion. It is crucial to have a set of known fibers from a reputable source to assist in the comparison and identification of these fibers

B. The microscope shall be adjusted to provide Kohler illumination

6 Procedure

1. Scrape the exterior of the technical fiber with a scalpel blade and mount the residue in Permount for a microscopic examination.

2. Label mounts with case number, item number, examiner initials, and date.

3. Microscopically examine the residue for the presence of crystals and document their shape.

4. Clear the technical fiber by mounting it in a glycerin/alcohol solution and applying low heat. Microscopically observe the fiber morphology and crystals.
5. Apply a few drops of Phloroglucinol solution to a portion of the technical fiber and document the reaction.
   a) Of the bast fibers, unbleached jute shows the highest degree of lignification and is stained bright red.
   b) Leaf fibers [e.g., manila (Musa textilis) and sisal (Agave sisalana)] are usually moderately to highly lignified.

6. Perform **Cross-Sectioning of Fibers** on the technical fiber.
   a) Document the microscopic characteristics of the cross-section, including the shape of the cross-section, thickness of the cell walls, and the lumen size.

7. Process the technical fiber to obtain fiber ultimates:
   a) Macerate the technical fiber by heating it in a solution of glacial acetic acid:hydrogen peroxide (1:1) [prepared fresh] in a water bath until the ultimates separate. **Do not allow the solution to evaporate to dryness: it may be explosive.**
   b) Wash the fiber ultimates with water and mount in Glycerin/Alcohol.

8. Examine the fiber ultimates microscopically and characterize the length of the fiber ultimates, the presence of dislocations and nodes and the ratio of the lumen diameter vs. the fiber ultimate diameter.

9. Perform the Herzog test on the fiber ultimates:
   a) Align the fiber ultimate in a north/south position on the polarized light microscope with crossed polars.
   b) Observe the retardation color.
   c) Insert a full-wave compensator and observe the retardation color:
      i. Vegetable fiber with an “S” twist will have subtraction of retardation, resulting in an orange color.
      ii. Vegetable fiber with a “Z” twist will have addition of retardation, resulting in a blue color.

7 **Interpretation**

A. A combination of vegetable fiber keys and examination of the characteristics of the questioned vegetable fiber with the in-house standard collection or other references is the best way to identify the genus or species of a questioned vegetable fiber.

B. Vegetable fibers, except for cotton, should be identified by genus/species.

C. There are a few common natural fibers (cotton, for example) that can be identified without the use of keys or extensive examination.

D. Unless the characteristics are distinctly typical of a certain genus or species, the examiner should not attempt to identify the genus or species of a vegetable fiber.

8 **Literature References Supporting Documentation**


TE-07-08 PHLOROGLUCINOL SOLUTION

1 Scope
Phloroglucinol solution is used to detect lignin in vegetable fibers.

2 Specifications
Phloroglucinol Solution

3 Related Chapters
Reagents

4 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Wear disposable gloves and protective eyewear during preparation

5 Equipment and Materials
- Graduated cylinder, 25 mL
- Amber dropping bottle
- Ethanol
- Concentrated Hydrochloric Acid

6 Instructions
1. Prepare a saturated solution of Phloroglucinol in 5 mL ethanol.
2. Add equal volumes of the Phloroglucinol solution and Hydrochloric Acid.

7 Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes specification above, initials, and date prepared.
B. Store in an amber dropping bottle.
C. Prepare fresh every six months.
D. Prior to casework, verify and document in the case record that the solution reacts properly by testing unbleached jute. Unbleached jute is stained bright red by Phloroglucinol solution.
TE-07-09  COMPARISON OF THREAD, YARN AND CORDAGE

1 Scope
The comparison of thread, yarn, or cordage to its suspected source requires comparing the dimensions and construction of the questioned sample to the known sample.

2 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Biohazard precautions, if applicable

3 Equipment and Materials
Stereomicroscope

4 Standards, Controls, and Calibration
None

5 Procedure
1. Document the observed physical characteristics of the thread/yarn/cordage:
   a) Diameter and length
   b) Twist ("S" or "Z")
   c) Number of crests and troughs per unit length
   d) Condition (presence of knots, cut or frayed ends, presence of fibers or other evidence, etc.)
2. Compare the physical characteristics of the known and questioned items.

6 Interpretation
A. If meaningful differences are observed in the characteristics of the questioned and the known sample, then the examiner can conclude that the questioned sample did not originate from the known sample
B. If no meaningful differences are observed in the characteristics of the questioned and the known sample, analyses of the constituent fibers, as outlined in Fiber Initial Examination and Overview, is required before a conclusion can be determined.

7 Literature References and Supporting Documentation

TE-07-10 SODIUM HYDROXIDE SOLUTIONS

1 Scope

0.75 M Sodium Hydroxide solution is used in the extraction of dye from wool fibers.
3 M Sodium Hydroxide solution is used in the extraction of dye from cotton fibers.

2 Specifications

0.75 M Sodium Hydroxide
3 M Sodium Hydroxide

3 Related Chapters

Reagents

4 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Wear disposable gloves and protective eyewear during preparation

5 Equipment and Materials

- Balance suitable for weighing 1-100 g
- Volumetric flasks, 100 mL
- Beakers, 100 mL
- Deionized water
- Sodium hydroxide, reagent grade or better

6 Instructions

6.1 0.75 M Sodium Hydroxide

1. Dissolve 3 g sodium hydroxide in approximately 50 mL water.
2. When cool to the touch, transfer into a 100 mL volumetric flask.
3. Dilute to volume using deionized water.

6.2 3 M Sodium Hydroxide

1. Dissolve 12 g sodium hydroxide in approximately 50 mL water.
2. When cool to the touch, transfer into a 100 mL volumetric flask.
3. Dilute to volume using deionized water.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.
B. Store in sealed plastic containers at room temperature.
C. Prepare fresh solutions after one month.
TE-07-11  CITRIC ACID SOLUTION

1  Scope
0.3 M Citric Acid in Methanol solution is used in the extraction of dye from wool fibers.

2  Specifications
0.3 M Citric Acid in Methanol

3  Related Chapters
Reagents

4  Safety
   A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
   B. Wear disposable gloves and protective eyewear during preparation

5  Equipment and Materials
   • Balance suitable for weighing 1-100 g
   • Volumetric flasks, 100 mL
   • Beakers, 100 mL
   • Citric Acid, reagent grade or better
   • Methanol

6  Instructions
   1. Dissolve 6.3 g citric acid in approximately 60 mL methanol.
   2. Transfer to a 100 mL volumetric flask and dilute to volume using methanol.

7  Testing, Storage, Expiration, and Disposal
   A. Minimum labeling includes specification above, initials, and date prepared.
   B. Store at room temperature in a sealed container.
   C. Prepare fresh solution after one month.
TE-07-12 ACETIC ACID SOLUTION

1 Scope
0.5 M Acetic Acid solution is used in the extraction of dye from cotton fibers.

2 Specifications
0.5 M Acetic Acid

3 Related Chapters
Reagents

4 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Wear disposable gloves and protective eyewear during preparation

5 Equipment and Materials
- Pipet, 3 mL
- Volumetric flasks, 100 mL
- Glacial Acetic Acid
- Deionized Water

6 Instructions
1. Pipet 3 mL glacial acetic acid into a 100 mL volumetric flask.
2. Dilute to volume using deionized water.

7 Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes specification above, initials, and date prepared.
B. Store in a sealed container.
C. Prepare fresh solution after one month.
TE-07-13 CELLULASE SOLUTIONN

1 Scope
Cellulase solution is used to destroy the cellulase in cotton fibers, thus releasing the dyes for thin layer chromatography analysis.

2 Specifications
Cellulase Solution

3 Related Chapters
Reagents

4 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Wear disposable gloves and protective eyewear during preparation

5 Equipment and Materials
- Balance suitable for weighing 50 mg - 1 g
- Beakers: 50 mL, 100 mL
- pH meter
- Pipet, 100 mL
- Volumetric flask, 50 mL
- Sodium Acetate, reagent grade or better
- Glacial Acetic Acid
- Cellulase (retain frozen until needed)
- Deionized water

6 Instructions
6.1 Sodium Acetate solution (0.1 M)
1. Dissolve 0.82 g sodium acetate in 100 mL deionized water.
2. When dissolved, adjust the pH to 5 with glacial acetic acid.

6.2 Cellulase Solution
1. Dissolve 80 mg Cellulase in 40 mL 0.1 M sodium acetate solution.
2. Transfer to a 50 mL volumetric flask and dilute to volume using the 0.1 M sodium acetate solution.

7 Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes specification above, initials, and date prepared.
B. This solution shall be prepared fresh daily, as needed.
TE-07-14 SOLUBILITY TESTING OF DI-ACETATE AND TRI-ACETATE FIBERS

1 Scope

Solubility is a destructive examination, however; solubility testing can provide supplemental information to nondestructive methods. Possible reactions of fibers include partial and complete solubility, swelling, shrinking, gelling, and color change. It is recommended to examine known and question fibers simultaneously when comparing their solubilities.

2 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials

- Stereomicroscope
- Glass slides and cover slips or spot plate
- 75% aqueous acetone
- Chloroform

4 Standards, Controls, and Calibration

Sample preparation shall be identical for all known and questioned fibers being compared using solubility tests.

5 Procedure

1. Cut small portions of the known and questioned fibers and place them in spot plates or on a microscope slide under a cover slip.
2. Add a drop or two of the microchemical test reagent (75% aqueous acetone or chloroform). Observe and record the reaction with the aid of the stereomicroscope.
3. Document the reaction

6 Interpretation

A. Diacetate fibers dissolve in 75% acetone, while the triacetate fibers do not. Triacetate fibers may swell to as much as twice their diameter but do not lose their crenulated appearance.
B. Triacetate fibers are immediately soluble in chloroform. Diacetate fibers will retain some crenulation for at least 15 seconds in chloroform, eventually swelling 5 to 10 times their normal diameter.

7 Literature References and Supporting Documentation

08 GUNSHOT PRIMER RESIDUE

TE-08-01 GUNSHOT RESIDUE ANALYSIS BY SCANNING ELECTRON MICROSCOPY-ENERGY DISPERSIVE SPECTROMETRY

1 Scope
Gunshot residue (GSR) particles can be detected and identified based on morphology and elemental composition using automated Scanning Electron Microscopy-Energy Dispersive Spectrometry (SEM-EDS) analysis. Gunshot residue particles detected during the automated analysis is confirmed by the analyst.

2 Related Chapters
Laboratory Equipment
Scanning Electron Microscopy-Energy Dispersive Spectrometry

3 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials
- Scanning Electron Microscope-Energy Dispersive Spectrometer
- Elemental standard(s): as referenced in local policies/instrument logbooks
- Gunshot residue standard
- Blank environmental stub

5 Standards, Controls, and Calibration
A. The previous SEM-EDS calibration is verified prior to each software run by acquiring the spectrum from an elemental standard.
   1. A copy of the calibration is placed in the case record. Calibration results are documented in the Equipment Log (LAB-405).
   2. If the X-ray detector will not acquire the correct spectrum of the elemental standard, the automated analysis of GSR will not proceed. Contact the manufacturer to correct this situation.
B. Analysis of GSR stubs requires a known GSR sample and an environmental blank. Data from this analysis is placed in the case record.
C. The brightness and contrast settings of the backscatter detector should be set to detect known GSR particles of appropriate size.
   1. If GSR particles of appropriate size are not detected on the GSR standard during a run, adjust the backscatter detector levels and restart the GSR software.
   2. Results of known GSR sample analysis are documented in the Equipment Log (LAB-405).
D. If GSR particles are detected on the environmental blank, the results obtained from the evidence stubs should be considered suspect.
   1. The results may be reported, but a qualifying statement shall be included in the report for each case analyzed that is associated with that blank.
2. In addition, steps should be taken to identify and eliminate the source of GSR particles detected on the environmental sample before further casework samples are analyzed.

6 Procedure

6.1 Sample Preparation

1. A blank stub will be exposed to the environment while evidence stubs are removed from their packaging and processed.
   a) A blank stub will be used if casework stubs require carbon coating.
   b) This stub will be the first one loaded for carbon coating and the last stub removed.

2. Uniquely mark each evidence stub for identification.

3. The evidence stubs may be examined by stereomicroscope for the presence of gunpowder flakes. Document the findings.

4. Place the sample stubs into the SEM chamber. A map may assist in tracking each stub location.

5. Activate the SEM-EDS.

6.2 Sample Analysis

1. Initiate the GSR analysis software.

2. Adjust the brightness and contrast settings of the backscatter electron detector using the appropriate instrument imaging standards.

3. Manual confirmation of characteristic and/or indicative GSR particles is required by the examiner.

4. Place the X-ray spectra and an image of all confirmed characteristic or indicative GSR particle, with the elemental peaks labeled, in the case record.

7 Interpretation

A. Morphology:

1. GSR particles detected and analyzed using this method are often spheroidal, non-crystalline particles between 0.5 um and 5.0 um in diameter; the remainder are irregular in shape and/or vary from 1 to 100+ um in size.

2. In general, it is not consistent with the mechanisms of GSR formation to commonly find particles with crystalline morphology, however; such particles have occasionally been observed in known primer (GSR) residues. Since morphology can vary greatly, it should never be considered as the only criterion for identification of GSR.

B. Elemental Composition:

1. Characteristic GSR particles are composed of lead, barium, and antimony.

2. Indicative GSR particles are composed of lead and barium, lead and antimony, or barium and antimony.
C. Occasionally, GSR particles with apparent unusual elemental compositions may be encountered in case work. In this circumstance, these particles may be reported as GSR if they can be shown to be consistent with GSR particles from the weapon and/or ammunition in question.

8 Literature References and Supporting Documentation


09 IMPRESSION
TE-09-01 KNOWN FOOTWEAR AND TIRE TRACK IMPRESSIONS

1 Scope
The purpose of footwear and tire track impression examination is usually to determine if a particular shoe or tire made a questioned impression. Known footwear or tires are photographed, imprinted, and/or cast to perform the comparison.

2 Related Chapters
Comparison of Impression Evidence
Casting of Impression Evidence
Digital Imaging and Processing of Impression Evidence

3 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Biohazard precautions, if applicable

4 Equipment and Materials
- Powder of contrasting color to substrate (fingerprint powder, talcum, etc.)
- Coating substance (silicone spray, petroleum jelly, etc)
- Brush
- Roll of white paper at least 12 inches wide
- Board at least 12 inches wide and 10-12 feet long
- Ink pad
- Inkless impression kit
- Photocopier with transparencies
- Black ink
- Paper

5 Standards, Controls, and Calibration
None

6 Procedure
6.1 Initial examination of shoe/tire
1. Prior to making test prints, inspect the exhibit for other evidence.
   a) If there is embedded material in the tread, it should be left embedded for the initial comparison. The embedded material can be removed later if deemed necessary.
   b) Otherwise, collect and preserve adhering debris and trace evidence from the shoe or tire.
2. If blood or other biological evidence is present on the shoe or tire, document, collect, and preserve it.
6.2 Test print preparation

Test prints from footwear and tires can be produced using the following techniques:

A. Preparation of inked test prints
   1. Apply ink to the tread with an inked pad or roller.
   2. Press the inked tread onto a flat, smooth paper surface to the extent that all edges or relevant portions of the tread come into contact with the paper to produce a test print. Several test prints of the same object may be made.
   3. Document the case, exhibit number, tread, date, and examiner on each test print.
   4. Transparency overlays of the test print(s) may be made on a photocopier.

B. Preparation of powder test prints
   1. Footwear
      a) Apply a light coating of a substance which will provide sufficient detail when a powder is applied to the test print.
      b) Press the prepared tread onto a flat, smooth paper surface to the extent that all edges or relevant portions of the tread come into contact with the paper to produce a test print. Several test prints of the same shoe may be made.
      c) Apply contrasting powder to the paper to visualize the tread pattern of the test print.
      d) Document the case, exhibit number, date, and examiner on each test print.
      e) Transparency overlays of the test print(s) may be made on a photocopier.
   2. Tire
      a) Roll a length of white paper onto a smooth, flat surface such as a board or cement floor. The paper should be long enough to contain one revolution of the tire and wide enough to contain the entire tread width.
      b)Apply a light coating to the circumference of the tire tread of a substance which will provide sufficient detail when a powder is applied.
      c) Roll the tire on the paper, marking where one revolution of the tire begins and ends.
      d) Apply contrasting powder to the paper to visualize the tread pattern of the tire. Several test prints of the same tire may be made.
      e) Document the case number, exhibit number, position of tire, direction of travel, outer/inner edge of tire, date and examiner initials on the test print.
      f) Document the manufacturer, size, and DOT # of tire as appropriate.
      g) Transparency overlays of the test print(s) may be made on a photocopier.

C. Preparation of inkless test prints
   1. Place exemplar on the pad-like dispenser.
   2. Place the exemplar onto the chemically treated paper.
   3. Test print will become dark.
   4. Preserve a copy of the test print.
5. Document the case, object, date, and examiner on each test print.
6. Transparency overlays of the test print(s) may be made on a photocopier.

6.3 Preparation of three-dimensional impressions

1. Evenly press the shoe into an impression material (such as moist soil) to record both the weight-bearing and non-weight-bearing surfaces of the sole.

2. Examination quality photographs may be made of the impression and the photographs used in the comparison process. See Digital Imaging and Processing of Impression Evidence.

3. A cast of the impression may be made and used for comparison with the questioned cast. Perform Casting of Impression Evidence.

7 Interpretation

A. The footwear/tire and the test prints/casts are examined for general and individual characteristics

B. Compare the known prints/casts to the questioned samples as in Comparison of Impression Evidence.

8 Literature References and Supporting Documentation


TE-09-02 DETECTION OF IMPRESSION EVIDENCE BY OBLIQUE LIGHTING

1 Scope
Impression evidence from footwear and tires is often found at the scene of a crime and can be used to associate footwear or tires from a suspect/victim to that particular location. The primary method of detecting impression evidence on areas such as floors at crime scenes and on evidence submitted for examination within the laboratory, is the use of oblique light.

Latent impressions may be found in dust or dirt on a surface. Because the dust particles are very small, they do not cast a shadow when light strikes them from above. However, when the light is incident from a low angle, the small particles will cast shadows and the impression becomes visible. After detection, examination quality photographs may then be taken of the impression to allow a comparison to be made.

2 Related Chapters
Comparison Of Impression Evidence

3 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment
B. Biohazard precautions, if applicable

4 Equipment and Materials
Flashlight or other light source

5 Standards, Controls, and Calibration
None

6 Procedure
1. Examine the questioned surface in a darkened area by shining a light at various oblique angles across the surface to visualize any impressions present.
2. Document the location of the latent impressions.
3. Take examination quality photographs to capture maximum detail of any detected impressions. See Digital Imaging and Processing of Impression Evidence.

7 Interpretation
A. Examine the photographs of the questioned impression for general and individual characteristics
B. Following photo documentation, attempts to recover the latent impression may be made as in Recovery of Impressions Using Electrostatic Lifting.
C. Compare the questioned impression to the suspected footwear/tire as in Comparison Of Impression Evidence.

8 Literature / Supporting Documentation
TE-09-03 COMPARISON OF IMPRESSION EVIDENCE

1 Scope

The comparison of a questioned impression to footwear or tire is performed to determine if the footwear or tire made that impression.

The comparison involves the examination of the following characteristics exhibited by the questioned impression and its suspected source:

A. **Class characteristics** (also called general characteristics) are characteristics that are shared by a finite number of shoes (tires) and serve to classify a shoe print (tire track) as belonging to a select group. Examples of class characteristics include tread pattern and approximate size. These characteristics are created during the manufacture of the shoe (tire).

B. **Randomly acquired characteristics** are characteristics that come from the environment the shoe (tire) has been subjected to since its manufacture. A randomly acquired characteristic is the result of something added to or taken away from a shoe sole (tire tread). Examples are cuts, scratches, or gouges in the sole and rocks embedded in the tread. The position, orientation, size, and shape of these characteristics contribute to the uniqueness of a shoe sole (tire tread). Randomly acquired characteristics are essential for an identification of a particular shoe (tire) as the source of an impression.

C. **Wear pattern** is the change in the tread pattern that occurs due to friction. This is a characteristic beyond the scope of class characteristics but does not constitute a basis for a positive association.

2 Related Chapters

Case Reviews

3 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials

Ruler

5 Standards, Controls, and Calibration

None

6 Procedure

1. Examine both the questioned impression (print/cast/examination quality photograph/recovered lift, etc.) and known exemplar (test prints, transparency overlays, casts, etc.) at the same scale, when possible. Document the applicable class characteristics:
   a) Physical dimensions
   b) Shape
   c) Design of the tread pattern
   d) Design element size
2. Document the similarity of the class characteristics exhibited by the questioned impression to those of the known shoe (tire).
   a) If the class characteristics are different, the shoe (tire) can be eliminated as the source of the impression and examination can stop at this point.
   b) If the class characteristics of the impression and the suspected shoe sole (tire track) are similar, the comparison proceeds with the examination of any randomly acquired characteristics that may be present.

3. Compare the randomly acquired characteristics found in the known shoe (tire) to the questioned impression. Randomly acquired characteristics should be confirmed on the actual shoe (tire) when available.

4. Document the results.

7 Interpretation
   A. A general match is attained when the impression exhibits the same class characteristics as the suspected shoe (tire). This means the impression could have been made by the suspected shoe (tire), or by any other shoe (tire) having the same class characteristics as the suspected shoe (tire) and having access to the location of the impression.
   B. A positive identification is achieved when the impression exhibits the same class characteristics and at least one well-defined randomly acquired characteristic as the suspected shoe (tire). A positive identification means that the impression was made by the suspected shoe (tire).
   C. Verification of all positive identifications will be documented by a second authorized examiner.
   D. An elimination is achieved when the impression exhibits different class characteristics as the suspected shoe (tire), or, in some instances, when the impression exhibits the same class characteristics but different randomly acquired characteristics as the suspected shoe (tire). It should be noted that a randomly acquired characteristic may occur on a shoe after that shoe made an impression. This shall be considered before an elimination can be determined.
   E. An unsuitable for comparison result is achieved when the impression lacks sufficient detail to compare class characteristics. This may occur with partial impressions having limited characteristics, faint or poorly defined impressions or impressions that are inadequately documented.

8 Literature References and Supporting Documentation


TE-09-04  FABRIC IMPRESSION EVIDENCE

1  Scope

Among the types of impression evidence that may connect a suspect, victim, and/or a crime scene are fabric impressions. These impressions may be on paint, metal, glass, plastic, or other surfaces in dust or blood. Fabric impression comparison is a procedure requiring observation of characteristics according to size, shape, and spatial relationship.

The comparison involves the examination of the class characteristics and any randomly acquired characteristics exhibited by the questioned impression and its suspected source:

A. **Class characteristics** (also called general characteristics) are characteristics that are shared by a finite number of fabrics and serve to classify an impression as belonging to a select group of fabrics. An example of a class characteristic includes weave pattern. These characteristics are created during the manufacture of the fabric.

B. **Randomly acquired characteristics** are characteristics that come from the environment the fabric has been subjected to since its manufacture. An example is a cut in the fabric. The size, shape, and location of these characteristics contribute to the uniqueness of that fabric.

2  Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Biohazard precautions, if applicable

3  Equipment and Materials

- Stereomicroscope
- Fingerprint ink or washable paint
- Ink pad
- Paper
- Margarine
- Silicone spray
- Petroleum jelly
- Lift tape or gel lifter(s)
- Magna brush
- Magnetic fingerprint powder

4  Standards, Controls, and Calibration

None

5  Procedure

5.1  Examination

1. Photograph or photocopy the impressions and examine for any adhering fibers. If present, remove the fibers and preserved for further examination and comparison.

2. Document and preserve any substance on the suspected fabric that may be associative evidence.
4. Document the pattern observed in the questioned impression and in the test prints of the suspected fabric.
5. Compare the class and randomly acquired characteristics of the questioned impression to the test prints.

5.2 Preparation of Test Fabric Impressions

Test prints of the suspected fabric may be produced using techniques including, but not limited to:

A. Inked test prints
   1. Apply the fabric to an inked pad, or alternatively, apply a thin layer of ink to the fabric.
   2. Press the inked fabric onto a flat, smooth paper surface to produce a test print. Several test prints may be made.
   3. Place documentation identifying the case, item number, date, and examiner initials on each test print.
   4. Transparency overlays of the test print(s) may be made on a photocopier.

B. Powder test prints
   1. Place a piece of white paper onto a smooth, flat surface. The paper should be large enough to contain the desired size test print.
   2. Apply a light coating of the chosen impression medium (ex. margarine, silicone spray, petroleum jelly) to the fabric.
   3. Press the fabric onto the paper.
   4. Apply black fingerprint powder to the paper with a Magna brush to visualize the fabric weave. Several test prints may be made.
   5. Place documentation identifying the case, item number, date, and examiner initials on the test print.
   6. Transparency overlays of the test print(s) may be made on a photocopier.

6 Interpretation

A. A general association is attained when the impression exhibits the same class characteristics as the suspected fabric. This means the impression could have been made by the suspected fabric, or by any other fabric having the same class characteristics as the suspected fabric.

B. A positive association (identification) is achieved when the impression exhibits the same class characteristics and at least one well-defined randomly acquired characteristic as the suspected fabric. A positive association means that the impression was made by the suspected fabric.

C. Verification of all positive associations will be documented by a second authorized examiner.

D. An elimination is achieved when the impression exhibits different class characteristics as the suspected fabric, or, in some cases, when the impression exhibits the same class characteristics but different randomly acquired characteristics as the suspected fabric.
E. Any unexplained differences between the impression and the fabric would eliminate that fabric as making that impression.

7 Literature References and Supporting Documentation


TE-09-05  RECOVERY OF IMPRESSIONS USING ELECTROSTATIC LIFTING

1  Scope
Impressions created on dry dust or dry impressions on relatively clean surfaces may be recovered with an electrostatic lifting device. Surfaces should first be examined using Detection of Imprint Evidence by Oblique Lighting. Examination quality photographs should be taken before attempting to lift any detected impression.

Impressions recovered by using an electrostatic lifter may exhibit more detail than observed in photographs due to increased contrast. The electrostatic lifter may be used to conduct a “blind search” of an area where a suspect likely walked and may recover impressions that would otherwise go undetected.

2  Related Chapters
Detection of Imprint Evidence by Oblique Lighting
Comparison of Impression Evidence

3  Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. When using the electrostatic lifting device, it is possible to receive electrical shocks from the lifting film, the ground plate and the metal probe. These shocks can be avoided by not touching those parts when the current is on and by allowing the probe to remain on the metalized portion of the lifting film for at least 5 seconds after the unit is turned off, thus allowing any remaining charge to dissipate.

4  Equipment and Materials
- Electrostatic lifting device
- Lifting film

5  Standards, Controls, Calibration
None

6  Procedure
1. Position the ground plate. The ground lead of the electrostatic lifting device shall be attached to the ground plate. In the case of a metal object, connect the ground lead to that object. Plug the other end of the ground lead into the voltage source.
2. Carefully position a piece of lifting film over the impression with the black side facing the impression.
   a) The lifting film should not touch any part of the ground plate. It may be necessary to place a piece of clean chart board between the item being processed and the ground plate or to make other adjustments so that the film and the ground plate are not in contact with one another.
   b) In cases where the surface is metal, it may be necessary to carefully place a piece of clear, very thin (1 or 2 mil) Mylar or polyester over the impression. Then place a slightly smaller piece of lifting film, black side down, over the Mylar.

Printed copy is uncontrolled. Refer to electronic copy for current version.
Mylar should be larger than the lifting film to ensure that none of the black lifting film is touching the metal surface.

i. Continue with the lifting procedure as outlined; however, remember that the lifted impression will now be on the Mylar.

ii. The Mylar and the black lifting film can be lifted and kept together to provide the necessary contrast.

c) Mark the lifting film and the surface to later facilitate the orientation of the lifted impression.

3. Ensure the tip of the hand-held probe touches an edge of the metalized backing of the lifting film. It should remain in contact with the film during the remainder of the procedure.

4. Turn on the power and slowly increase the voltage. The application of sufficient voltage will cause the lifting film to be pulled down tightly against the impression.

a) Air bubbles may be trapped beneath the film. These will often disappear in a few seconds.

b) If any air bubbles remain trapped beneath the film, they may be very gently rolled out by lightly passing a clean fingerprint roller over the film.

5. Turn the power off. Allow the probe to remain in contact with the film for approximately 5 seconds to discharge the film. When this is done, the film can be seen to relax as the charge leaves it. Failure to discharge the film with the probe will result in a static shock to the person who lifts the film off the impression!

6. Remove the film by carefully peeling it off.

7. In darkness, examine the film carefully with oblique light to see if an impression has been transferred to it. If this is not possible at the crime scene, then all lifts should be saved until they can be examined in total darkness.

8. Label each lift (or its sealed container) with case number, item number, date, and examiner’s initials.

9. The lifting film should be protected immediately after being removed from the impression. To properly preserve and store the lifting film, it should be taped inside a folder or in a shallow box. Never store lifted impressions in plastic or in the case folder.

10. The lifts shall be photographed to preserve the impression.

7 Interpretation

A. The lifts and photographs of the impression are examined for general and randomly acquired characteristics

B. Compare the impression to the suspected shoes as in Comparison of Impression Evidence.

8 Literature References and Supporting Documentation

TE-09-06 CASTING OF IMPRESSION EVIDENCE

1 Scope

Footwear and tire impressions are often found in soil. These three-dimensional impressions should always be properly photographed before any attempt is made to cast them. Casts are capable of exhibiting additional characteristics that may not be revealed through photography.

A cast is made to document and to aid in the comparison of the impression to the footwear or tire suspected of making the impression. Casts are also made using the footwear or tire suspected of making the impression.

2 Related Chapters

Comparison of Impression Evidence

3 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials

- Dental stone (pre-measure two pounds of dental stone into a gallon sized ziplock bag)
- Hair spray (optional)
- Water
- Gallon sized ziplock bags

5 Standards, Controls, Calibration

None

6 Procedure

1. Do not remove any debris that is part of the impression. If the soil is loose, the examiner may choose to gently apply hair spray to the soil to help preserve the impression.

2. Add water (approximately 5 ounces of water per pound of dental stone) to the dental stone and mix thoroughly for 3-5 minutes.

3. Gently pour the dental stone onto the ground at the edge of the impression, allowing the dental stone to flow into and fill the impression. On a slope or uneven ground, a form may be used to control the flow of the dental stone.

4. Allow 30-45 minutes for the cast to set.

5. Gently remove the cast from the ground and place into a paper bag.

6. Label the cast with case number, item number, date, and examiner initials.

7. The cast will continue to harden for about 48 hours and air must be allowed to circulate around the cast during this time.

8. After the cast has hardened, it will be cleaned to remove any adhering soil, sand and debris.
   a) The examiner may choose to photograph the cast prior to cleaning to document its condition.
   b) Loose soil or sand can be removed by rinsing the cast surface with water and by using a soft brush.
7 Interpretation
   A. The cast is examined for general and randomly acquired characteristics.
   B. Compare the questioned cast to the known footwear/cast (or vice versa) as in Comparison of Impression Evidence.

8 Literature References and Supporting Documentation
TE-09-07 ENHANCEMENT OF IMPRESSIONS USING DIAMINOBENZIDINE (DAB)

1 Scope

Diaminobenzidine (DAB) treatment of suspected bloody impressions can be used on porous and non-porous surfaces to enhance and develop the impressions.

DAB is converted to a dark brown insoluble product in the presence of hydrogen peroxide as a result of the peroxidase activity of the hemoglobin derivatives in blood. It is particularly useful on porous items such as sheets and other fabrics as it causes minimal or no staining of the material.

It is strongly recommended that every attempt be made to retrieve the original items bearing the bloody impression so that they may be treated in the laboratory.

DAB has no effect on the Amido Black process, however DAB cannot be used after an item has been treated with Amido Black or Luminol.

2 Specifications

DAB Stock Solution
DAB Working Solution

3 Related Chapters

Reagents
Sulfosalicylic Acid Fixer Solution
Comparison of Impression Evidence
Digital Imaging and Processing of Impression Evidence

4 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Biohazard precautions, if applicable

5 Equipment and Materials

- Graduated cylinders
- Balance
- Volumetric Flasks
- Trays of miscellaneous sizes
- Beakers
- Wash bottle
- 2% 5-Sulfosalicylic Acid Fixer
- Commercially available pH 7.4 phosphate buffer
- 3% Hydrogen Peroxide or 30% Hydrogen Peroxide
- 3,3'-Diaminobenzidine tetrahydrochloride
- Water (de-ionized or distilled)
6 Standards, Controls, and Calibration

Known bloodstain

7 Procedure

7.1 Solution Preparation

A. DAB Stock Solution: dissolve 1 g 3,3’-Diaminobenzidine tetrahydrochloride in 100 mL of water.

B. DAB Working Solution
1. Mix 20 mL commercially available pH 7.4 buffer with 160 mL water.
2. Add 20 mL DAB stock solution to the 180 mL diluted buffer solution.
3. Add 1 mL 30% Hydrogen Peroxide or 10 mL 3% Hydrogen Peroxide.

7.2 Application
1. Ensure examination quality photographs of the impression are taken before any enhancement is attempted.
2. Demonstrate that the DAB working solution is working properly by testing a known bloodstain for a dark brown positive result. Document the results.
3. Saturate the item with 2% 5-Sulfosalicylic Acid Fixer for 4 minutes.
4. Rinse the item thoroughly with water.
5. Saturate the item with the DAB working solution. Keep the specimen wet with the DAB working solution until the impression fully develops (approximately 4 minutes).
6. Rinse the item thoroughly with water.
7. When dry, take examination quality photographs of the processed impression.

8 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes reagent name, initials, and date prepared.
B. Store the DAB stock solution in the freezer for six months. The DAB working solution is prepared fresh for use.
C. The DAB stock and working solutions cannot be disposed of by pouring down the drain. Dispose of solutions through external service provider. Refer to the SDS of 3,3’-Diaminobenzidine tetrahydrochloride for additional information.

9 Interpretation

A. The enhanced impression is examined for general and randomly acquired characteristics
B. Compare the enhanced impression to the suspected footwear as in Comparison of Impression Evidence.

10 Limitations

DAB cannot be used after an item has been treated with Amido Black or Luminol.
11 Literature References and Supporting Documentation


TE-09-08  SULFOSALICYLIC ACID FIXER SOLUTION

1 Scope
Sulfosalicylic Acid solution is used to fix bloody impressions to ensure that the impression is not dissolved or washed away during processing.

2 Specifications
2% 5-Sulfosalicylic Acid Fixer

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Balance
- Volumetric flask
- Beaker
- 5-Sulfosalicylic Acid
- Water (distilled or de-ionized)

6 Instructions
1. Dissolve 20 g 5-Sulfosalicylic Acid in approximately 500 mL water.
2. Pour into a 1000 mL volumetric flask.
3. Dilute to volume using water.

7 Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes reagent name, initials, and date prepared.
B. The reagent shall be prepared fresh.
C. The solution can be disposed of by pouring down the drain.
TE-09-09 ENHANCEMENT OF IMPRESSIONS BY AMIDO BLACK

1 Scope
Amido Black is a protein stain that will react with a bloody impression to produce a dark blue-black color. This staining may cause a latent bloody impression to become visible and provide additional characteristics of the impression for comparison purposes.

It is strongly recommended that every attempt be made to retrieve the original items bearing the bloody impression so that they may be treated in the laboratory.

2 Specifications
Amido Black-Methanol Based solution
Amido Black-Aqueous Based solution
Amido Black Rinse solution

3 Related Chapters
Reagents
Digital Imaging and Processing of Impression Evidence

4 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Biohazard precautions, if applicable
C. Good ventilation

5 Equipment and Materials
- Balance
- Graduated cylinders
- Bottles
- Trays of miscellaneous sizes
- Wash bottle
- Amido Black (also known as Amido 10B or Naphthalene Black)
- Glacial Acetic Acid
- Methanol
- 5-Sulfosalicylic Acid
- Water (distilled or de-ionized)

6 Standards, Controls, and Calibration
Known bloodstain

7 Precautions
DAB processing will not be possible after treatment with Amido Black.
8 Procedure

8.1 Solution Preparation

A. Amido Black-Methanol Based solution
   1. Mix 100 mL Glacial Acetic Acid with 900 mL Methanol in a 2 L bottle.
   2. Dissolve 2 g Amido Black in the solution.

B. Amido Black-Aqueous Based solution
   Dissolve 2 g Amido Black and 20 g 5-Sulfosalicylic Acid in 1 L water.

C. Amido Black Rinse Solution
   Mix 100 mL Glacial Acetic Acid with 900 mL Methanol.

8.2 Application

1. Ensure examination quality photographs of the impression are taken before any enhancement is attempted.
2. Demonstrate that the Amido Black Solution is working properly by testing a known bloodstain for a positive blue result. Document the results.
3. Test a small area of the item with the Amido Black-Methanol Based solution, away from the impression, to check for background staining and/or distortion. Document the results.
   a) If the background staining can be rinsed away by the Rinse solution, and if the paint on the item does not distort, proceed with the Amido Black-Methanol Based solution.
   b) If background staining cannot be rinsed away by the Rinse solution, or if the paint on the item distorts, test another small area with the Amido Black-Aqueous Based solution.
   c) If background staining cannot be rinsed away by water, use a different enhancement technique.
4. Apply the selected Amido Black solution to the impression by immersion or by direct application. Keep the impression wet with the Amido Black solution until the impression fully develops (approximately 3 minutes).
5. Rinse the specimen thoroughly, using the Rinse solution for the Amido Black-Methanol Based solution or water for the Amido Black-Aqueous Based solution.
6. When dry, take examination quality photographs of the enhanced impression.

9 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes reagent name, initials, and date prepared.
B. Store in a sealed bottle at room temperature for up to two years.
C. Dispose by pouring down the drain.

10 Interpretation

A. The enhanced impression is examined for general and randomly acquired characteristics
B. Compare the enhanced impression to the suspected footwear as in Comparison of Impression Evidence
11 Literature References and Supporting Documentation


TE-09-10 DETECTION OF BLOOD IMPRESSIONS BY LUMINOL

1 Scope
Luminol is used in the laboratory and at crime scenes to detect latent bloodstain patterns, footwear impressions, and latent prints. Luminol undergoes a catalytic reaction by the heme group of hemoglobin to produce luminescence. It is of sufficient sensitivity to luminesce in the presence of blood concentrations that are far more dilute than could be visually detected.

2 Specifications
Luminol, Reagent A
Luminol, Reagent B
Luminol Solution

3 Related Chapters
Reagents
Comparison of Impression Evidence

4 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Biohazard precautions
C. Gloves, dust mask, and eye goggles

5 Equipment and Materials
- Graduated cylinder, 250 mL
- Balance to weigh 0.5-25 g
- Beakers, 500 mL
- Sodium carbonate
- Sodium perborate
- 3% Hydrogen peroxide (H₂O₂)
- Luminol (3-aminophthalhydrazide; 5-amino-2,3-dihydro-1,4-phthalazinedione sodium)
- Water (distilled or de-ionized)
- Spray bottle

6 Standards, Controls, and Calibration
A. A known bloodstain is used to demonstrate the luminol will properly luminesce with the blood.
B. A ruler with fine copper wire spaced at one inch intervals can be used as a photographic scale.
7 Procedure

7.1 Solution Preparation

A. General Considerations

1. The Luminol solution is unstable and shall be mixed immediately before use.

2. Reagents A and B can be stored separately at 2-8°C for up to 8 weeks.
   a) If stored, the label must include at a minimum, “Luminol, Reagent A” or “Luminol, Reagent B” as appropriate, initials, and the date prepared.

B. Method 1

1. Reagent A
   a) Dissolve 25 g sodium carbonate in 250 mL water.
   b) Dissolve 0.5 g luminol in the solution.

2. Reagent B
   Dissolve 3.5 g sodium perborate in 250 mL water.

3. Luminol solution
   Immediately prior to use, mix equal volumes of Reagent A and Reagent B. This mixture is stable for approximately one hour.

C. Method 2

1. Reagent A
   a) Dissolve 25 g sodium carbonate in 450 mL water.
   b) Dissolve 0.5 g luminol in the solution.

2. Reagent B
   Combine 50 mL 3% H₂O₂ and 45 mL water.

3. Luminol solution
   Immediately prior to use, prepare and mix equal volumes of Reagent A and Reagent B. This mixture is stable for approximately one hour.

7.2 Examination

1. In near total darkness and after the eyes have become adjusted, spray a known bloodstain and observe for luminescence to ensure that the Luminol Solution is working properly. Document the results.

2. Lightly spray the Luminol Solution on the items or areas to be examined for possible bloody impressions.

3. Document with photographs any luminescent patterns, including a scale for reference.

4. Mark areas of possible impressions which may be enhanced with other techniques.
8 **Interpretation**

A. The photographs of the impression are examined for general and randomly acquired characteristics.

B. Compare the impression to the suspected shoes as in *Comparison of Impression Evidence*.

9 **Literature References and Supporting Documentation**


TE-09-11 GELATIN LIFT

1 Scope
Impressions created in dry dust or dry impressions on relatively clean surfaces may be recovered with a gelatin lift. Impressions created by wet footwear can also be developed by a powdering process and recovered by a gelatin lift.

Gelatin lifts are generally used if the item bearing the imprint cannot be removed from the scene, electrostatic lift was unsuccessful, or when the imprint is discovered as a result of powdering the scene.

Impressions recovered by using a gelatin lift may exhibit more detail than observed in photographs due to increased contrast.

Surfaces should first be examined for impressions using Detection of Impression Evidence by Oblique Lighting.

2 Related Chapters
Detection of Impression Evidence by Oblique Lighting
Comparison of Impression Evidence
Digital Imaging and Processing of Impression Evidence

3 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials
Gelatin lift (Black, White, or Translucent)

5 Standards, Controls, and Calibration
A. The lift should be larger than the impression being recovered.
B. The background of the lift should contrast the impression or powder.

6 Procedure
1. Ensure examination quality photographs are taken before attempting to lift any detected impressions.
2. Obtain the proper size gelatin lift to use after viewing the area and size of the impression.
3. To obtain the lift:
   a) Peel the separator from the adhesive side of the gelatin lift and set aside.
   b) Place the adhesive side gently over the impression.
   c) Gently press the lift with an ink roller or the ball of a finger.
   d) Remove the lift in a steady, even motion.
   e) Replace the plastic cover by starting at one edge and rolling it across to the other, making sure to not leave air bubbles.
4. Mark each lift with case number, item number, date, and examiner's initials.
5. Photograph or scan the lifts to preserve the impression.
7 Interpretation
   A. The lifts and photographs of the impression are examined for class and randomly acquired characteristics.
   B. Proceed to Comparison of Impression Evidence.

8 Literature References and Supporting Documentation
TE-09-12 ENHANCEMENT OF IMPRESSIONS BY LEUCOCRYS TAL VIOLET

1 Scope

Leucocrystal Violet (LCV) and hydrogen peroxide react with hemoglobin or its derivatives to produce a violet color. This staining may cause a latent bloody impression to become visible and provide additional characteristics of the impression for comparison purposes.

It is strongly recommended that every attempt be made to retrieve the original items bearing the bloody impression so that they may be treated in the laboratory.

Any developed impressions shall be photographed as soon as possible because photoionization may occur, resulting in unwanted background development.

Amido black can be used after treatment with LCV to increase the contrast.

2 Specifications

Leucocrystal Violet Solution

3 Related Chapters

Reagents

Comparison of Impression Evidence

Digital Imaging and Processing of Impression Evidence

4 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Biohazard precautions, if applicable

C. Good ventilation

5 Equipment and Materials

- Spray Bottle
- Weighing balance
- Graduated cylinder
- Light sensitive storage bottle
- Hydrogen peroxide 3%
- 5-sulfosalicylic acid
- Sodium acetate
- Leucocrystal violet

6 Standards, Controls, and Calibration

Known bloodstain
7 Procedures

7.1 Solution Preparation

1. Combine chemicals in the following order in a light sensitive storage bottle.
   a) 1000 mL Hydrogen Peroxide 3%
   b) 20 g 5-sulfosalicylic acid
   c) 7.4 g Sodium acetate
   d) 2 g Leucocrystal violet

2. Place bottle on stirring device for approximately 30 minutes.

7.2 Application

1. Ensure examination quality photographs of the impression are taken before any enhancement is attempted.

2. Demonstrate that the Leucocrystal violet solution is working properly by testing a known bloodstain for a positive violet result. Document the results.

3. Test a small area of the item with the Leucocrystal Violet solution, away from the impression, to check for background staining and/or distortion.
   a) Document results.
   b) If background staining occurs, a different chemical enhancement method should be considered.

4. Spray the bloody impression with the Leucocrystal Violet solution using the finest mist possible. The development should occur within 30 seconds.

5. If necessary, blot the area with a tissue or paper towel. When the area is dry, the preceding step can be repeated to possibly improve contrast.

6. Photograph the enhanced impression as soon as possible to avoid background staining due to photoionization. Photographs shall be examination quality.

8 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes reagent name, initials, and date.

B. Any unused Leucocrystal violet solution shall be stored in dark bottles and refrigerated.

C. Discard after one year.

D. Dispose by pouring down the drain.

9 Interpretation

A. The enhanced impression is examined for general and randomly acquired characteristics.

B. Compare the enhanced impression to the suspected footwear as in Comparison of Impression Evidence.

10 Literature References and Supporting Documentation


TE-09-13 DIGITAL IMAGING AND PROCESSING OF IMPRESSION EVIDENCE

1 Scope
Digital processing of impression evidence can be a valuable tool for the forensic examiner. Digital processing can be used as a stand alone technique when other processing methods are not suitable. It can also be used before, after, or in conjunction with other development techniques such as chemical processing. Proper image capture, processing, preservation, and storage of the digital images shall be considered.

2 Related Chapters
Comparison of Impression Evidence
Fabric Impression Evidence

3 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials
- Digital camera [8 megapixel minimum (12-24 megapixel is preferable)]
- Interchangeable lenses for camera
- Detachable flash and/or flash with remote capabilities
- Level
- Media card(s)
- Tripod or stand
- Light sources
- Scaling devices
- Scanner
- Printer
- Electronic storage media (i.e. CD-R and DVD-R)
- Digital image processing software

5 Definitions
The following definitions pertain only to the methods outlined in this chapter.

**Calibration** – The processing of an image to create a 1:1 representation.

**Camera RAW** – The raw image data as it comes directly off the imaging sensor. In order for it to be viewed it shall be converted into a conventional image format using appropriate software. The original image data remains unchanged while a great degree of flexibility exists to process the data including white balance, exposure latitude, and color correction. Due to the nature of camera RAW images, any processing performed to the image shall be saved into a secondary format such as TIF.

**Canon Raw (.crw) or Canon Raw Version 2 (.cr2)** – The proprietary RAW format used in Canon cameras. Depending on the model of Canon camera used, the color range is either 12 bits (.crw) or 14 bits (.cr2).
Processing – Any process or technique intended to improve the visual appearance of an image. Processed images are referred to as working images.

JPEG Standard (.jpg) – Joint Photographic Expert Group is a file format used in digital imaging to compress images. Image compression reduces the size of an image file, saves storage space, and reduces the time needed to transmit an image from one location to another. The JPEG format is subject to degradation and loss of data each time it is saved.

Lossless Compression – Compression in which no image data is lost and the image can be retrieved in its original form.

Nikon Electronic File Format (.nef) – The proprietary RAW format used in Nikon digital cameras.

Original Image – An accurate and complete replica of the primary image, regardless of media.

Tagged Image File Format (.tif, .tiff) – Tagged Image File Format is a file format used to store digital processed images. TIF files can use a lossless compression process, but the original data can be restored without any loss of data.

Working Image – Copy of an original image that may be calibrated or processed for the purpose of comparative analysis. Enhanced images are considered working images.

6 Procedure

6.1 Images Obtained By the Laboratory

A. Examination Quality Photographs

1. Photographs should be taken with the camera mounted on a tripod or other stand to ensure that there is no movement.

2. Include a scaling device in the photographs. The scale should be placed along the length of the impression at the same plane as the bottom of the impression.

3. Include case information (laboratory case number, item number, date, and initials) in or on the photograph.

4. Ensure the impression fills the field of view of the camera and is in focus.

5. Overlapping exposures for large impressions may be necessary. Overlapping exposures can also be used for smaller impressions.

6. Illuminate the impression using appropriate lighting techniques. Light sources, angles, and directions can all be adjusted to capture details in the impression(s). Additional photographs can be taken for each lighting adjustment. Camera settings may need to be adjusted for each lighting scenario.

7. Photographs should be taken at 90° to the impression. A leveling device may assist in this determination.

8. Capture the impression with a digital camera in the highest resolution lossless format such as RAW or TIF.

   a) Grayscale digital imaging should be captured at a minimum of 8 bits

   b) Color digital imaging should be captured at a minimum of 24 bits

9. Immediately record photographed images onto a memory card (or other storage device) or send to a workstation.
10. If the impression is processed (i.e. chemical processing), the impression should be re-photographed as above.

B. Scanner
   1. Place impression evidence onto a flatbed scanner.
   2. Include a scaling device with the evidence.
   3. Include case information (laboratory case number, item number, date, and initials) with the item or on the scanned image.
   4. Acquire image 1:1 in the highest resolution lossless such as TIF.
      a) Grayscale digital imaging should be captured at a minimum of 8 bits
      b) Color digital imaging should be captured at a minimum of 24 bits
   5. Immediately record scanned images directly onto the workstation to which it is connected.

6.2 Images Submitted to the Laboratory
   A. Examination Quality Photographs
      1. Ensure submitted image is in focus, taken at 90° to the impression, and contains an appropriate scale.
      2. Submitted photographs that do not meet these criteria may not be suitable for further examinations.

6.3 Digital Image Processing
   1. Perform any digital image processing on a working copy of the image(s). Do not overwrite the original image file.
   2. Document the software application and version used for digital processing in the case folder.
   3. Ensure that history tracking is enabled on the software so that any changes that are made to the image will be embedded within the processed image file. These changes can be later retrieved through the software.
      a) The history of the final image(s) may also be printed and included with the case folder. If necessary, additional notes may accompany the history log.
      b) If a history tracking feature is unavailable in the software, thorough notes shall be taken to permit another authorized examiner to understand the sequence of processing steps taken, the techniques used, as well as the settings and parameters used to extract comparable information from the image(s).
   4. Process the working image(s) using approved techniques which include, but are not limited to,
      a) Color Processing,
      b) Levels,
      c) Curves,
      d) Color Balance,
      e) White Balance,
f) Grayscale Conversion,
g) Hue/Saturation,
h) Brightness/Contrast Adjustment,
i) Inversion,
j) Resizing,
k) Image Sharpening,
l) Noise Reduction,
m) Color Channel Selection,
n) Subtraction, and
o) Fast Fourier Transform (FFT) Filters.

5. Tools and/or techniques that may potentially add or delete content from an image are prohibited and include, but are not limited to,
   a) Rubber Stamp,
   b) Airbrush,
   c) Paintbrush,
   d) Paint Bucket, and
   e) Eraser.

6. If necessary, calibrate and print the image(s) 1:1 for comparison.

6.4 Storage
A. The original image(s) and working image(s) will either be stored on non-rewriteable electronic media (i.e. CD-R or DVD-R) and will be considered evidence, or uploaded onto an appropriate data server, such as Foray.

B. Printed copies of the original image(s) and processed image(s) may be included in the case folder and/or stored as physical evidence and should be clearly labeled (original copy vs. processed copy).

7 Interpretation
If the digital image is suitable for comparison, the examiner will proceed to Comparison of Impression Evidence or Fabric Impression Evidence for analysis and comparison.

8 Literature References and Supporting Documentation


10 CHEMICAL SCREENING TESTS

TE-10-01 SILVER NITRATE TEST

1 Scope
This test is used for the presumptive detection of halides. With the exception of fluoride, halides will form a white precipitate upon application of the reagent.

2 Specifications
Silver Nitrate Solution

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Silver nitrate
- De-ionized water
- Nitric acid
- Graduated cylinders
- Balance
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Amber glass bottle

6 Procedure

6.1 Reagent Preparation
1. Mix 5 g Silver Nitrate in 100 mL water
2. Store in amber glass bottle at room temperature.
3. The reagent shall be prepared fresh.
4. Verify and document the quality of the reagent with a chloride containing salt (positive control) and water (negative control).

6.2 Sample Preparation
1. Liquid samples can be tested directly.
2. If the evidence is a stain, a portion of the stained area and an equal portion of the unstained area are extracted separately with the same minimum volume of water.
3. If the evidence is dried residue, it can be dissolved in a minimum of water.
6.3 Application
1. Place 5-6 drops of the solution in a test tube.
2. Add 1 drop of Silver Nitrate reagent.
3. Document the reaction.

7 Interpretation
The presumptive presence of silver nitrate will yield a white curdy precipitate with chloride, bromide, and iodide.

8 Limitations
A. Silver nitrate gives no precipitate with fluoride.
B. Silver chloride, silver bromide and silver iodide are isomorphous and are similar in crystallization. Their solubility does not differ enough so that any dependable separation may be obtained by taking advantage of this property.
C. Sulfide and high concentrations of chromate will also react.

9 Literature References and Supporting Documentation
TE-10-02  BARIUM CHLORIDE TEST

1 Scope
This test is used for the presumptive detection of sulfate.

2 Specifications
Barium Chloride Reagent

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Barium chloride
- De-ionized water
- Graduated cylinders
- Balance
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Amber glass bottle

6 Procedure
6.1 Reagent Preparation
1. Dissolve 5 gm Barium Chloride in 100 ml water
2. Store in amber glass bottle at room temperature
3. The reagent shall be prepared fresh
4. Verify and document the quality of the reagent with a 1% aqueous sodium sulfate solution (positive control) and water (negative control)

6.2 Sample Preparation
1. Liquid samples can be tested directly.
2. If the evidence is a stain, a portion of the stained area and an equal portion of the unstained area are extracted separately with the same minimum volume of water.
3. If the evidence is dried residue, it can be dissolved in a minimum of water.

6.3 Application
1. Place 5-6 drops of the solution in a test tube.
2. Add 1 drop of Barium Chloride reagent.
3. Document the reaction.
7 **Interpretation**

A white precipitate is formed to indicate the presumptive presence of sulfate.

8 **Literature References and Supporting Documentation**


TE-10-03  DIPHENYLAMINE TEST

1 Scope
This test is used for the presumptive detection of nitrocellulose, nitric acid residue, nitrates, nitroglycerin, and other organic nitrates.

2 Specifications
Diphenylamine Reagent

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Diphenylamine
- Sulfuric acid
- Acetic acid
- Balance
- Beakers
- Pipettes
- Dropper bottle
- Microscope slides
- Spot plate

6 Procedure
6.1 Reagent Preparation
1. Mix 20 mL Sulfuric Acid and 10 mL Acetic Acid and allow to cool before use
2. Dissolve 0.3 g Diphenylamine in the acid mix
3. Verify and document the quality of the reagent with nitrocellulose paint or an inorganic nitrate (positive control) and sodium chloride or non-nitrocellulose paint (negative control)
4. The reagent shall be replaced after one year, when it becomes so discolored that any color reaction is hidden, or when it fails to yield a strong positive result with a positive control; whichever comes first.

6.2 Sample Preparation
1. Liquid samples can be tested directly
2. If the evidence is dried residue, it can be tested directly

6.3 Application
1. Place a small amount of the sample on a glass slide or in a spot plate well
2. Add 1 drop of Diphenylamine reagent
3. Document the reaction in the case record
7 Interpretation
   A. A presumptive positive reaction is the production of an intense dark blue color within 5 seconds of application of the Diphenylamine reagent.
   B. Nitrocellulose paints, inorganic nitrates, nitroglycerin and other organic nitrates will give a positive result.

8 Limitations
   A. Chlorates will also produce the dark blue color.
   B. Fiegl reports that nitrites, bromates, iodates, chromates and other oxidizing agents will produce the blue color.

9 Literature References and Supporting Documentation
TE-10-04 AMMONIUM MOLYBDATE TEST

1 Scope
This test is used for the presumptive detection of phosphate.

2 Specifications
Ammonium Molybdate Solution

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Nitric acid
- Molybdic acid
- Ammonium hydroxide
- Phosphoric acid
- De-ionized water
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Hot plate
- Spot plate
- Amber glass bottle

6 Procedure
6.1 Reagent Preparation
1. Mix 3 mL Nitric Acid in 10 mL water to make 3 M Nitric Acid
2. Dissolve 2 g Molybdic Acid in a mixture of 6 mL water and 3 mL Ammonium Hydroxide. Add this solution slowly, and with stirring, to a mixture of 23 ml water and 10 mL Nitric Acid to form the Ammonium Molybdate solution
3. Verify and document the quality of the test with a 10 mL 1% Phosphoric Acid solution (positive control) and water (negative control)
4. The reagent shall be prepared fresh.

6.2 Sample Preparation
1. Liquid samples can be tested directly.
2. If the evidence is a stain, a portion of the stained area and an equal portion of the unstained area are extracted separately with the same minimum volume of water.
3. If the evidence is dried residue, it can be dissolved in a minimum of water.
6.3 Application

1. Mix 4-5 drops of the sample solution in a test tube.
2. Acidify with 3 M Nitric Acid.
3. Add 3-4 drops of the Ammonium Molybdate solution and mix.
4. Heat almost to boiling for 2 minutes.
5. Document the reaction.

7 Interpretation

A. Formation of the finely divided yellow precipitate, the ammonium salt of phosphomolybdic acid, indicates the presumptive presence of the phosphate ion.

B. Precipitation takes place most readily at a temperature of 60° C in the presence of excess Nitric Acid.

8 Limitations

Both phosphates and arsenates react with ammonium molybdate to form insoluble yellow precipitates.

9 Literature References and Supporting Documentation


TE-10-05 ZINC CHLORIDE TEST

1 Scope
This test is used for the presumptive detection of hypohalites. The most common application is to test for hypochlorite due to the presence of bleach.

2 Specifications
Zinc Chloride Reagent

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Zinc chloride
- Potassium iodide
- Starch
- De-ionized water
- Bleach
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Amber glass bottle
- Cotton swabs

6 Procedure
6.1 Reagent Preparation
1. Dissolve 2 g Zinc Chloride, 0.1 g Potassium Iodide and 0.2 g Starch in 10 mL water to form the Zinc Chloride reagent.
2. Verify and document the quality of the reagent with a 10% bleach solution (positive control) and a chloride containing salt (negative control).
3. Discard the prepared reagents after use.

6.2 Sample Preparation
1. Liquid samples can be tested directly.
2. If the evidence is a stain, a portion of the stained area and an equal portion of the unstained area are tested directly, if allowed. Otherwise, swab the stain and the unstained areas with separate cotton swab moistened with water.
3. If the evidence is dried residue, it can be tested directly or swabbed with a cotton swab moistened with water.
6.3 Application

1. Place the sample (2-3 drops of the liquid sample or the stain/swab) in a spot plate.
2. Add 1 drop of the Zinc Chloride reagent.
3. Document the reaction.

7 Interpretation

A. The formation of a blue-black color indicates the presumptive presence of a hypohalite (hypochlorite, hypobromite, hypoiodite). Due to the interaction between the hypohalite ions, a mixture of them can never be expected. It would be exceedingly rare to find hypobromite or hypoiodite in a normal household environment.

B. The instability of the hypohalites is such that they may be characterized more by their oxidizing power and by their decomposition product than by the reaction of the hypohalites themselves. Solutions of hypohalites ordinarily smell of the free halogen. The characteristic odor of household bleach may be the only indicator of the presence of hypochlorite.

8 Limitations

A. Some samples may be oxidized to a different color by a hypohalite.

B. This test will react with oxidizers other than bleach, such as hydrogen peroxide.

9 Literature References and Supporting Documentation


TE-10-06 RESORCINOL TEST

1 Scope
This test is used for the presumptive detection of sugar.

2 Specifications
Resorcinol Reagent

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Resorcinol
- Sulfuric acid
- De-ionized water
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Amber glass bottle

6 Procedure
6.1 Reagent Preparation
1. Dissolve 0.1 g Resorcinol in 10 mL water.
2. Verify and document the quality of the reagent with sucrose (positive control) and sodium chloride (negative control).
3. The reagent shall be prepared fresh

6.2 Sample Preparation
1. Liquid samples can be tested directly.
2. If the evidence is dried residue, dissolve it in a minimum of water.

6.3 Application
1. Place about 1 mL Sulfuric Acid in a test tube.
2. In a separate tube, mix 2 drops of Resorcinol reagent and 2 drops of sample.
3. Add the Resorcinol/sample mix, one drop at a time, by allowing it to flow down the side of the tube and stratify above the Sulfuric Acid.
4. Document the reaction.
7 Interpretation
   A. A cherry red color appearing within 1 minute indicates the presumptive presence of a sugar.
   B. Starch and cellulose produce an orange color.

8 Literature References and Supporting Documentation
TE-10-07  1-NAPHTHOL TEST

1  Scope
This test is used for the presumptive detection of sugar.

2  Specifications
1-Naphthol Reagent

3  Related Chapters
Reagents

4  Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5  Equipment and Materials
- 1-Napthol
- Ethanol
- Sulfuric acid
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Amber glass bottle

6  Procedure
6.1  Reagent Preparation
1.  Dissolve 3.0 g 1-Naphthol in 20 mL Ethanol.
2.  Verify and document the quality of the reagent with sucrose (positive control) and sodium chloride (negative control).
3.  The reagent shall be prepared fresh

6.2  Sample Preparation
1.  Liquid samples can be tested directly.
2.  If the evidence is dried residue, dissolve it in a minimum of water.

6.3  Application
1.  Place 2 drops of the sample solution in a test tube.
2.  Add 2 drops of 1-Naphthol reagent.
3.  Add 2 drops of Sulfuric Acid.
4.  Document the reaction.
7 Interpretation

A. A purple color will result from un-charred sugar.
B. A blue or purple-blue color is obtained from partly charred sugar.
C. Starch produces a purple color.

8 Literature References and Supporting Documentation

TE-10-08 EXPLOSIVE AND EXPLOSIVES RESIDUES SCREENING WITH EXPRAY KIT

1 Scope
The Expray kit is used to test samples for the presumptive presence of explosives and explosives residues.

2 Safety
   A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
   B. Biohazard precautions, as applicable

3 Equipment and Materials
Expray kit

4 Standards, Controls, and Calibration
   A. The kit contains RDX-impregnated verification strips.
   B. A strip is tested on the day of use to demonstrate the sprays react as expected and results shall be documented in the case record.

5 Procedure
   1. Wipe the sample with a collection wipe contained within the kit. Alternatively, a cotton swab may be used.
   2. Spray the wipe with the Step 1 spray. Note any color reaction that occurs within a few seconds.
   3. If there is no color change with the Step 1 spray, spray the wipe with the Step 2 spray. Note any color reaction that occurs within a few seconds.
   4. If there is no color change with the Step 2 spray, spray the wipe with the Step 3 spray. Note any color reaction that occurs within a few seconds.

6 Interpretation
   A. Step 1 spray will react with Group A explosives (nitro-aromatics such as TNT and TNB) as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Expected Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT, TNB</td>
<td>Dark brown-violet</td>
</tr>
<tr>
<td>DNT</td>
<td>Blue-green</td>
</tr>
<tr>
<td>Trinitro-Naphthalene</td>
<td>Violet</td>
</tr>
</tbody>
</table>

   B. Step 2 spray will react with Group B explosives (nitro-esters and nitro-amines such as RDX, Semtex, PETN, dynamite and smokeless powder) to give a pink color.
   C. Step 3 spray will react with inorganic nitrate-based explosives to give a pink color.
   D. The samples may be tested further with instrumental analysis.
7 Literature References and Supporting Documentation

Manufacturer's instructions for the Expray kit, developed by Mistral Group and sold by Symann Security.
TE-10-09  ANILINE SULFATE TEST

1  Scope
This test is used for the presumptive detection of chlorates.

2  Specifications
Aniline Sulfate Reagent

3  Related Chapters
Reagents

4  Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5  Equipment and Materials
- Aniline sulfate
- Sulfuric acid
- De-ionized water
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Amber glass bottle

6  Procedure
6.1  Reagent Preparation
1. Dissolve 0.1 g Aniline Sulfate in 10 mL water.
2. Verify and document the quality of the reagent with a 10 mL 1% aqueous sodium chlorate solution (positive control) and a chloride containing salt (negative control).
3. The reagent shall be prepared fresh.

6.2  Sample Preparation
1. Liquid samples can be tested directly.
2. If the evidence is dried residue, it can be tested directly.

6.3  Application
1. Place 3-4 drops of the sample in a test tube.
2. Add an equal amount of the Aniline Sulfate reagent.
3. Tilt the tube and slowly add 2-4 drops of Sulfuric Acid, allowing it to run down the side of the tube to make a separate layer.
7 Interpretation

After approximately one minute, the formation of a blue-violet color indicates the presumptive presence of chlorates.

8 Literature References and Supporting Documentation

TE-10-10 PYRIDINE TEST

1 Scope
This test is used for the presumptive detection of elemental sulfur.

2 Specifications
10% Sodium Hydroxide

3 Related Chapters
Reagents

4 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 Equipment and Materials
- Pyridine
- Sodium hydroxide
- De-ionized water
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Hot plate
- Amber glass bottle

6 Procedure
6.1 Reagent Preparation
1. Dissolve 1 g Sodium Hydroxide in 10 mL water to make 10% Sodium Hydroxide.
2. Verify and document the quality of the reagent with sulfur (positive control) and sodium sulfate (negative control).
3. The reagent shall be prepared fresh

6.2 Sample Preparation
1. Liquid samples shall be dried before testing.
2. If the evidence is dried residue, it can be tested directly.

6.3 Application
1. Place a small amount of the sample in a test tube.
2. Add 1 mL Pyridine.
3. Gently heat the tube without boiling.
4. Add 2-3 drops of 10% Sodium Hydroxide.
5. Document the reaction.
7 Interpretation

A blue to green color indicates the presumptive presence of elemental sulfur.

8 Literature References and Supporting Documentation

TE-10-11  THYMOL TEST

1  Scope

This test is used for the presumptive detection of nitrates and organic explosives.

2  Specifications

None

3  Related Chapters

Reagents

4  Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

NOTE: Chlorates will react vigorously with acid. The sample should be tested for chlorates prior to performing the Thymol test.

5  Equipment and Materials

- Thymol
- Ethanol
- Sulfuric acid
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Amber glass bottle
- Glass rod

6  Procedure

6.1  Reagent Preparation

1. No reagent preparation.
2. Verify and document the quality of the reagent with sodium nitrate (positive control) and sodium chloride (negative control).

6.2  Sample Preparation

1. Liquid samples can be tested directly.
2. If the evidence is dried residue, it can be tested directly.

6.3  Application

1. Mix a small amount of the sample and milligram quantities of Thymol in a spot plate using a glass rod.
2. Add 3 drops of Sulfuric Acid.
3. Add 5 drop of Ethanol.
4. Document the reaction.
7 Interpretation
   A. A green color indicates the presumptive presence of nitrates.
   B. A rose color indicates the presumptive presence of RDX.

8 Literature References and Supporting Documentation

1 **Scope**
This test is used for the presumptive detection of sucrose and dextrose.

2 **Specifications**
Potassium Hydroxide Reagent
Cobalt Acetate Reagent

3 **Related Chapters**
Reagents

4 **Safety**
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

5 **Equipment and Materials**
- Potassium hydroxide
- Cobalt acetate
- De-ionized water
- Balance
- Graduated cylinders
- Beakers
- Pipettes
- Test tubes
- Spot plate
- Hot plate
- Amber glass bottle

6 **Procedure**
6.1 **Reagent Preparation**
1. Dissolve 1.0 g Potassium Hydroxide in 10 mL DI water.
2. Dissolve 1.0 g Cobalt Acetate in 10 mL of DI water.
3. Verify and document the quality of the reagents with sucrose and/or dextrose (positive control) and sodium chloride (negative control).
4. The reagent shall be prepared fresh.

6.2 **Sample Preparation**
Dried residues or crystalline samples can be tested directly.

6.3 **Application**
1. A small amount of sample is added to a test tube.
2. Add one drop of DI water and one drop of potassium hydroxide solution to the test tube.
3. Heat the liquid until boiling.
4. Add one drop of cobalt acetate solution to the test tube and lightly mix the solution.

5. Document the reaction.

7 Interpretation
   A. A blue precipitate will form in the presence of sucrose.
   B. A green precipitate will form in the presence of dextrose.
   C. If the precipitate does not remain after light mixing then the result of the test is negative.

8 Literature References and Supporting Documentation
11 FIRE DEBRIS ANALYSIS

TE-11-01 HANDLING OF FIRE DEBRIS ANALYSIS ITEMS

1 Scope
This chapter outlines the specific procedures for the handling of evidence submitted for Fire Debris analysis. The procedures and appropriate facilities for avoiding deterioration, loss, or damage to Fire Debris analysis evidence during storage, handling, preparation, and analysis are also summarized.

2 Related Chapters
Instrumental Analysis of Fire Debris
Ignitable Liquid References, Standards and Materials

3 Safety
Follow general safety rules regarding materials in question. Refer to Instrumental Analysis of Fire Debris, CLS, and Safety manuals for safety procedures.

4 Equipment and Materials
- Evidence Tape (use green evidence tape, if available, to differentiate fire debris section seals)
- Awl (A metal tool with a sharp, pointed tip used for piercing a hole in an object)
- Fume Hood

5 Standards, Controls, and Calibration
None

6 Procedure
6.1 Receipt of Evidence
A. Evidence packages containing evidence with possible ignitable liquid content shall be sealed in such a way as to prevent spillage, breakage, or contamination of evidentiary materials.
B. During the receiving process, if the containers/cans appear to be ‘under pressure’ (i.e. the lids appear to be ‘bowing’ out, more convex than flat), evidence technicians may contact a Fire Debris analyst to relieve the pressure after assigning a case number, but before storing the evidence in the main vault.
   1. The analyst is responsible for ensuring evidence possession has been transferred to him/her.
   2. The analyst will take the evidence container to the designated fume hood and put on gloves. Making sure the hood is on and air is flowing, puncture the lid of the can while inside the hood using one of the sterile awls located inside the hood.
   3. Once the pressure has been released (should be an obvious release of air), the analyst will properly seal the hole with either packing tape or evidence tape.
   4. The analyst will return the evidence to the original receiving evidence technician. The analyst will ensure that this action is documented. (i.e. ensure that either...
documentation is made in the LIMS data file or a Laboratory Information Sheet (LIS) is added to the case record).

6.2 Storage and Transfer of Evidence

A. Fire debris evidence items will be stored in flammable cabinets or in refrigerated locations, where practicable.

B. Analysts are responsible for transporting evidence from the Evidence Coordination section and securing it in the appropriate locations of the fire debris analysis work areas.

C. Evidence items are stored in such a way as to ensure that there is limited and authorized access, while preserving the nature of the evidence. Where practicable, analysts will strive to complete sample run and return evidence to the Evidence Coordination Section (ECS) within 10 days.

D. If evidence is unusual or submitted under unusual circumstances, it may be left in the fire debris fume hood located in a secured area of the laboratory, if deemed necessary by the section supervisor.
   1. Examples may include evidence saturated with unknown liquids, odd-shaped packaging, leaking cans, etc.
   2. Evidence may be stored on a cart in the secured oven room of the fire debris analysis section, if deemed necessary, under similar unusual circumstances (i.e. unusually large packaging, during times of backlog, or rush case processing, etc.)

6.3 Evidence Storage During Analysis

A. Evidence for fire debris analysis that has been transferred from the main evidence vault located in the Evidence Coordination section (ECS) for processing will be stored in designated flammable cabinets in the GC/MS instrument room.

B. After samples have been prepared for processing and analysis, the evidence cans (or other evidence containers) can either be returned to the flammable cabinets or stored on a cart in the oven room until analysis has been completed.

C. Evidence that has been analyzed (completed) will be properly sealed by the analyst assigned to the case and stored in that analyst's assigned locked flammable cabinet. Evidence will remain in this secured area until it is ready for transfer back to ECS.

D. Evidence may not be left in unsecured and/or non-work areas unattended. Evidence may not be left or secured in office or cubicle areas.

6.4 Access to Evidence

A. Analysts are responsible for securing all evidence and work areas in the fire debris section of the laboratory at the close of business each day.

B. Where practicable, fire debris evidence assigned and in possession of an analyst should be secured in his/her designated flammable cabinet or in secure work areas while analysis is being completed.
   1. If the seal on the evidence has been broken for analysis procedures and the analyst must leave the immediate vicinity of the evidence, the analyst will ensure the door of the flammable cabinet is locked.
   2. The analyst should also lock evidence in a flammable cabinet if absent for an extended period of time.
C. If evidence is not actively being processed, it shall remain either in the main evidence vault with ECS, locked in the analyst’s assigned flammable cabinet, or the Fire Debris section biohazard refrigerator. If evidence is undergoing the analysis process (e.g. opened and preparation/analysis begun), but awaiting final steps, it should be stored in either the analyst’s flammable cabinet or on a cart in the secured oven room.

6.5 Return of Evidence

A. During analysis, extraction vials are created as a work product per sample. These may be stored in the analyst’s assigned flammable cabinet, or in designated cabinets in secured work areas of the fire debris section of the laboratory until such time as the required packaging is created to house the extraction vial(s) along with the LIMS generated barcode.

B. Although analysts are not required to return these labeled vial packages/envelopes to ECS at the same time as the associated evidence cans, it is the responsibility of the analysts to not only ensure that vial envelopes and barcodes are created per case vial set (in order to track this work product and to create a chain of custody) but to inform ECS that these vial envelopes need to be returned along side the physical evidence in the case. Therefore, ‘returning evidence under separate cover’ shall include all evidence in the submission and their accompanying extraction vials, where practicable.

7 Literature References and Supporting Documentation

ASTM 1459-13 Standard Guide for Physical Evidence Labeling and Related Documentation

ASTM 1492-11 Standard Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

NFPA921: Guide for Fire and Explosion Investigations (most current edition)

Equipment Manuals

Software Application Manuals
TE-11-02 INSTRUMENTAL ANALYSIS OF FIRE DEBRIS

1 Scope
To provide instruction regarding the procedures employed in the preparation and instrumental analysis of fire debris evidence, where the presence and identification of ignitable liquids is determined.

2 Related Chapters
Handling of Fire Debris Analysis Items
Fire Debris Analysis Equipment
Ignitible Liquid References, Standards and Materials

3 Safety
Universal safety precautions and general laboratory safety guidelines are to be followed, which may include use of PPE and fume hood. Procedure cites particular safety precautions.

4 Equipment and Materials
- Activated charcoal strips (ACS) also referred to as “Activated Carbon Strips” or “C-strips”
- Hooks (clean from manufacturer, unused, and quality tested for contamination)
- Screw top vial caps with septa and small sample vials (with appropriate measurement markings and writing space)
- Solid vial caps
- GC/MS solvent and wash vials
- Pry (a screwdriver or paint can lid removing tool)
- Awl (A metal tool with a sharp, pointed tip used for piercing a hole in an object)
- Laboratory Tweezers/Forceps (small: one flat & straight; one curved & bent)
- Silver spatula
- Mallet/Lid press
- Solvent (pentane or carbon disulfide [CS2])
- Solvent pump dispenser, variable pipettor, and/or glass pipettes with rubber bulb aspirator
- Sharpies, black and dark blue to indicate CS2, if available
- Empty/unused evidence cans (from laboratory stock, that have been quality tested)
- White paper (unused, from laboratory supply)
- Other laboratory supplies, as needed (e.g. sterile gauze, small scissors, box cutters, beakers, etc.)
- GC/MS

5 Standards, Controls, and Calibration

5.1 Ignitable Liquid Known Samples
A. To establish ignitable liquid comparison standards, at least one sample from each class of known ignitable liquids should be run on each active GC/MS. (TE-13-04)
B. These known samples shall be run under the same conditions and methods on the same GCMS used for case sample analysis, prior to and within no more than one year of the sample run. Known samples, include but are not limited to:

1. Gasoline (fresh and 75% evaporated)
2. Light Petroleum Distillate
3. Medium Petroleum Distillate
4. Heavy Petroleum Distillate
5. Isoparaffinic Products
6. Naphthenic-Paraffinic Products
7. Isopropyl Alcohol
8. Ethanol
9. Acetone (where practicable)
10. Limonene
11. 2-butoxyethanol (where practicable)

C. Additional knowns may be included at the discretion of the analyst. However, if changes in the GC/MS retention times are significant (exceeding 0.5 minutes) and/or sensitivity has significantly changed, then known samples must be rerun.

5.2 ASTM Test mix

A. The laboratory will use ASTM E1618 standard test mix for fire debris analysis from an approved vendor for performance checks (see TE-13-03)

B. The test mix shall be run on each GC/MS being used for analysis on a bi-weekly schedule. The required ASTM test mix for fire debris analysis is described below:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CAS Number</th>
<th>Target Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Decane</td>
<td>124-18-5</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Dodecane</td>
<td>112-40-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Eicosane</td>
<td>112-95-8</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>o-Ethyltoluene</td>
<td>611-14-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>m-Ethyltoluene</td>
<td>620-14-4</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>544-76-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>110-54-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Octadecane</td>
<td>593-45-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Octane</td>
<td>111-65-9</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Tetradecane</td>
<td>629-59-4</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>Toluene</td>
<td>108-88-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>1,2,4-Trimethylbenzene</td>
<td>95-63-6</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>106-42-3</td>
<td>0.05 %v/v</td>
</tr>
</tbody>
</table>

C. Analysts may need to run this test mix in CS2 prior to running actual case samples in CS2 (see TE13-04 for preparation of test mix)
5.3 Controls

A. A batch of samples (sequence) will have both positive and negative controls designed to provide quality assurance and quality control information.

B. The lots of pentane solvent as well as each new stock of cans and hooks are analyzed separately for quality and the results are maintained in an appropriately labeled location in the instrument room.

C. Activated charcoal strips and carbon disulfide (solvent) are also analyzed for quality and results are retained on a list alongside other consumable quality controls.

D. The negative control (blank) is created by hanging a c-strip in a contamination free can. This can is then heated alongside the sample cans to be run in the same sequence/batch.
   1. All consumables used in the blank for a particular sequence (e.g. hook, c-strip, vials/caps, and solvent) shall come from the same lot numbers of those being used in the evidence samples.
   2. This c-strip blank will be prepared in a vial using the same preparation method as the case samples (and same vial/cap lot numbers) and be run between each sample of that sequence. The vial should be labeled as “blank” with the date of creation.
   3. The negative control is run alongside the evidence samples from preparation to heating to instrumental analysis. The c-strip from this control (blank) is prepared in a separate, marked vial with solvent (all from the same stock that will be used in the accompanying evidence) and run before every sample in that batch sequence. A “clean” blank result provides information that:
      a) Laboratory cans from the stock used are uncontaminated
      b) Hooks from the lot used in the batch are uncontaminated
      c) C-strips from the lot used in that batch are uncontaminated
      d) Vials/caps from lot used in that batch are uncontaminated
      e) Pentane (or carbon disulfide) from lot used in that batch is uncontaminated
      f) No cross-contamination occurred during oven heating
   4. Lot numbers of all the aforementioned items used in an analyzed batch will be recorded on the LIMS analysis worksheet.

E. The positive control is known as the SIM (Standard Ignitable Mix) and will be prepared in two ways: dilution and heated c-strip extraction. Both SIMs will be run at the beginning and end of each sequence.
   1. The SIM consists of 50% gasoline (fresh) and 50% diesel fuel. *Vial of SIM should be shaken before use.
   2. The diluted positive control SIM is created simply by filling a 1 mL sample vial with solvent and adding a drop (~3-4 µL) of SIM from laboratory supplies. The vial should be labeled with “SIM” and date of creation.
   3. The extraction version of the SIM is created by placing sterile gauze, lab wipe, or Kimwipe® into a quality checked can and adding 1-3 drops (~3-9 µL) of SIM to this gauze. A c-strip (from the same lot being used for case samples) is then hung in the SIM prepared can, which is heated alongside the evidence samples (and the
negative control can) for the sequence/batch in which this positive control is being used.

a) After heating, c-strip is transferred and prepared similar to case sample prep: placed in a 1 mL vial with the solvent of choice added to it and labeled as “SIM” with date of creation.

b) The number of drops used is dependent on the solvent: if pentane is used, 1-2 drops (~4-8 µL) is sufficient whereas carbon disulfide requires at least 3 drops of the SIM (~12 µL).

c) The diluted SIM and extracted SIM, although labeled similarly, are distinguishable simply by observation of c-strip in extraction vial (i.e. the extracted SIM will obviously contain a c-strip while the dilution will not). However, analysts should label them using distinguishing notations, such as SIMD (dilution) and SIME (extracted).

5.4 Quality Control

A. In the event that a positive control gives a negative result, there will be an assessment of other samples run in the batch.

1. If every sample in the batch (including controls and samples) is negative, then a Quality Incident will be initiated.

2. Alternate positive controls and case work samples will be assessed for positive results.

3. Prior to the next batch being run, diluted and extracted positive controls will be prepared and analyzed using the same process, materials, and instrumentation that were used in the batch with the failed control.

   a) If the results indicate negative results from either control, a Quality Incident will be initiated.

   b) If the results indicate expected positive results, casework continues.

4. With an alternate acceptable positive control result, positive samples in the batch can be reported.

5. For batches with a failed positive control, negative casework samples will be reported as inconclusive with a qualifying statement that there was insufficient data to support a negative result.

6. If one or both of the positive controls differ from start to end (i.e. SIM at the beginning of a sequence is higher in abundance than the SIM at the end of the sequence or vice versa, the SIM at one end of the sequence failed while the other passed, etc.), the analyst will assess the situation based on the outcome of all the negative controls and evidence samples in the sequence. If a reasonable explanation cannot be reached as to the discrepancy in the SIM(s), the analyst will defer to the section supervisor for guidance.

B. In the event that a negative control gives a positive result, a process analysis will be conducted.

1. Instrumental data is assessed to determine if there are indications of carryover.

2. Further testing is conducted to isolate the source of the contaminant using the same process, materials, and instrumentation. All components of the process will
be assessed including the quality-checked consumables (e.g., pentane, c-strip, blank can, hook, etc.).

3. Any identified sources of contaminated consumables are removed and replaced.

4. Positive results in the batch are assessed for suitability of reporting.

5. The initiation of a Quality Incident is considered at all points of the investigation.

C. Sensitivity test mix:

1. In order to establish a minimum, all compounds in the 0.005% (v/v) ASTM Standard test mix shall be identified. To ensure accuracy of measurement, a new (dilution) vial of the ASTM Standard Test Mix for Fire Debris Analysis (E1618) should be prepared for each of the bi-weekly sensitivity test runs and by lab personnel only.

2. A separate mix of compounds may be run as a sensitivity check in addition to the aforementioned ASTM Standard Test mix at the discretion of the analyst. For example, a mixture of methyl alcohol, ethyl alcohol, acetone, and isopropyl alcohol in CS2 may also be run if desired. Because the laboratory generally uses pentane as a solvent except in cases where acetone is suspected, a quality check of the GC/MS in CS2 may be necessary before a sample is run in CS2.

3. The test mix is to be prepared by lab personnel from the known reference sample inventory. This sample will establish the consistency of compound retention times with the previously analyzed batch. These mixes also provide the benchmarks necessary for correcting possible deviations in instrument performance. Additionally, it shows that the instrument is operating properly.

D. Quality Assurance of Evidence Cans:

1. The laboratory performs quality checks on one-gallon and one-quart sized metal cans for laboratory use (blank cans).

2. The same performance check is performed on cans used in evidence by submitting agencies, per agency request. This service is offered to submitting agencies to alleviate submission of a control can per case. The procedure for this quality check is as follows:

3. The can is submitted using the appropriate form available on the crime lab website.

4. Cans are submitted separately (not with criminal case evidence) and properly sealed with can lot # written on can itself. The size can and lot number should be identified on the submission form.

5. The form and cans are received into the lab per evidence receiving protocols.

6. The resulting report should identify that this service follows accepted practices for testing consumable stock and clarify if the can is clear for use in evidence collection. Further, it is communicated that the lab is not responsible for the quality of the remaining stock of cans under the submitted lot number should the submitting agencies fail to maintain proper storage protocols to keep the other cans contamination free.
6 Procedure

6.1 Case Assignment

A. Generally, the section supervisor will assign cases based on an even distribution of samples, paying special attention to assign cases with soil and/or biohazard samples first.
   
   1. Where practicable, the section supervisor will assign all biohazard samples to one analyst and make every effort to rotate case sets so that biohazard sample sets are distributed equally among available analysts.
   
   2. Consult section supervisor if case assignment is unclear.

B. Analysts should strive to prepare samples for extraction within 5 business days after their receipt. Smaller batch sizes may need to be employed.
   
   1. Under unusual circumstances (e.g. loss of full-time authorized analyst, backlog, etc.), the section should strive to analyze samples within 30-60 days of receipt.
   
   2. If extreme circumstances prevent analysis of samples within 60 days of their receipt, it is up to management to appropriately address the issue.

C. Soil samples or samples suspected to have acetone or isopropyl alcohol shall be prioritized and worked as soon as practicable.
   
   1. Evidence marked as biohazard will be given next priority during times of backlog.
   
   2. The evidence will be flagged by receiving personnel upon inspection of submission form or upon review by the section supervisor while assigning cases.

D. The section supervisor may choose to reassign cases due to analyst absence (court, travel, illness, vacations, etc.) or to expedite analysis.

6.2 Receipt of Evidence

A. Once a case is assigned, create a worklist of these cases in LIMS.

   Note: All evidence marked as ‘biohazard’ shall be prepared in the designated Trace Section hood located on the first floor. Prior to analysis, biohazard samples may be stored in the designated biohazard refrigerators located in the secure oven room. After analysis, analysts may determine whether or not refrigeration of evidence is still needed.

B. The analyst should inspect each can to ensure that all cases are present, correctly identified, and properly sealed. The condition of the seal shall be documented on the worklist.

C. If the evidence is NOT properly sealed, the analyst shall make a note of this next to the appropriate case and item number on the worklist. In the case of missing initials on seal, the analyst will initial, date, and write ‘seal check’ or ‘seal √’ across existing intact tape seals prior to opening the can.

D. For evidence with unusual packaging (e.g. 5-gallon metal cans, paper bags, etc.) that would disrupt efficient flow of evidence preparation, set aside until all other cans have been prepared.

E. The analyst should note if the evidence container is breached in any way (i.e. holes, lid popped off, lid not completely seated). If the analyst determines that the breach is significant to evidence loss or contamination, the analyst shall issue a Closed without Analysis (CWA) Report and document the reason.
F. If the evidence container is breached and the analysis was performed for whatever reason:
   1. Analysts shall take photos of the breach and upload to LIMS. Breaches shall be properly sealed prior to heating the sample (e.g. lids seated correctly, holes taped over with evidence tape and initialed/dated, etc.)
   2. Analyst must document the reason for continuing with analysis. Examples may include:
      a) **Holes are small and appear to be corrosion that does not breach the inside of the can**, 
      b) **Lids may have come unseated between receiving and transfer to the evidence vault**, 
      c) **Only one can present in properly sealed box (limiting exposure), etc.**

G. Evidence needs to be properly sealed and secured in either of the storage locations in preparation for return to ECS. It is at the discretion of the analyst to determine the necessity of a proper seal on the damaged portions/holes in the evidence packaging.

### 6.3 Sample Preparation

A. Wipe down and clean general work space.

B. Remove appropriate number of c-strips from the bulk packaging (jar) and place on sterile surface. Ensure that bulk packaging is properly closed and returned to storage area. Only half a strip is needed per extraction of a sample (e.g. half a strip per evidence can/container).

C. Using sterilized laboratory scissors, cut a c-strip in half width wise to produce strips that are approximately 6 mm x 20 mm. A slight diagonal cut will provide a strip that can be more easily placed into a sample vial following extraction.

D. Punch a hole through the center of each c-strip using the hook itself or a sterilized lab awl. Pass one end of the hook through the hole to affix the c-strip to it, being sure to press the hook closed to “lock” c-strip in place. Place the same number of c-strips on hooks as there are cans of evidence (on a sterile surface), being sure to add one for the blank can and one for the extracted SIM can.

E. The c-strips on hooks may be prepared in advance and stored in an appropriately labeled c-strip jar or in one of the assigned, sterile c-strip drawers for each analyst located in the secured oven room on the second floor.

F. Cans must be opened (and closed) one at a time. As c-strips are placed inside each can, analysts will make note of contents of can as quickly and accurately as possible in order to minimize the time the sample is exposed/open. Analysts will document observations on the pre-printed worklist.

G. At times, a ‘bowing’ effect on a can lid will be apparent due to pressure build-up of can contents. If this is evident, the analyst may use a sterilized awl to punch a hole in the lid and release this pressure prior to opening the can for c-strip placement
   1. The analyst must immediately and properly re-seal this hole with either packing tape or evidence tape
   2. This pressure is indicative of high volatility of contents which may cause the lid to be expelled during heating. The analyst will take proper precautions (such as
taping the lid down with packing tape or ‘nesting’ the can in another can and sealing the outer can’s lid with tape, etc.) to prevent lid expulsion.

3. It is not always possible to predict this event and other steps may need to be taken to address this issue (see section 6.3 L).

H. For each item of fire debris evidence, a prepared c-strip is to be suspended in the “vapor space” of the container.

1. This is accomplished by hanging the hooked c-strip on the inside lip of the evidence can and then either immediately hammering down or using a lid press to reseat the can lid back in place.

2. The analyst may choose to leave a portion of the hook visible (e.g. slightly sticking out from underneath the lid) after securing the lid back in place, but shall ensure that this is done in such a way so as not to cause injury when retrieving or moving cans.

3. Note that in most cases, even if the evidence container is not a can, hanging a c-strip from a hook in the container can still be employed. See section supervisor for any issues with non-traditional containers.

I. All evidence samples will be analyzed using c-strips hung in the “vapor space” of a container and heated for approximately 2 hours at 80°C - 90°C ±10 °C. Note that for rush cases, heating time may be altered to approximately one hour at the higher temperature range.

1. After heating, remove evidence samples from the oven and allow cooling to ambient temperature (~1 hour).

2. While cans are cooling, GCMS vials should be labeled with the analyst's initials and corresponding lab case and item number for that sequence of cans.

3. Once cooled, the evidence containers are opened one at a time for c-strip transfer into the corresponding, labeled vials. Transfer step must be completed one can at a time; closing one can (& vial) prior to moving to the next can.

4. After placing the c-strip into the vial, add an aliquot of approximately 500 µL to 1 mL of the appropriate laboratory solvent, being careful not to cause ‘splashback’ of solvent and avoid touching the tip of the dispenser to the c-strip. This aliquot may also be added to each pre-labeled vial prior to transfer of the c-strip. *Note: this step shall be performed in the designated hood for all biohazard evidence and/or use of carbon disulfide as solvent.

5. Solvents are to be quality tested before use in analysis and it is the responsibility of the analyst to verify quality control records on a solvent before use.

J. If the oven minimum/maximum temperature of 80°C - 90°C ±10 °C should fall below the desired range during the ~2 hour heating time, the analyst shall leave the cans in the oven and monitor the temperature until it falls within this temperature range.

1. Once the analyst confirms that the approved temperature range has stabilized, the cans will be heated for approximately one additional hour from the time the temperature stabilized. The analyst is responsible for ensuring that the temperature remains within the required range for that hour by checking the oven temperature every ~20 minutes.
2. In order to verify that the extended heating time was sufficient for analysis, the analyst should run the heated SIM sample alongside the diluted SIM in a GCMS while cooling the cans from this set (prior to transfer of the c-strips into their vials).

3. If both SIMS are as expected, the analyst may continue with the steps for the GCMS run portion of the analysis process. However, if the heated SIM fails, the analyst must refer to the section supervisor to assess the appropriate steps.

K. If the oven minimum/maximum temperature of 80°C - 90°C ±10 °C should rise above the desired range during the ~2 hour heating time, the analyst shall remove the cans immediately from the oven and record the temperature.

   1. In order to verify that the extreme temperature did not negatively affect samples for analysis in this set, the analyst should run the heated SIM sample and the blank c-strip alongside the diluted SIM in a GCMS while cooling the cans from this set (prior to transfer of the c-strips into their vials).

   2. The analyst should then stabilize the oven temperature (TE-13-03)

   3. If both SIMS and blank are as expected, the analyst may continue with the steps for the GCMS run portion of the analysis process. However, if the heated SIM fails (missing a large portion of the "light" peaks) or the blank is compromised, the analyst must refer to the section supervisor to assess the next appropriate steps.

L. Due to the high heating temperatures, it is **not** uncommon for the lid of a can to be expelled during this process. Should this occur, the analyst will determine the best course of action to take.

   1. If the c-strip was not ejected from the can and the other can lids are intact, the analyst may continue with processing the evidence for run on the GC/MS per protocol.

   2. The analyst will make note if contamination occurred in the blank for that heated set.

      a) **If no contamination appears in the blank, the analyst may continue with analysis for the sample per normal procedure.**

      b) **If contamination does appear in the blank, documentation must be made on the worklist or worksheet of each sample in that sequence to explain the incident**

   3. If the sample that expelled the lid has affected the blank, assess possible contamination in the case samples. If the samples were affected, the analyst will make the determination with this contamination in mind and document on the worksheet that possible contamination from a sample with an expelled lid was the cause.

   4. If the c-strip was expelled from the can and/or the can contents were ‘spilled’ out of the can, a QI will be initiated and the proper course of action will be determined with section supervisor guidance.

M. Due to the evaporative nature of this type of evidence, it is ideal to transfer the c-strip to the sample vials as the sample cans have cooled down (~1 hour after removal) if not, the same day as heating.

   1. Every attempt should be made to transfer the c-strips to their designated vials on the same day as the samples were heated, at which point analysis can be
postponed (i.e. sealed vials can be stored in a secured location until they can be run in a GCMS).

2. If, for whatever reason, the c-strips could not be transferred in the same day, the cans containing the c-strips (lids firmly seated) should be stored in a secured location and should be transferred to their designated vials the very next day or as soon as possible. NOTE: once heated, c-strips should be transferred within 5 days, no later.

6.4 Instrumental Analysis

A. The GC data should produce a chromatogram with baseline separation of the majority of the components of the test mixture. (Exceptions are ortho and meta xylene which co-elute, acetone and isopropyl alcohol which are not baseline resolved, pentadecane which tends to co-elute with substituted alkanes; and tridecane and 1-methylnaphthalene which often co-elute even with optimized columns)

B. SIMS

1. Both SIMs shall be run at the beginning and end of every sequence.
2. Diluted SIMs should have a new vial prepared at least once a week to alleviate evaporation issues.
3. The extracted SIM should be created for every new sequence/batch.
4. The analyst is responsible for monitoring the sensitivity of both SIMs to ensure efficacy and proper intended use.
5. No more than an average of 20-25 samples per GCMS sequence/batch run is recommended. If an occasion arises where the number of samples run in a single sequence exceeds this recommended number, both SIMs should be run again within the sequence. For example, if the sequence batch consists of 40 sample vials, run both SIMs again after the first 20-25 case samples have been run before continuing the sequence run on the remaining evidence samples.

C. Blanks

1. To ensure quality, a blank shall be run before each evidence sample in a sequence.
2. A blank consists of a vial with a sample of solvent containing a control c-strip that was placed in a quality checked metal can and heated alongside the evidence being run in that particular sequence.
3. Consumables in the prepared blank-can shall come from the same lot # as those used in the evidence samples for which the blank was created. This includes the solvent, (control) c-strip, hook, vial, and vial caps.
4. The lot numbers for the aforementioned consumables, including the blank-can itself, should appear in the analysis worksheet/LIMS data for that case in which they were used.
5. The expected outcome is a flat baseline (i.e. no contamination present). Occasionally, blanks appear to be contaminated with peak show through. It is the responsibility of the analyst to address any peaks that appear in the blanks. These are usually ‘background noise’ and the analyst may document that no cross-contamination has occurred on either the analysis worklist or worksheet.
6. The solvent blank injected between case samples within a sequence must be clean of any carry-over contamination.

a) Analysts will examine blanks before and after the sample run to identify contamination by an ignitable liquid or other interference compounds in any blanks. If the contaminant in a particular blank is determined to have originated from the prior sample, this is to be noted in the case information. Ensure that the contamination has not affected the actual case sample.

   i. If the contamination appears to have affected the sample, the analyst will take this into consideration when making a determination and document this carry-over on the analysis worksheet

   ii. If the contamination does not affect a positive result, the analyst will document why the call can still be made

b) If the contamination has persisted to the next blank (and sample), analysts may proceed with one of the following steps:

   i. If the contamination is such that it does not interfere with accurate analysis of sequences samples, continue with analysis, making sure to note the contamination (with no adverse effect) in the case file information.

   ii. Otherwise, assess each additional blank and make the appropriate notation regarding the carry-over contamination. If this carry-over is observed while the sequence is still running, the blank may be replaced with a new blank from the same consumables lot number as the sequence samples’ being run. Documentation of this replacement will be made on the analysis worksheet for each case sample that was affected. The analyst should assess each case sample to determine if samples were also affected by carry-over and appropriate steps are to be taken.

7. If contamination is observed in the blanks throughout the sequence, the analyst may proceed with one of the following:

a) If the blank contamination is such that it does NOT impede accurate analysis of samples in that batch run (e.g. peaks are significantly smaller than sample peaks, peaks appear in areas before RT2 or after RT14, peaks in blanks do not appear in samples, etc.), the batch sample results can still be used for analysis, but a notation in the case information for each case in that sequence shall be made explaining that the blank contamination had no adverse effects on the sample results.

b) If the blank contamination throughout the entire sequence is such that it is difficult to determine adverse effects on the samples in that batch run, the batch shall be re-run with a clean blank to determine the extent (if any) of the contamination. A fresh blank (i.e. new pentane, vial/cap, and c-strip, etc. from the same lot/stock as the previous blank and its associated batch) will be created and run with the affected batch to eliminate a consumables contamination cause. If the blanks appear correct after the second run and the samples appear to be similar as the previous run, or greatly improved, the second run’s set of data and chromatograms will be used for analysis. The chromatograms from the original run need to also be retained in the affected case file and documentation of these corrective sets will be recorded for each case in the affected sequence.

c) If an issue persists (e.g. blanks still contaminated, sample results seem to be affected, etc.), the analyst shall confer with the section supervisor to determine
the best corrective action. Options from re-running the sequence in a different GC/MS to initiating a QI will be discussed.

d) If the baseline of a blank should become skewed (e.g. have increased “noise” or be raised or lowered at an extremely different level than previous blanks), the batch should be halted and the blank checked. This can be done by either running the blank in a different GC/MS or running a vial of fresh pentane through the same GC/MS after changing a few key parameters. Note that a performance verification of the GCMS may need to be done prior to running the sequence again.

e) If the issue persists (despite blank change), all parameters and connections need to be checked, troubleshoot various GC/MS functions, and change/replace any key parameters/issues if deemed necessary. The GC/MS cannot be put back into use until blanks yield the proper, expected output and a performance verification is completed.

f) If the sequence run is completed and a baseline issue is observed in the blanks and samples of the entire sequence (i.e. issue was not discovered before completion of the run), confer with the section supervisor to determine best course of action.

D. Sequence

1. Before placing the vials into the autosampler, the analyst shall enter the sequence data on the GC/MS. This is done by either editing an existing sequence or by starting a new sequence.

2. Select “Blank” for type of run on all blanks and “Sample” for each evidence sample. Note that the SIM shall be run as a “Sample.”

3. Select the current method on all instruments (except if using CS2 as a solvent, then use the most current CS2 method), for each sample.

4. Place the SIM and blank vials in desired locations and enter the vial location numbers for the SIM and Blank vials in the appropriate field on the sequence list. Vial case numbers should be placed in the vial locations to match the laboratory case number used in the file name.

5. Ensure that the number of injections is “1.” Save the sequence under the directory name using the year-month-day format, making sure that the GC/MS being used appears in the directory name.

6. For example: “C:\MSDCHEM\4\DATA\2019 CASEWORK\JANUARY\2019_01_17” directory name indicates that this sequence was run on GC/MS4 (number following “C:\MSDCHEM\” in the “Date File Directory:” field) on January 17, 2019.

7. Analyst initials should appear in the “Operator Name” field of the “Run Sequence” interface on the GC/MS computer interface. (refer to Figure 1 for interface).
8. The following naming conventions should be followed.

9. Convention for sequence (directory) naming: **YYYY_MM_DD**
   
   Where YYYY is the four digit year, MM is the two digit month and DD is the two
digit day. For single digit months/days, a “0” should be in the first position and the
year, month date shall be separated by underscores. Therefore, for sequences run
on January 15, 2019, a variation of “2019_01_15” or “20190115” should appear in
the sequence name

10. Sample File Name assigned to the sequence blank before each sample:
   
   **BAUS-YYMM-NNNNN#n**
   
   Where B identifies the blank YY = two digit year, MM = two digit month,
   NNNNN = sequential laboratory case number, n = item number;

   *Note that the ‘#’ sign denoting item number may be replaced by a single space or
   an underscore so long as the other case numbering conventions remain the same,
such as **BAUS-2004-07389_1** in place of **BAUS-2004-07389#1**

11. Data File Name assigned to the sequence blank before each sample:
   
   **bNNNNN#n**
   
   Where b identifies the blank NNNNN = last 5 digits of the laboratory case number,
   n = item number (example: b07389_1)

   *Note that the first 7 letter-number identifiers of the laboratory case number are left
off the sample name due to space constraints in the software and, as described
previously, a single space or underscore may take the place of the # sign.

   Follow the naming convention described above for sample vials EXCEPT without
the “B/b.” Sample and Data File names without a “B” or “b” indicates actual sample
extractions (example: **AUS-2004-07389#1 and 07389#1, respectively**)

12. For labeling of GCMS sample vials, case numbers must be written in their entirety
   (i.e. no abbreviating) along with the item number and the analyst initials
6.5 Data Analysis

A. Chromatograms are examined by the analyst who will determine the presence (or absence) of ignitable liquid residues based on the current ASTM E1618 standard. The results are recorded in LIMS and any pertinent observations are entered on the worksheet or worklist.

B. Follow the identification nomenclature for the different ignitable classes (see Casework Documentation section).

1. Do not use ‘gas’ as an abbreviation for ‘gasoline’ as this term is open to interpretation. Other abbreviations (i.e. IPA for Isopropyl Alcohol) are not acceptable for final analysis results/conclusions. These may be used for shorthand in worksheet notes, but the full name shall be used in final analysis result reporting.

2. LPD, MPD, and HPD may be used for Light Petroleum Distillate, Medium Petroleum Distillate, and Heavy Petroleum Distillate, respectively. Full-name/identifier is preferred in the final analysis report.

3. Although ignitable liquid names shall be used in their entirety on the final analysis report, universally accepted abbreviations (such as IPA, ETOH, MEK, etc.) may be used when identifying these peaks on multiple pages of the same or similar chromatographic data, as long as the full name has been defined somewhere in the case record data.

C. Per the procedure identified in the ASTM E1618 standard, additional criteria for identification of positive results should be outlined and used in conjunction with the standard in order to make a final determination. These are:

1. For LPD’s, at least one verifiable n-alkane in the C4-C9 range (range specified in ASTM Standard E1618). It is preferable to have 2 consecutive n-alkanes verified but if only one n-alkane is verifiable, a strong pattern match and/or ion match to a known LPD sample; and/or high abundance are required for a final LPD call.

2. For MPD’s, at least two GC/MS verified n-alkanes (within the specified ASTM E1618 range of C9 to C14) shall be present and consecutive. A library search printout for these n-alkanes shall be included in the case record to prove this verification.

3. For HPD’s, whether narrow range or otherwise, the library search report of the n-alkanes used in the criteria to support an HPD call shall also be included in the case record.

   a) The laboratory requires 3-5 consecutive n-alkanes within the range indicated by ASTM guidelines.

   b) Although ASTM Standard 1618 indicates that n-alkanes for HPD’s can be in the range of C9-C20, for purposes of additional criteria, n-alkanes in HPD’s should fall in the range of C11-C16, as this is typical.

   c) This is the laboratory’s basic range for HPD identification. Generally the laboratory does not identify anything past C19 due to GC/MS limitations.

   d) Any n-alkanes below C11 may be indicative of an LPD-HPD or MPD-HPD range versus solely an HPD product.

   e) In rare instances, an HPD may still be called even if there is a gap in the consecutive n-alkanes as long as the missing n-alkane is properly documented.
For example, an HPD with a range of C12-C16 may still fit the narrow range HPD criteria if the C15 cannot be resolved due to co-elution issues (see 4 below)

4. Should issues arise regarding either non-consecutive n-alkanes, n-alkanes that cannot be identified at better than 70% quality, or any other identifier issues, a petroleum distillate may still be called so long as the pattern or extracted ions matches a known ignitable liquid sample and a reasonable explanation (i.e. co-elution, possible evaporation, etc.) can and shall be documented on the worksheet or worklist.

5. For gasoline, at least one library search printout shall be included in the case record for each verified C2* (if present), C3, and C4 alkylbenzenes.
   a) These alkylbenzenes should be further identified by examining the key retention times (~9.5 to 12.25 minutes) and printing a landscape expansion of the pattern on which the key alkylbenzenes will be identified/numbered.
   b) Although the analyst is required to label only one of each type of alkylbenzene, it is the responsibility of the analyst to verify each of the required alkylbenzenes prior to calling the gasoline. If 1-2 of these peaks cannot be identified at better than 70% quality, a gasoline may still be identified so long as a reasonable explanation (i.e. co-elution, evaporation/weathering, matrices type, etc.) is documented on the worksheet or worklist. If 3 or more alkylbenzenes cannot be identified at greater than 70% quality, the analyst should call a ‘negative’ sample result and document ‘possible gasoline’ in the case notes.
   c) Refer to the Gasoline Peak Comparison and Nomenclature informational sheet that accompanies the gasoline known chromatographic data.

*C2 is not a definitive requirement for a gasoline identification, but can be present; therefore, its library search report sheet is not mandatory.

6. In the case of single compound identifications, mass spectra and retention time comparisons to known standards shall be shown. For single non-oxygenated peaks, the following criteria are required:
   a) The peak shall be at least 10X the abundance of the next significant peak present. A significant peak is defined as a peak that is significant to a positive result (e.g. a peak that is either an ignitable liquid on its own or part of significant peaks found in ignitable liquid residues).
   b) The absorbance of this peak shall be at least 1 million (from the baseline).

7. The final decision for an identification is at the discretion of the analyst.
   a) If any part of either the ASTM criteria or additional laboratory is not met, the analyst shall document valid reasons for continuing with a positive identification.
   b) Conversely, if the chromatographic data appears to have met the criteria, but the analyst chooses to make a negative finding, this shall also be documented in the case record.

6.6 Casework Documentation

A. Analysts shall ensure that both tunes prior to the sequence run were successful. Additional, analysts should confirm that the start and end SIMs were successfully completed. Printouts of the tunes and Full Scale Chromatograms of the SIMs, along with a printed copy of the sequence shall be included with the chromatographic generated by the sample run of each case record.
1. Analyst will include copies of these documents in each case record listed in that sequence after ensuring analyst initials and corresponding case number has been added to these documents. The sequence list should be numbered in a 1 of 2, 2 of 2 (1/2, 2/2, etc.) format. The SIMS and tunes are understood to only be produced on a single page. This numbering format is also not required on the chromatographic data generated from the sample run.

2. If there are issues producing any of these documents, this shall be documented on the LIMS worksheet in every case record of that sequence that was affected. A proper substitute for these documents shall also accompany the explanation for their absence.

B. A Full Scale Chromatogram (FSC) of each sample and accompanying blank shall be included in the case record.

1. This is accompanied by library search printouts of the 3 peaks with the highest abundance, regardless of their quality. Due to software constraints, the program does not allow for the page 1 of 2, 2 of 2 format. Therefore, this numbering format is not required for chromatographic data. The software generates the FSC as page number 1 followed by the three peaks with the highest abundance, in retention time order, with page numbering of 2, 3, and 4 respectively.

2. If an FSC is generated with peaks present, but the above referenced library searches are not automatically generated, the analyst should manually print up to three peaks present on the FSC and include this data with the case file. The library search does not have to be at >70% quality since this is done to verify that any peaks present are not significant. Manually generated library search reports do not have page numbers and analysts should add the laboratory case number and place them in the case file in retention time order.

C. The Total Ion Chromatogram (TIC) is created by using the “bigscreen” macro and is printed in landscape format. The analyst should include, where practicable, an additional landscape TIC, “zooming into” target peaks/retention times for clarity of analysis. Where appropriate, the target peaks should be identified and labeled. These pages are also understood to only be one page each (i.e. no page numbers present)

D. An Extracted Ion Chromatogram (EIC) shall also be included in the case record for each sample TIC.

1. At minimum, individual extracted ion chromatograms representing “aromatic” and “alkane” compounds shall be verified. Examples of EIC summed ions with the compound classes of interest follows:
   a) m/z 43, 57, 71, 85, 99 alkane
   b) m/z 91, 92, 105, 106, 119, 120, 133, 134 aromatics-alkylbenzenes
   c) m/z 104, 117, 118, 132, 146 alkylstyrenes
   d) m/z 178, 192, 206 alkylanthracenes
   e) m/z 55, 69 alkene and cycloalkane compounds
   f) m/z 82,83 n-alkylcyclohexane compounds
   g) m/z 154, 168, 182, 196 alkylbiphenyls/acenaphthenes
   h) m/z 43, 58, 72, 86 ketones

Printed copy is uncontrolled. Refer to electronic copy for current version.
i) \( m/z \) 31, 45 alcohols

j) \( m/z \) 117, 118, 131, 132, 145 indanes

k) \( m/z \) 128, 138, 142, 156, 170 alkylnapthalenes (condensed ring aromatics), decahydronaphthalene (trans-decalin)

l) \( m/z \) 93, 136 terpene compounds

2. These are also printed on a single page (no page number) with multiple EIC in portrait format

   a) Due to data size and conservation of supplies, the EIC printout will display only the following ions from the list above as they are found to be common to the ignitable liquid residues submitted to the laboratory and in the known ignitable liquid samples retained by the laboratory:

      i. \( m/z \) 57, 71, 85, 99 alkanes

      ii. \( m/z \) 91, 105, 119, 133 aromatics

      iii. \( m/z \) 117, 131, 145 indanes

      iv. \( m/z \) 55, 69, 83 alkenes/cycloalkanes

      v. \( m/z \) 128, 142, 156 naphthalenes (PNAs)

   b) It is the responsibility of the analyst to review additional ions as described in section C1 above to verify criteria is being met for a particular ignitable liquid and print out any additional data to support this finding.

E. For positive identifications, a printed portrait TIC with ‘zoom’ in landscape version of this TIC for a known reference sample should be included in the case file record for comparison. Additionally, a portrait printout of the known ignitable liquid reference EIC with the same formatting as the sample EIC shall be included.

1. ASTM E1618, section 9.1 Initial data analysis consists of a visual comparison of the total ion chromatograms to reference ignitable liquid chromatograms as described.

2. A page, for comparison, (one portrait and one landscape ‘zoom in’) of the TIC for a reference sample shall be included in the case record

3. A page, for comparison, of the relevant extracted ion chromatograms of a reference sample shall be included (portrait format EIC). Each page should also show the comparison mass spectrum of selected compound(s) from the known reference sample.

4. Display range (intensity, retention time) is at the discretion of the analyst, but should be consistent for all items in the case.

F. Known ignitable liquid reference sample chromatographic data

1. The reference sample shall be from the same ASTM E1618 class and sub-class as the reported class of the unknown sample. Exceptions include

   a) Uncommon commercial products which may be composed of the ending components of one sub-class of an ASTM E1618 class and the beginning components of the next sub-class of the same ASTM E1618 class.
b) When the unknown is a mix of two or more ASTM E1618 classes. In this instance, the mixture will be displayed against each of the reference standards seen in the mixture.

2. Retention time ranges of extracted ion chromatograms should be consistent throughout the case record whenever possible.

3. There are times when the retention time range for only one sample or comparison needs to be different than the TIC’s in the rest of the file. Document the TIC with time ranges consistent with the file, then document an additional TIC with the different time ranges. This also applies to EIC retention times.

4. Ion ranges of mass spectra should be consistent throughout the case record whenever possible. There are times when the ion range for only one sample or comparison needs to be different than the ion ranges in the rest of the file. In this case, first document the mass spectra with ion range consistent with the file, then document an additional mass spectra with the different ion ranges. Ion ranges for oxygenates are usually distinct from ion ranges for other classes of compounds.

5. Chain of Custody is documented by the LIMS. Analysts will ensure this remains intact by following CLS Manual procedures for possession of evidence and marking physical evidence per protocols above when in the process of analysis.

G. Once results have been entered, transfer the case folder(s) to the section supervisor or designated analyst to be distributed for administrative and technical review.

7 Records

Equipment Log (LAB-405)
Worksheet/LIMS data file
Laboratory Information Sheet
Worklist
Instrumental sequence

Instrumental method(s)

8 Literature References and Supporting Documentation

American Society for Testing and Materials:

- E1412, "Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal"
- E1618 "Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry"

NFPA921: Guide for Fire and Explosion Investigations (most current edition)

Equipment Manuals
Software Application Manuals
TE-11-03 FIRE DEBRIS ANALYSIS EQUIPMENT

1 Scope
To establish a plan for safe handling, transport, storage, use, and maintenance (including calibration) of measuring equipment and appropriate use of quality controls to ensure proper functioning, in order to prevent contamination and/or deterioration.

2 Related Chapters
Handling of Fire Debris Analysis Items
Instrumental Analysis of Fire Debris
Ignitable Liquid References, Standards and Materials

3 General Requirements for Analytical Equipment

3.1 Overall Requirements for Handling and Use of Equipment

A. All handling, transport, storage, packaging, preservation, and delivery of equipment is verified by laboratory personnel using the appropriate standard operating procedures or manufacturer’s specifications.

B. To ensure the proper environment is maintained to prevent contamination or deterioration, the analysis work areas have been assessed and are cleaned both before and after preparation of analysis items.

C. QC procedures verify the performance of an instrument prior to its operation and may reveal the occurrence of measurement anomalies which require correction (see Instrumental Analysis of Fire Debris)

D. Special attention is paid to the required environmental conditions (e.g. temperature, humidity, etc.) for optimal performance from all equipment and instrumentation involved in analysis of evidence and to ensure accurate, contamination free analysis procedures.

E. Only authorized laboratory personnel may operate fire debris analysis equipment.

F. The guidelines for all significant laboratory equipment maintenance and corrective action documentation and labeling requirements are covered in the CLS Manual.

G. Generally, all maintenance/repair of analysis equipment is performed on site. If any significant equipment requires servicing or calibration off-site, the laboratory will ensure the safe handling, transport, storage, use and planned maintenance of the equipment to ensure proper functioning and in order to prevent contamination or deterioration. This will be done by contracting only approved vendors following CLS Manual guidelines and ensuring proper transport and completion of a performance verification prior to use in casework.

H. Significant equipment that is determined to be malfunctioning or interfering with analysis of evidence shall be removed from service, marked as “out of service” and serviced (see CLS procedures). This equipment cannot be returned to service until it has successfully passed a performance verification. Refer to CLS Manual for procedure details and use of the Equipment Out of Service Incident Form (LAB-410).

I. All performance checks and verifications shall be completed with appropriate reference standards. If during analysis the equipment is discovered to be giving questionable results, analysis should be halted and any impacted samples shall immediately be re-examined, where appropriate, to ensure the integrity of analysis results.
J. Equipment and instruments that can be adjusted shall have sufficient controls in place or other measures shall be taken to indicate if an adjustment occurred (e.g. run a positive control on a GC/MS to show that the retention times are consistent from beginning to end of an analytical sequence). In order to safeguard against unintentional changes, the laboratory has adopted the following measures:

1. Positive and negative controls shall be run at the beginning and end of each instrumental run or analytical sequence, with additional negative controls (blanks) between each sample item, and/or
2. Tamper proof seals may be placed over adjustment points, and/or
3. Only trained laboratory personnel shall be authorized to operate the equipment.

3.2 General Maintenance

A. At the end of each month, where practicable, the analyst will gather all case and QA data from a single month and create a folder on the C: drive of their GC/MS with the month and year to house all data from that month and year. QA data can be transferred to its own folder within that calendar year. At the end of a calendar year, all “monthly” folders will then be placed in a folder named by that calendar year, e.g. “2018 Casework” (or similar name).

1. Analysts are generally assigned specific GC/MS’s to monitor but are expected to help with the other GC/MS’s during times of understaffing or absence of another analyst.

B. When the instrument is not in use, keep the GC/MS oven at ~35°C to 40°C. The following additional maintenance may be needed and logged:

1. Replace injector liners every 3 months (quarterly) as practicable
2. Replace filaments as indicated by GC/MS
3. Run weekly autotune on all GC/MS’s
4. Run ASTM Standard (Sensitivity) Test Mix on GC/MS bi-weekly (See TE-13-04 Section 3.3)
5. Each GC/MS has a maintenance log book. All general maintenance and trouble-shooting procedures are to be recorded, dated and initialed by the analyst performing the maintenance in the log book as it is performed for each instrument (LAB-405).

C. Modifications to active instrumental or data processing methods in use for analyses are not permitted until after the modified method has been tested and validated and the results approved by the QA system in place and undergone proper approval protocols.

D. Any repair, replacement, or cleaning of key components of Mass Spectral Detectors (ion sources, photomultipliers, filaments, etc.) should be well researched prior to replacement. Any replacements should be documented in that instrument’s corresponding maintenance log book. A performance verification may need to be conducted prior to placing the instrument back in use (LAB-408).

NOTE: For septa and liners, it is at the analysts’ discretion to determine whether these need to be change ahead of this schedule or not at all based on how often samples are run on the instrument.
E. Unusual samples, which require method development utilizing different parameters, should be recorded in the instrument log book and shall have prior method validation following validation requirements defined in the CLS Manual. A new/modified method should be saved under a different name from that of other active methods.

   1. Once the sample(s) have been analyzed by a new method, return the instrument to its original settings.

F. Software: Acquisition and display of chromatographic data are controlled by software applications unique to the manufacturer of the instrument in use. Other software applications may be used for viewing and interpretation of analyzed samples and collected data.

   1. Each software program has specific procedures for setting methods, writing sequences, naming files, and storage of the data. The specific instructions as to the operation of the software will be found in the tutorials and user guides for the software found with the instruments themselves. General use of these programs will be covered during analyst training.

   2. In general, the software should be capable of acquiring data from the separation and detection of the complex mixtures of organic compounds found in ignitable liquids. Once acquired, the data should be available as a uniquely named data file that can be archived and recalled for post-acquisition analysis (see section 6.3).

3.3 General Housekeeping

   A. Analysts are required to properly clean analysis work stations both before and after preparation of samples for analysis, as necessary.

   B. Analysts should wipe down (and allow to air dry) preparation tools with pentane or isopropyl alcohol both before and after use.

   C. Used hooks should be cleaned by collecting them in a glass beaker after analysis, rinsing them in pentane, and heating in the oven overnight.

   D. General cleaning of the instrument room and hood area will be performed on a monthly basis.

4 Significant Equipment

Service of equipment is generally maintained by performing cleaning and safety checks as necessary. Performance checks will be necessary where the setting can significantly affect the analysis or analytical result (i.e. the flow rate of a gas chromatograph, the alignment of spectrometer optics, the tuning of mass spectrometers, etc.).

4.1 List of Significant Equipment

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

   B. NIST Traceable Thermometer

4.2 Gas Chromatograph/Mass Spectrometer (GC/MS)

   A. The Fire Debris section has three gas chromatograph/mass spectrometers: GCMS1, GCMS2, and GCMS4. Note that GCMS4 can be converted into a GC/FID (Gas Chromatograph-Flame Ionization Detector).

   B. The GC/MS is the primary instrument used for fire debris analysis, along with approved analysis methods. GC/MS methods are written based on instrument parameters.
determined by both manufacturer and those previously validated for fire debris analysis to ensure optimal detection of ignitable liquids. These validated methods are maintained by running ASTM standard/sensitivity test mixes (ASTM E1618 Test Mix for Fire Debris Analysis) on the GC/MS on these methods as well as GC/MS runs of known Reference Ignitable Liquid samples to test detection efficiency and consistency.

C. Quality Control Procedures

1. Regular performance checks are carried out by running full MSD tunes and tune evaluations prior to running sample sequences. The mass spectrometer should be tuned within a 24 hour period prior to use. Perfluorotributylamine (PFTBA) is currently used. According to Hewlett Packard and ASTM guidelines, the operational parameters should be adjusted to produce a mass fragmentation pattern of PFTBA such that mass 69 is the base peak and the listed criteria ranges are obtained (see figure 1 below).

   a) If either of these repeat tunes confirms an out of range value, the analyst shall troubleshoot the GCMS until the tunes pass. If they do not pass, the equipment will be removed from service.

   b) For unstable instruments (i.e. instruments taken temporarily out of use), a aforementioned performance check (see 7 below) will be conducted prior to each new series of analyses until such time as the chromatographic data shows detection stability (See Instrumental Analysis of Fire Debris).

   Figure 1

2. Scheduled performance checks are completed bi-weekly by running the ASTM E1618 Test Mix for Fire Debris Analysis on each active GC/MS. These are documented in the equipment log associated with each GC/MS (LAB-405).

   a) The GC/MS has passed this performance test by clearly resolving all peaks in the test mix (see TE-13-04 Section 3.3 D). The relative abundance of these peaks may vary and these particular hydrocarbons were chosen for clear separation (i.e. no co-elution should occur between peaks) and to cover the retention times of expected elution from ignitable liquids.

   b) If repeat runs of the ASTM Test Mix should yield unexpected results or lack of expected peak pattern, the analyst will troubleshoot the GC/MS until the expected test mix results are passing. If the peaks do not resolve as expected, the equipment will be removed from service.

   c) For unstable instruments (i.e. instruments taken temporarily out of use), a aforementioned performance check (see 7 below) will be conducted prior to each new series of analyses until such time as the chromatographic data shows detection stability (See Instrumental Analysis of Fire Debris).
3. Known ignitable liquid samples can be run to provide baseline, sensitivity and retention time guidelines for analysis. This also serves to verify that the instrument is performing at the expected levels of detection.

4. Instrument performance checks and validations are conducted under the same instrumental and chemical conditions as those that will exist during the analysis process. These conditions are parameters that have been set in each GC/MS based on historical empirical guidelines for optimal analysis of fire debris and are documented on the chromatographic data output (see GCMS stamp). If these parameters change for whatever reason, protocol for validation of new parameters will be followed.

D. Maintenance

1. Each GC/MS maintenance logbook shall contain an Equipment Log (LAB-405) including date records of all maintenance performed.

2. Where practicable, preventive maintenance will be performed by an authorized fire debris analyst on an annual basis. All maintenance is documented on the LAB-405.

   a) This includes, but not limited to, replacing inlet liners, o-rings, gold seals, ferrules, syringes (if necessary), and filaments; trimming column (if necessary), cleaning the MS ion source; replacing split-vent traps and gas filter (if necessary)

   b) A performance check is performed following preventive maintenance.

3. Preventive maintenance (PM) services may also be scheduled with the instrument manufacturer or approved vendor and a printout describing services, any parts changes or recommended parts change shall be included in the maintenance logbook.

4. After the preventive maintenance is completed, the GC/MS itself should be marked in some way to indicate the date the PM was performed. This can be done with a label sticker or a PM sticker from the manufacturer. Information may include but is not limited to:

   a) GC/MS # (i.e. GCMS1, GCMS2, GCMS4)

   b) GC/MS S/N

   c) Title of “PM” or “Preventive Maintenance”

   d) Date and initials or name(s) of person(s) performing PM

5. Other unscheduled maintenance repairs occurring due to broken or faulty parts will be documented on the LAB-405 in the maintenance logook.

   a) A performance check is conducted after maintenance is performed.

   b) If the instrument is removed from service, a performance verification (LAB-408) is conducted prior to returning the instrument to service.

6. Instrument maintenance logs are retained within their appropriately labeled binders (i.e. GCMS1, GCMS2, GCMS4). Along with their preventive maintenance records, a log indicating changes in consumables done per instrument is kept bearing the analyst initials and date of when this was performed.

7. Maintenance performed outside of the annual PM, including, but not limited to, septa changes, liner changes, filter changes, syringe changes, column
replacement, cleaning of ion source, flow change, etc. are to be logged. These are also completed on an “as needed basis” and can be determined by daily performance checks:

a) **Physical damage** – Dropped, dinged, broken, etc. in some fashion.

b) **Poor chromatography** – permanent abnormal peaks, trailing peaks, ghost peaks, abnormal RT shifting, no peaks, high baseline etc.

c) **Low sensitivity** – auto tune cannot pass, abnormal readings in auto tune, low/no peaks or ions detected, etc.

d) **Leaks** – O2, N2, H2O detected in high concentrations.

e) **Abnormal readings** – Reads abnormally high, low, or no reading

E. Removal of Instrument from Service:

1. If the performance check fails or the instrument is not performing as expected and the analyst cannot determine which of the consumables may be the source of the unexpected outcome, the GC/MS used will be removed from service following CLS procedures until the issue can be resolved.

2. The vials in the sequence run with the failed controls will be re-run on a different GC/MS that has passed all controls while the analyst troubleshoots the GC/MS alongside further investigation of possible consumables contamination.

3. When the results of a control are unsatisfactory, all cases run since the last acceptable result of that control should be reviewed.

4. A performance verification shall be completed prior to returning the instrument to service.

4.3 **NIST Traceable Thermometer**

A. All thermometers for critical measurement must be either calibrated or their accuracy must be verified to ensure the temperature is being measured +/-1.0oC.

B. Thermometers used to monitor set points will be verified with a NIST traceable thermometer at least annually at the appropriate temperature.

C. If the thermometer varies greater than +/- 1.0oC, then the amount of deviation will be noted.

D. If the deviation of the thermometer is greater than 2.0oC, then the thermometer must be replaced.

5 **Non-Significant Equipment**

5.1 **Oven**

A. The acceptable temperature range for heating evidence samples is between 80˚C - 90˚C ±10˚C. Although the ideal temperature is approximately 85˚C, this temperature range has been validated and any temperature within this range is sufficient for extraction of ignitable liquid residue from samples heated for approximately 2 hours.

B. The oven is equipped with a NIST certified thermometer.

1. The average oven temperature will be documented prior to heating samples.
2. The minimum and maximum temperature reading on the NIST thermometer will be documented after heating samples for the requisite ~2 hour time span. Analysts make note of heating start time on worklist.

C. The oven is a constant temperature chamber that does not contain a heating coil and heats samples using hot air. Therefore, no ignition source is present within the heating chamber. The temperature is controlled by both a temperature setting gauge and a damper:

1. The temperature gauge knob is located on the front, lower portion of the oven and is set around ~100 °F. However, adjusting this control may not be sufficient to reach ideal temperatures and the damper may need adjustment.

2. The damper knob is located on the left side of the oven (when facing front door) and the width of the opening can be adjusted by turning the knob clockwise or counterclockwise.

3. The size of the damper opening aids in lowering or raising the internal temperature of the oven: a wider opening will cool the oven temperature whereas a smaller opening will allow temperatures to increase.

D. Maintenance

1. Preventive maintenance is performed as needed. Since the temperature is monitored on a regular basis, if the temperatures fall consistently (3+ days in a row) outside of the desired temperature range for heating samples, casework will be halted until the temperature has stabilized and the desired temperature is maintained for at least 2 days in a row.

2. Cleaning the inside of the oven is completed monthly.
   a) The inside is wiped down with a diluted bleach spray.
   b) Analysts may opt to follow-up with a pentane wipe down should they deem this necessary.
   c) The oven should also be wiped down on an as needed basis.

5.2 Fume Hood

A. A fume hood is available for use in preparation of evidence with biohazard materials. Accompanying PPE should be used when preparing biohazard case samples.

B. Ensure that the hood fan is on and a proper flow rate is observed.

5.3 Volumetric Equipment

A. Flasks, cylinders, pipettes, burettes

B. The analyses conducted by this laboratory are qualitative and the use of volumetric equipment is not critical to analyses.

5.4 Other Equipment and Tools

A. The Fire Debris section is equipped with sterile scissors, tweezers, awls and hooks for use in preparing charcoal strips for extraction of evidence. These are sterilized before and after preparation of cans with c-strips for heating. Jars containing quality certified c-strips shall be sealed immediately after removal of number of c-strips needed for sequence being worked.
B. Can lid presses are used to aid in ease of reseating lids. A ‘pounding mat’ and mallets may also be used. Be cautious of the noise and vibrations which may interrupt surrounding analyses occurring nearby.

C. Checks are performed of the following equipment and/or conditions in both the instrument room and oven room and are logged:
   1. Weekly refrigerator and freezer temperature checks
   2. Certification or QC analysis records on stock of the following consumables (as needed per new lot):
      a) Hooks
      b) Cans (blank)
      c) Solvent (pentane and carbon disulfide)
      d) C-strips
      e) Vials/caps
      f) Solvent dispensers (as part of solvent QC)

6 Records

Relevant Equipment Logs (including LAB-405)
Validation/Verification Form (LAB-408)

7 Literature References and Supporting Documentation

Equipment Manuals
Software Application Manuals
TE-11-04 IGNITABLE LIQUID REFERENCES, STANDARDS AND MATERIALS

1 Scope
To describe the use of ignitable liquid references samples, standards, and materials as well as to outline safe handling, transport, storage, and use of reference samples, standards and materials in order to prevent contamination or deterioration and in order to protect their integrity.

2 Related Chapters
Handling of Fire Debris Analysis Items
Instrumental Analysis of Fire Debris
Fire Debris Analysis Equipment

3 Practices
3.1 General
A. Storage
1. All ignitable liquid known reference samples shall be stored in their designated area, either in a refrigerator or flammables cabinet. They may be temporarily moved to the appropriate work stations or fume hood while reference sample preparations, dilutions and/or mixtures are being prepared.
2. Known reference samples (standards), solvents, and test mixes are stored and used in a manner consistent with preserving the integrity and in accordance with manufacturer guidelines.

B. Labeling
1. All known reference samples, reference standards and materials prepared in the lab will be labeled on the date of preparation with:
   a) Name of chemical/solvent and concentration (if appropriate)
   b) Date prepared and initials of who prepared it
   c) Signal Word (where practicable, such as WARNING or DANGER)
   d) Hazard Statements (if space allows)
   e) Pictograms (where practicable)
   f) Expiration date (if any)
2. Exceptions to this labeling method is when vials from manufacturers received are too small to include all the aforementioned information. Minimally, and if space allows, the smaller vials shall be labeled with the following:
   a) Name
   b) Signal Word (where practicable, such as WARNING or DANGER)
   c) Analyst initials and date
3. The working standard or known reference sample is labeled immediately after preparation with its identity, creation date, expiration date (if any), and the person who prepared it.
4. Date of receipt and initials of the person receiving or creating stock of solvent, test mixes or known reference samples may be handwritten on the labels/containers of the commercially purchased stock.

5. SIMs and ASTM Test Mix labels are affixed to their containers and prepared in the fume hood for analytical/verification purposes or performance measures. With the exception of the 1 mL autosampler vials, the vial label shall contain the mixture name, date prepared, lot number and initials of analyst preparing the mixture.

6. All test mixes and all chemicals/solutions/solvents prepared at the lab that are removed from their original container for use in the lab in an alternate container (other than an autosampler vial or vial with a volume less than 5 mL) must have an identification label.

7. Lot numbers can be found on original containers, where practicable. Other information and more in depth descriptions can be found on the safety data sheets.

C. Inventory

1. All chemicals, reference standards, and reference samples are listed on the appropriate inventory list. Upon receipt, all reference samples/standards and materials are added to the appropriate inventory list.

2. Any additional known reference samples and/or standards may be added to the Ignitable Liquids Reference Library, per section supervisor approval.

3. An inventory of known ignitable liquid reference materials shall be conducted at least once per year and will be assigned by the section supervisor. The section supervisor shall ensure the inventory is properly updated, re-stocked where needed, and information uploaded/saved to the proper medium per laboratory protocol.

4. Inventory of all newly received known ignitable liquid reference materials will include the following information:
   a) Chemical name
   b) Source
   c) Who received/added reference
   d) Date it was received/added
   e) Concentration
   f) Expiration date (if any)
   g) Notes
   h) Environmental Hazard Code

3.2 Ignitable Liquid Known Samples and Sensitivity Reference Standards

A. Ignitable liquid known reference samples are "pure" (packaged from the manufacturer) standards of hydrocarbons, aromatics, alcohols, or other organic classes.

1. The known samples are prepared for use at a concentration of approximately 1% v/v in pentane when run on a GC/MS as a representative sample.

2. This is done by pipetting a tiny drop (~1-3 µl) of the known ignitable liquid into an autosampler vial with ~1 mL of the desired solvent.
B. Each GC/MS library shall have a current chromatogram (within one year of a sample run) of the most commonly identified ignitable liquids from this ignitable liquids reference library stock.

   1. “Fresh” known ignitable liquid samples from this library shall be run at the beginning of each calendar year, preferably in January but as soon as practicable.

   2. The more uncommon known reference samples, including certain naphthenic-paraffinics, single peak ignitables (e.g. MIBK, MEK), miscellaneous class ignitable liquids, etc., not identified in evidence on a regular basis will be run as needed for a known reference sample match and on the same GC/MS as the sample being identified.

C. If any significant change occurs on a specific GC/MS that may affect the overall appearance and/or retention time of a known ignitable liquid sample, a new set of known samples shall be run on that GC/MS.

   Note: a significant change is defined as a change to the overall appearance (including baselines and peaks) and retention time of a sample which is more noticeable when using a known sample or sensitivity reference standard.

3.3 Test Mixture External: ASTM Standard Test Mix

A. This is a reference standard test mixture commercially bought and prepared in a 1:10 dilution with pentane (ASTM E1618 for Fire Debris Analysis). Higher n-alkanes may be present in the commercial mix as well, but are not required.

B. The standard mix typically arrives in an ampoule which must be broken open and transferred to a vial for dilution into a stock solution. The standard mix solution is to be stored in the reference refrigerator prior to use and may be temporarily stored in the analysis laboratory after the ampoule is opened and transferred to a vial.

C. The stock solution may be used for multiple performance checks when verifying sensitivity of a GC/MS after changes or maintenance has been performed on it. This depends on the concentration of the original commercial mixture which may vary depending on supplier availability. However, for the required GC/MS bi-weekly (ASTM) sensitivity test, a new ampoule shall be opened. Its dilution can be shared among all instruments.

D. A stock of the ASTM Test Mix solution is prepared by mixing approximately 1 mL of the test mix and 9 mL of pentane. Once mixed, this stock test mix solution must be immediately used for the bi-weekly sensitivity checks of the GC/MS. It need not be saved after the sensitivity test run but, if saved, is to be stored in the Reference Sample refrigerator.

   1. For use as the QA/QC Bi-weekly Standart Test Mix run for sensitivity on all GC/MS’s, the “ASTM Test Mix” (external) is used at this dilution to establish the consistency of compound retention times, provide the benchmarks necessary for correcting possible deviations in instrument performance, and to show that the instrument is operating properly.

      a) Purchased standard (ASTM) test mixes (external) should not be used more than two years past its received date.

      b) Prepared sensitivity reference standards solutions from the ASTM Standard Test Mix may be used past a year from its preparation date IF the resulting chromatographic output remains consistent (e.g. the expected output shows a significant absorbance and the EIC is still a strong representation of that mix
especially in comparison with the original run) and only if a test needs to be done immediately but no “unexpired” ASTM Standard Test Mix is available.

c) These mixtures come with quality checked paper work and need not be verified until their first use in the Bi-weekly Standard Test Mix run for sensitivity check. The ASTM Standard Test Mixes may be run alongside a SIM for performance measures or verification purposes.

d) The ASTM Standard Tes Mix is composed of the following:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CAS Number</th>
<th>Target Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Decane</td>
<td>124-18-5</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Dodecane</td>
<td>112-40-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Eicosane</td>
<td>112-95-8</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>o-Ethyltoluene</td>
<td>611-14-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>m-Ethyltoluene</td>
<td>620-14-4</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>544-76-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>110-54-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Octadecane</td>
<td>593-45-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Octane</td>
<td>111-65-9</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>n-Tetradecane</td>
<td>629-59-4</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>Toluene</td>
<td>108-88-3</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>1,2,4-Trimethylbenzene</td>
<td>95-63-6</td>
<td>0.05 %v/v</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>106-42-3</td>
<td>0.05 %v/v</td>
</tr>
</tbody>
</table>

3.4 Standard Ignitable Mix (SIM).

A. The SIM is composed of a 50:50 mix of gasoline and diesel.

B. A new SIM shall be prepared at the beginning of the work week. In order to continue use of the prepared SIM throughout the week, the analyst must recap SIM vial with new, unpunctured sept cap or solid blue cap. Subsequent creation of a SIM solution may need to be prepared throughout the work week, as necessary.

C. The SIM is prepared using the pre-prepared SIM stock by diluting ~1-3 µl of the SIM in ~1 mL of solvent (pentane or carbon disulfide). If either instance of the SIM (or ASTM Standard Test Mix) does not yield the expected results, the standards will be checked by creating a new SIM (diluted and/or heated) and running protocols for checking parameters/connections on the GC/MS, as well as quality checks on the known reference samples used to create the SIM (to check for contamination or loss/evaporation).

D. To preserve both the SIM and ASTM Standard Test Mix, analysts should recap vial with new septa cap immediately after injection into the GC/MS. For long-term preservation, a solid cap should be used.
3.5 Quality Control for ASTM Test Mixes, SIMs, and Solvent Blanks

A. Internal and External Test Mix(es).

1. The retention times for the shared components of test mixes (internal and external) should be consistent between the bi-weekly sensitivity test mix runs.

2. The GC/MS can cause a slight variation in retention times of test mix components over time. Should a variation greater than 0.3 minutes be noted between a bi-weekly sensitivity test mix runs, GC/MS performance checks should be performed.

3. If the retention times of test mix components shift by more than 0.3 minutes on the same GC/MS over a six month period, that GC/MS’s analytical column may be replaced, instrument parameters reset, and connections checked.

4. Note that an analyst may decide to make the aforementioned changes even if the retention time shift is less than 0.3 minutes.

B. Sequence/Batch SIM.

1. The retention times for SIMs should be consistent between different batches run on the same GC/MS using the same SIM.

2. The GC/MS can cause a slight variation in retention times of the SIM components over time. Should a variation greater than 0.5 minutes be noted between the retention times of the SIM and the one immediately preceding it, the analytical batch should be halted and instrument parameters and connections checked.

3. If the issue cannot be resolved, the instrument is removed from service following CLS procedures.

4. If the time differential between the retention times of SIM components shift by more than 0.5 minutes over a six month period, the analytical column may be replaced, instrument parameters reset, and connections checked.

5. If the SIM itself is contaminated, non-existent (i.e. SIM vial was empty or contained only solvent), or its absorbance level is so low that its chromatographic pattern is not clear, and the sequence samples and blanks have distinct chromatographic data, several options can be taken

   a) If the issue was caught early in the sequence run, the sequence can be halted and the entire batch re-run with a new SIM.

   b) Otherwise, a SIM from a batch run previous to the current batch and a SIM from a batch run after the current batch should be included in the place of, or in addition to the unusable SIM chromatogram. Note that the individual analyst may decide to perform any of the aforementioned corrections even if the retention time shift is less than 0.5 minutes.

4 Records

Inventory logs (includes Reference Materials)
Certificates of Manufacture (if provided)
Certificates of Purity (if provided)
Purchasing records (copies of receipts, packing lists, invoices per DPS protocol)
Safety Data Sheets
Ignitable Liquid Reference Library List and Inventory

Individual Reference Sample/Standard Labels

5 Literature References and Supporting Documentation


12 MISCELLANEOUS

TE-12-01 ANALYSIS OF SUBSTANCES BY GC OR GCMS

1 Scope
Textiles (clothing, bedding), swabs, and other samples are occasionally submitted for analysis to determine the presence of a lotion, lubricant, or other compound. Unless a component of specific interest can be identified, this analysis requires the submission of the suspected source in order to perform a comparison to evidence stains. The analysis of serological evidence may be hindered by this procedure.

Examples of the application of this procedure include:

A. Testing a stain for components of a lotion or lubricant
B. Identification of oleoresin compounds from suspected pepper spray
C. Identification of 1-methylaminoanthraquinone (MAAQ) from a dye pack
D. Identification of an unknown stain (non-biological)

2 Related Chapters

3 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment
B. Biohazard precautions, if applicable
C. High temperatures and compressed gases are encountered with gas chromatography

4 Equipment and Materials

- Polilight, Lumalite or other alternative light source
- Gas chromatograph mass spectrometer
- A chromatography column suitable for the application
- Known sample(s) of the component(s) of interest
- Compressed gases (air, hydrogen, nitrogen or helium)
- Screw-top vials or other sealed glass containers
- Cyclohexane, methanol, hexane and other solvents as necessary

5 Standards, Controls, and Calibration

A. If available, a sample of the suspected source is analyzed to demonstrate the procedure will detect components of the source.
B. The successful detection of the component(s) of interest should be demonstrated prior to the analysis of the evidence. A review of the literature often reveals a useful analytical scheme.
C. Known oleoresin standards such as capsaicin and dihydrocapsaicin can be prepared in methanol as a 1mg/mL solution. This can then be diluted approximately 1:10 for final analysis.
D. Blanks will be run at the same conditions as the samples. A blank will be run between each sample.
6 Procedure

6.1 Routine Sample Analysis

A. Solid Phase Microextraction (SPME)
   1. Place a small amount of liquid sample in a glass vial and cap securely.
   2. Gently heat the liquid sample to promote vaporization, if necessary.
   3. Run a blank of the SPME fiber before analysis of the sample.
   4. Expose the SPME fiber to the headspace or liquid of a sample. Exposure times may vary.
   5. Insert the SPME probe directly into the gas chromatograph and allow the SPME fiber to be exposed for desorption and analysis.
   6. Alternatively, a “dirty needle” technique may be used to capture the volatile component(s) of a sample.
      a) Proceed with steps 1-2 from SPME section.
      b) Run a blank of the injection needle before analysis of the sample.
      c) Draw a volume of the headspace into the injection needle. Alternatively, draw a volume of the solvent into the needle and expel.
      d) Insert the injection needle directly into the gas chromatograph and inject the sample for analysis.

B. Liquid (Solvent) Extraction
   1. Extractions of the unadulterated sample are routinely performed, however, acid-base extractions may also be considered.
      a) Weak acids like citric acid, phosphoric acid, or diluted sulfuric acid are used for adjusting to moderately acidic pH values and hydrochloric acid or more concentrated sulfuric acid is used for adjusting to strongly acidic pH values.
      b) Weak bases like ammonia or sodium bicarbonate (NaHCO₃) are used for adjusting to moderately basic pH values while stronger bases like potassium carbonate (K₂CO₃) or sodium hydroxide (NaOH) are used for adjusting to strongly alkaline conditions.
   2. Sample concentration and substrate may require that extractions are performed under a variety of parameters.
   3. Questioned, known, and/or control samples should be treated in the same manner and the extraction parameters should be documented. The following factors may be considered:
      a) Extraction time and agitation/vortexing.
      b) The extract(s) can be filtered to remove fibers and other debris prior to analysis.
      c) The extract(s) can be dried with an appropriate drying compound (i.e. Sodium Sulfate).
      d) The extract(s) may be concentrated by evaporation of the solvent prior to injection to aid in the detection of compounds found in low concentrations.
C. A volume of the extract(s) is injected into the gas chromatograph and the analysis run is begun. The parameters of the gas chromatograph may be adjusted to accomplish the separation and detection required for the application.

D. The mass spectra of peaks of interest can be obtained and compared to known mass spectra generated from the reference material or in literature/MS library databases.

6.2 Specific Evidentiary Sample Analysis

The following procedures have been validated, however they may be modified according to case circumstances, the availability of laboratory equipment, and the examiner’s preference.

A. Lotion/Lubricant/General Unknown

1. Extraction from a Suspected Liquid
   a) Extract a volume of the suspected liquid with an equal volume of an appropriate solvent.
   b) Proceed with steps B-D from Section 6.1.

2. Extraction from Clothing and Other Substrates
   a) A search of the clothing is made using visual light and/or an alternate light source to locate stains. Stains are outlined with a permanent marker.
   b) A portion of each stain is collected and individually placed into a glass vial.
   c) A portion of an apparent unstained area of the item, if present, is also collected and placed into a glass vial.
   d) A portion of a swab sample suspected of having a compound present can be removed from the shaft and placed into a glass vial. A portion of a control swab should be removed and treated in the same manner.
   e) A volume of cyclohexane or other suitable solvent sufficient to cover the sample is added to the vial.
   f) Proceed with steps B-D from Section 6.1.

B. Pepper Spray/Lacrymators

1. Extraction from a Suspected Liquid
   a) Extract a volume of the suspected liquid with an equal volume of 5% aqueous Sodium Hydroxide.
   b) The aqueous extract is washed two times with hexane.
   c) The extract is acidified with Hydrochloric Acid and extracted with chloroform.
   d) The chloroform is evaporated to dryness and the residue dissolved in methanol.
   e) Alternately, the liquid sample can be diluted approximately 1:10 in methanol and analyzed directly.
   f) Proceed with steps C-D from Section 6.1.

2. Extraction from Clothing and Other Substrates
   a) A search of the item is made using visual light, UV light, and/or an alternate light source to locate stains. Stains are outlined with a permanent marker. The absence of visible staining on the clothing/substrate may impede the detection of oleoresin compounds.
b) A portion of the stain is collected and individually placed into a glass vial.

c) A portion of an apparent unstained area of the item, if present, is also collected and placed into a glass vial.

d) The stained and unstained samples are extracted with hexane.

e) Extract the hexane with 5% aqueous Sodium hydroxide.

f) Proceed with steps b-d from Section 6.2 B.1.

g) Alternately, the samples can be extracted with a small amount of methanol (~1-2 mL) and analyzed directly.

h) Proceed with steps C-D from Section 6.1.

3. Pepper sprays and tear gas sprays typically contain capsaicinoids (80-95% of which are capsaicin and dihydrocapsaicin) as the active lachrymator ingredient.

C. 1-Methylaminoanthraquinone (MAAQ)

1. If reddish stains are present, examine under ultraviolet light. Rhodamine compounds and many inks will fluoresce while MAAQ does not.

2. MAAQ is soluble in acetone, chloroform, and methanol. A small section of stain is removed and tested with an appropriate solvent, preferably acetone. If soluble, a portion of the solvent extract is collected and placed into a glass vial.

   a) If the substrate is plastic, use chloroform or methanol instead of acetone.

3. Two current vendors of bank security dye packs currently use MAAQ in their devices. Older devices may have Solvent Red 1 instead of MAAQ. Bank security dye packs have serial numbers and markings to indicate composition and delay time.

7 Interpretation

A. A compound may be reported as identified if the mass spectrum and/or retention time criteria are met.

1. Criteria for Mass Spectrum Identification/Matches

   a) For compounds with several ions with a relative intensity greater than 20% of the base peak, all ions with a relative intensity greater than 20% of the base peak in the reference spectrum should be present in the sample spectrum.

   b) For compounds with a minimal number of ions with relative intensities greater than 20% of the base peak, compare relative intensities of all significant ions below 20% of the base peak before determining a match.

2. The mass spectrum is acceptable when the spectrum matches a reference spectrum from the instrument or if a matching spectrum is found from an outside reference and the source is specified.

3. The retention time of the compound is acceptable if it matches the retention time for a known reference standard with the same type of chromatographic column and conditions within +/- 0.2 minutes based on a plot of a major ion.
8 Literature / Supporting Documentation


1 Scope
This procedure provides the analytical approach for the identification of various acids, based upon
the results from a series of tests.

2 Related Chapters
Silver Nitrate Test
Barium Chloride Test
Diphenylamine Test
Ammonium Molybdate Test
Microchemical Analysis

3 Safety
Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

4 Equipment and Materials
pH paper covering the range of 0-14

5 Procedure
5.1 Sample Preparation
1. Test liquid samples directly.
2. If the evidence is a stain, extract a portion of the stained area and an equal portion
   of the unstained area separately with the same minimum volume of water.
3. If the evidence is dried residue, dissolve it in a minimum of water.

5.2 Determination of pH
1. Place a volume of the water-soluble fraction, the extraction liquid, and the
   substrate extract onto separate strips of pH paper.
2. Document the pH of the water-soluble fraction, the extraction liquid and the
   substrate extract.

5.3 Determination of Salt Form
1. If the pH is acidic, individually test the sample with the following reagents:
   a) Use the Silver Nitrate Test for Halides.
   b) Use the Barium Chloride Test for Sulfates.
   c) Use the Diphenylamine Test for Nitrates.
   d) Use the Ammonium Molybdate Test for Phosphates.
2. Document the formation of a precipitate or color.
3. Alternative tests for those listed above can be found in Microchemical Analysis
   and may also be used.
6 Interpretation

A. If Hydrochloric Acid is present, the solution will have a pH less than 7 and a white precipitate will be formed with the silver nitrate reagent.

B. If Sulfuric Acid is present, the solution will have a pH less than 7 and a white precipitate will be formed with the barium chloride reagent.

C. If Nitric Acid is present, the solution will have a pH less than 7 and a deep blue color will rapidly form upon addition of the diphenylamine reagent.

D. If Phosphoric Acid is present, the solution will have a pH less than 7 and the Ammonium Molybdate Test will form a yellow precipitate.

E. An acidic pH and negative results with the silver nitrate, barium chloride, diphenylamine and acetone/ammonium hydroxide indicates the presence of an unknown acidic substance.

F. A neutral or basic pH reading indicates acidic substances are not present.

7 Limitations

These tests are not specific for the indicated acid. Positive results are only indicative of the presence of the indicated acid.

8 Literature References and Supporting Documentation


TE-12-03  X-RAY DIFFRACTION

1 Scope
X-ray diffraction analysis can be used to uniquely identify materials having a crystalline structure.

2 Related Documents
Safety Manual: Radiation Safety

3 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. The operator's hands or face should never be in proximity to the x-ray beam ports during operation of the instrument.

4 Equipment and Materials
- X-ray diffraction instrument
- Oven
- Silicon wafer standard

5 Standards, Controls, and Calibration
A. Each day the instrument is used, a silicon wafer is analyzed to demonstrate proper operation.
   1. The lines on the diffraction pattern shall be marked and be located at 28.44° +/- 0.06°, 47.30° +/- 0.06° and 56.12° +/- 0.06° in order to “pass”.
   2. If the lines are not within this tolerance, adjust the goniometer and repeat the analysis of the silicon wafer. If the lines remain outside tolerance, advise the supervisor and/or the manufacturer representative before performing casework.
B. The standard diffraction pattern will be placed in the case record. The Equipment Log (LAB-405) will be completed and marked pass or fail.

6 Procedure
6.1 Sample Preparation
1. Some samples may require drying before analysis. To dry, place the sample in an oven set at 80-100°C for 2 hours.
2. Samples may need to be ground to a fine powder to assure a complete diffraction powder is obtained.
3. Load the sample in the appropriate holder.

6.2 Sample Analysis
1. Turn the instrument on.
2. Place the sample holder in the chamber and close the chamber door.
3. Initialize the goniometer in the manual measurement mode. Turn the x-ray beam ON.
4. Open the standard measurement program and select an appropriate measurement condition. Start the scan.
5. Open the diffraction pattern obtained from the scan with the JADE program. The peak locations can be marked (optional).

6. Print the diffraction pattern.

7. Mark the diffraction pattern with case number, initials, date, and sufficient information to identify the particular sample.

8. Document the identification and/or reference used.

7 Interpretation

A. The identification of a compound from its powder diffraction pattern is based upon the position of the lines (the d spacing) and their relative intensities.

B. The Powder Data File is searched with the JADE program. Possible compounds are identified from a search/match list.
   1. Overlay the sample diffraction pattern with the d-spacing marks of the possible compounds from the search/match list to identify the sample.
   2. Print the results of the search/match. The appropriate Powder Data File cards may be printed from the database (optional).

8 Literature References and Supporting Documentation


TE-12-04 COMPARISON OF PRESSURE SENSITIVE TAPE

1 Scope

The laboratory is generally requested to compare questioned tape, typically recovered from a crime scene or a victim, to a roll or pieces of tape that are associated with a suspect.

Pressure sensitive tape can consist of components such as release coat, paper or film backing, scrim fabric, and adhesive, among others. Comparison of the backing color, texture, width, thread count, weave uniformity, the characteristics and composition of the warp and fill fibers within the fabric (if present), and the color and composition of the adhesive can be used to assist the examiner in formulating a conclusion.

Before conducting a tape comparison, consider the need to preserve any latent prints, DNA, or trace evidence that may be on the tape.

2 Related Chapters

Microscopic Examination of Fibers
Cross-Sectioning of Fibers
X-Ray Diffraction
Fourier Transform Infrared Spectroscopy
Pyrolysis-Gas Chromatography Mass Spectrometry

3 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Biohazard precautions, if applicable

4 Equipment and Materials

- Stereomicroscope
- Compound microscope
- Polarized light microscope
- Polarizing filters
- Ruler
- Hexanes or other solvents
- Micrometer
- UV light source and barrier filter
- Compressed gas duster or liquid nitrogen
- Razor blade or scalpel
- Backlit light box or other light source

5 Standards, Controls, and Calibration

None
6 Procedure

6.1 Physical Characteristics

1. Perform Physical Comparison, documenting these characteristics where appropriate:
   a) Width of the tape
   b) Color of the backing
   c) Presence and shape of any markings on the backing
   d) Overall thickness and thickness of backing
   e) Uniformity of the fabric weave
   f) Number of threads per inch in the warp and fill directions
   g) Color of the adhesive
   h) Polymer orientation
   i) Number of layers

2. The existence of a physical match should be checked if adequate samples are submitted and the physical characteristics of the questioned and known tapes are similar by performing the Fracture Physical Match Comparison.
   a) If a fracture physical match exists between known and questioned tape, it is not usually necessary to continue with tape analysis.
   b) Heat, liquid nitrogen, and other solvents can be used to release the adhesive and allow the ends of the tape to be compared.

6.2 Sample Analysis

1. If present, cut small, separate sections of thread from the warp and from the fill directions.

2. Remove the adhesive from the backing to expose threads using a solvent and separately mount the warp and fill thread fibers.

3. On the threads, perform Microscopic Examination of Fibers, Cross-Sectioning of Fibers and Fourier Transform Infrared Spectroscopy, as necessary. Warp and fill fibers of the scrim can be examined using a UV light source, a forensic light source, and/or fluorescence microscopy.

4. A small portion of the adhesive may be removed and compared using Fourier Transform Infrared Spectroscopy. Some adhesives (such as dark colored adhesives) may be better examined using Pyrolysis-Gas Chromatography Mass Spectrometry.

5. A small portion of the backing may be identified and compared using Fourier Transform Infrared Spectroscopy.

6. The backing and adhesive portion of the tape may be analyzed and compared using X-Ray Diffraction.

7. Make a cross section of multilayered tape by freezing the tape with liquid nitrogen or compressed air to make the tape more rigid and cutting the edge of the tape. Examine cross sections microscopically.
8. The polymer orientation of polypropylene tapes can be determined by using polarizing filters. Using a backlit light box or other light source, sandwich the tape sample between two polarizing films. Observe the interference colors, patterns, and variations of the tape when rotating the films parallel and perpendicular to each other.

   a) Monoaxially oriented polypropylene will have one point of extinction, every 180º.
   b) Biaxially oriented polypropylene will have two points of extinction, every 90º.
   c) The angles of the crosshatches and extinction angles may vary from film to film but should be consistent within a single roll of tape.
   d) Some tape films may not exhibit total extinction. Irregularities or inclusions should be noted.
   e) Interference colors can also give an indication of film thickness and variations.

9. Compare the data from the sample analysis of the questioned sample and the known sample.

7 Interpretation

   A. Questioned and known tape samples which exhibit meaningful differences in the observed characteristics such as color, thickness, material composition, texture of the backing, width, thread count, uniformity of the fabric weave, adhesive color, adhesive composition or fiber composition leads to the conclusion that the questioned tape and known tape are from different rolls of tape.

   B. Questioned and known tape samples which exhibit no meaningful differences in the observed characteristics such as color, thickness, material composition, texture of the backing, width, thread count, uniformity of the fabric weave, adhesive color, adhesive composition or fiber composition leads to the conclusion that the questioned tape and known tape could have come from a common source.

   C. Questioned and known tape samples with a physical match leads to the conclusion that the questioned tape was once joined to, and subsequently removed from, the known tape.

8 Limitations

Tape may not be in its original state due to weathering, stretching, chemicals, etc. These changes may prevent a full range of examinations from being performed and, therefore, may limit the information obtained from the analyses.

9 Literature References and Supporting Documentation


TE-12-05 SCANNING ELECTRON MICROSCOPY – ENERGY DISPERSIVE SPECTROMETRY (SEM-EDS)

1 Scope

Scanning Electron Microscopy-Energy Dispersive Spectrometry (SEM-EDS) can be used to obtain magnified images of samples and to obtain elemental information based upon the energy of the X-rays emitted by the sample when bombarded by electrons. The SEM serves a dual role of providing the energy to generate X-rays and isolating small regions for analysis. The EDS collects and processes the X-rays for chemical element identification.

Bulk analysis is a type of SEM analysis that determines the average elemental composition of a material. The raster area of SEM is selected to yield the largest sample area possible. If it is not possible to select one large area, several small areas are analyzed and the data from each are summed.

Point analysis is generally performed in order to associate elements in a specific particle or structure which have been previously identified by bulk. It can also be useful for a determination of the presence of trace elements, should those elements be located in specific particles or structures. The electron beam of the SEM is directed onto the structure of interest, either by increasing the magnification or by placing the scan generator in “spot” mode.

2 Safety

Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

3 Equipment and Materials

- Scanning Electron Microscope-Energy Dispersive Spectrometer
- Elemental standard(s): as referenced in local policies/instrument logbooks.
- Organic, liquid, pressure sensitive adhesive, such as Duro-Tak 80-1061, (National Starch and Chemical)
- Stereomicroscope
- Image standard

4 Standards, Controls, and Calibration

A. The previous SEM-EDS calibration is verified prior to each software run by acquiring the spectrum from an elemental standard.
   1. A copy of the calibration is placed in the case record
   2. Calibration results are documented in the Equipment Log (LAB-405).

B. If the x-ray detector will not acquire the correct spectrum of the elemental standard, the EDS will be removed from service following the policy in CLS until appropriate corrections and/or repairs can be performed.
5 Procedure

5.1 Operating Conditions

A. The following suggested operating conditions are meant as general guidelines for starting conditions. Actual requirements may vary as determined by specific analytical needs.

- Beam voltage: 20-30 KV
- Beam current: Adjusted to about 35% deadtime
- Live count time: 10 seconds
- Pulse processor time constant: 3 or 4

B. Generally, changes in the suggested operating conditions are required under the following circumstances:

1. Beam voltage is increased when higher energy line excitation is required.
2. Beam voltage is decreased when greater spatial resolution is required.
3. Pulse processor time constant is lengthened when greater spectral resolution is required.
4. Pulse processor time constant is shortened when a greater count rate is required (e.g., for trace element analysis, or construction of elemental distribution maps).
5. Spectral energy display scale is expanded when sufficient detail is not evident.
6. Beam current is increased when the X-ray count rate is too low. Beam current may be increased by increasing the final aperture size or spot size.
7. Beam current is decreased when the X-ray count rate is too high. Beam current may be decreased by decreasing the final aperture size or spot size.

C. The operating conditions used in analysis are retained in the case record.

5.2 Sample Preparation

A. The choice of a specific method for sample preparation will depend on the size and condition of the specimen, as well as the analytical request. For the accurate comparison of elemental composition and structure, samples shall be prepared in as similar a manner as possible.

B. Samples are examined with a stereomicroscope.

1. It may be possible to remove any adhering extraneous material visible in the adhesive by picking or cutting it out with a fine tipped scalpel.
2. It may be possible to remove any extraneous material with a cotton-tipped applicator moistened with water.
3. When extraneous materials cannot be removed, their location should be noted during light microscopy and/or backscatter electron SEM observations. An attempt to avoid areas with extraneous material can then be made during subsequent analysis.
C. Samples are mounted onto sample stubs using conductive adhesive. A drawing of the surface of the stub assists in locating the samples within the SEM.

1. A sample stub is cleaned by wiping with an appropriate solvent (e.g., water, acetone).
2. The dilution of the adhesive is adjusted with acetone to achieve the desired thickness.
3. The adhesive can be applied to a stub as a spot, line, or thin film. A spot or line is applied with a capillary tube or micro-dropper. A thin film is applied by drawing a drop of adhesive across the stub with the leading edge of a coverslip. The adhesive is thickest in the spot or line.
4. Small samples or particles are attached to a thin film.
5. Larger samples are attached to a spot or line.
6. Alternatively, larger samples can be attached using double sided adhesive tape.

D. The sample stubs are placed into the chamber and the SEM-EDS is activated.

5.3 Sample Analysis

1. Determine the type of analysis to be conducted, either “bulk” or “point” depending on the sample type and nature of the request.
2. Calibrate the EDS with the appropriate elemental standard. Plot the results and mark the spectrum with case number, initials, and date.
3. Focus on an area of the sample from which to collect X-rays while maintaining the optimum working distance.
4. Activate the EDS system to collect an X-ray spectrum. Adjust the spot size and preset live time to optimize the collection of x-rays.
5. Examine the spectrum for peaks with characteristic energies, label the peaks and plot the results.
6. Mark the spectrum with case number, initials, date, and sufficient information to identify the particular sample.

6 Interpretation

A. An element is identified when:

1. A peak is statistically significant, and
2. The peak(s) has the characteristic energy for that element.

B. Only peaks which are statistically significant should be considered for identification.

1. The minimum size of the peak P should be three times the standard deviation of the background at the peak position, i.e., \( P > 3(N_B)^{1/2} \).
2. This peak height can be estimated directly on the EDS display from the statistical scatter in the background on either side of the peak. The “thickness” of the background trace due to statistical fluctuations in the counts is a measure of \((N_B)^{1/2}\). The peak, then, should be at least three time this thickness.
3. If it is difficult because of statistical fluctuations in the count to decide whether a peak exists above the continuum, then more counts should be accumulated in the spectrum to "develop" the peak.

C. The process is straightforward for the peaks of elements present in major amounts, and for non-overlapping peaks. Misidentifications or omissions of minor components are possible however, unless a systematic approach to elemental identification is used. This approach includes: consideration of X-ray line families, spectral artifacts, escape peaks, sum peaks, and overlaps.

D. Reference lines, or energies, may be obtained from several sources including energy slide rules, computer generated "KLM markers" that may be superimposed on the spectrum, and published tables. Additionally, manufacturers often provide an "automatic" element identification application. Often these aids are used in a complementary fashion.

E. Identification will begin with high energy peaks and major peaks. High-energy peaks will typically be less likely to be overlapped than lower energy peaks.

   1. If a major peak is present, then a complete family of peaks can generally be identified. Each line within the family is labeled with elemental symbols.

   2. Spectral artifacts, including sum peaks and escape peaks associated with major peaks, should be identified and labeled.

F. As spectral interpretation alternates between the identification of major and minor peaks, the vertical (counts) scale should be adjusted to reveal required detail.

   1. In addition to the higher energy peaks, note the presence of any lower energy families and their expected relative intensities.

   2. Individual asymmetric peaks and inconsistent peak ratios within a family may indicate a peak overlap. Elemental identification is aided by superimposing and scaling "KLM" reference lines on the spectrum, or referencing the actual spectrum of an elemental standard.

   3. The analyst should become familiar with the characteristic pattern and relative intensities of peaks of various atomic numbers.

G. Following the identification of major components, lower intensity peaks and overlapped peaks are identified. Identification of minor elemental components is often limited by the number of characteristic peaks present in a spectrum.

H. The presence of an element can be considered unequivocal only when a distinctive, unique set of lines is produced, or when a single peak occurs at an energy where it cannot be mistaken for another element or spectral artifact. Unequivocal identification may not be possible if an element is present in low concentration, or if lines required for confirmation are overlapped with the lines of other elements.

I. If an automatic identification application is used, peak identification shall be confirmed by the analyst.

J. If an identification is unequivocal, then each individual peak is labeled with the corresponding elemental symbol. If the identification is probable but not unequivocal, then the peak label should indicate so (e.g., such as by parenthesizing the elemental symbols).

K. For documentation, spectra shall be displayed on a scale that clearly demonstrates the peaks identified. Multiple scales may be necessary.
7 Limitations

A. Elements present below about 0.1% may not be detectable.

B. Although the natural X-ray line width is approximately 2eV, EDS resolution is generally no better than approximately 127eV. As a result, overlap of peaks in the EDS spectrum of materials containing several elements may occur. Some commonly occurring overlaps encountered in EDS are: Pb-Ma/S-Ka/Mo-La, Ti-Ka/Ba-La, Na-Ka/Zn-La, and Ca-Ka/Sb-La.

8 Literature References and Supporting Documentation


TE-12-06 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

1 Scope

Infrared spectroscopy is a nondestructive technique that allows the examiner to classify binder types and/or extenders in paint samples, identify the composition of man-made fibers and polymers (plastics and rubbers), and identify and compare organic and some inorganic compounds.

Infrared analysis of fibers can provide information about fiber polymer composition to supplement that obtained by microscopic and other methods. The American Society for Testing and Materials (ASTM) lists infrared spectroscopy as the preferred method of analysis for identifying man-made fibers (ASTM D276-87).

2 Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. Protective eyewear shall be worn while filling the detector with liquid nitrogen.

3 Equipment and Materials

- Fourier transform infrared instrument (may include microscope with MCT detector and/or other accessories and detectors, including ATR)
- Compression cell
- Stereomicroscope
- Infrared windows for use from 4000-600 cm\(^{-1}\)
- Roller, scalpel, forceps, tungsten probe
- Liquid nitrogen
- Polystyrene film

4 Standards, Controls, and Calibration

A. The infrared spectra of a polystyrene film will be obtained each day the instrument is used. The peaks at 3025, 1600, 1028 and 698 cm\(^{-1}\) shall be marked on the spectra and be within +/- 4 cm\(^{-1}\) of their expected value in order to pass.

B. If the value is not within acceptable limits, perform re-calibration to correct the problem.
   1. If re-calibration fails to produce an acceptable result, advise the supervisor. The instrument will need to be removed from service following CLS policy. Consultation with a service engineer may be needed.
   2. Casework cannot proceed until the polystyrene film passes.
   3. The Equipment Log (LAB-405) will be completed and marked pass or fail.

5 Procedure

5.1 FTIR Operating Conditions

1. The following parameters shall be utilized for infrared spectroscopy:
   a) Data collection with a resolution of 4 cm\(^{-1}\)
   b) The same number of scans (minimum 20 scans) shall be collected for each sample, reference, and background
2. Fill the infrared microscope detector Dewar with liquid nitrogen, if applicable
3. Perform the polystyrene quality control check on the day of use
4. Document the parameters on the printed polystyrene spectra.

5.2 Sample Preparation
1. Cut a thin portion of the sample and flatten it as necessary.
2. Place flattened sample directly on a window or sample surface:
   a) The thin peel may be compressed between two windows of the same material until optical contact is made between the sample and both windows.
   b) Alternatively, the thin peel may be run without a top window.

5.3 Sample Analysis
1. Collect background spectra
2. Collect sample spectra
3. Label spectra with case number, sample ID, date, and examiner's initials. The wave number marking of significant peaks is optional if a direct overlay is being performed. Print full spectra representative of the detector used to be included with the case file
4. If the spectral pattern is electronically altered, clearly indicate the manner of adjustment and include the original spectra in the case folder.
5. If possible, classify the binder type and/or extender pigments with peak values at +/- 10 cm\(^{-1}\).
6. All spectra of case exhibits shall be maintained in the case record.

6 Interpretation
6.1 Paint
A. A combination of diagnostic infrared peaks (see Table 12-06-A for paint binders/resins and Table 12-06-B for common pigments and extenders used in automotive paints), and comparison to spectra of known paint binder types and extender pigments is the best way to classify the paint binder type and the extender pigments.
B. Acrylic dispersion lacquer, acrylic-melamine enamel, alkyd-melamine enamel and polyester-melamine enamel are typical of original finishes on automobiles. (The “polyester” and “alkyd” binders may be further classified according to the format used in the Paint Data Query from the Royal Canadian Mounted Police laboratory).
C. Alkyd enamel, acrylic-alkyd enamel, and acrylic-urethane enamel are typical of refinishes.
D. Nitrocellulose lacquers are most likely refinishes.
E. Acrylic solution lacquer and flexible polyurethane enamel may be used for both original finishes and refinishes.
F. Polyvinyl acetate-acrylic and acrylic are the most common architectural latex paints. Other architectural paints include alkyd enamel, nitrocellulose lacquer, acrylic lacquer, acrylic enamel and urethane enamel.
G. Nitrocellulose lacquer, acrylic lacquer and acrylic enamel are often packaged as spray paint.

H. Unless the characteristics are distinctly typical of a certain binder type, the examiner should not attempt to define the binder classification of the paint.

I. Some paints may contain more than one binder type or extender pigment.

6.2 Fiber

A. The generic, and possibly the sub-generic, class of manufactured textile fibers can be unequivocally identified by comparison of the fiber spectrum with reference spectra, with the exception of rayon vs. lyocell.

B. Identification made by comparison to other than an in-house standard shall be noted. A copy of the reference standard should be placed in the case file.

C. Identification made via computer search shall be confirmed by visual comparison of the spectra.

6.3 Other Substances

A. Identification is made when the spectrum of a sample is the same as the spectrum of a reference sample.

B. Polymers such as plastics and rubbers may contain additives and components that can be identified using a combination of diagnostic infrared peaks (see Table 12-06-A for paint binders/resins and Table 12-06-B for common pigments and extenders used in automotive paints).

<table>
<thead>
<tr>
<th>Binder/Resin</th>
<th>Coding</th>
<th>Key Peaks (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic</td>
<td>ACR</td>
<td>1450 1380 1260 1170 1150</td>
</tr>
<tr>
<td>Orthophthalic Alkyd</td>
<td>ALK OPH</td>
<td>1450 1380 1270 1130 1070 740 700</td>
</tr>
<tr>
<td>Isophthalic Alkyd</td>
<td>ALK IPH</td>
<td>1475 1373 1305 1237 1135 1074 730</td>
</tr>
<tr>
<td>Terphthalic Alkyd</td>
<td>ALK TER</td>
<td>1270 1250 1120 1105 1020 730</td>
</tr>
<tr>
<td>Benzoguanamine</td>
<td>BZG</td>
<td>1590 1540 825 780 710</td>
</tr>
<tr>
<td>Cyano</td>
<td>CYA</td>
<td>2238</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>CYA NIT</td>
<td>2272</td>
</tr>
<tr>
<td>Isocyanate</td>
<td>CYA ICN</td>
<td>2272 (don't confuse with Ferrocyanide pigment peak at 2092)</td>
</tr>
<tr>
<td>Epoxy</td>
<td>EPY</td>
<td>1510 1240 1180 830</td>
</tr>
<tr>
<td>Melamine</td>
<td>MEL</td>
<td>1550 815</td>
</tr>
<tr>
<td>Nitrocellulose</td>
<td>NCL</td>
<td>1650 1280 840</td>
</tr>
<tr>
<td>Polybutadiene</td>
<td>PBD</td>
<td>970 915</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>PUR</td>
<td>1690 1530 1470 1250 1070</td>
</tr>
</tbody>
</table>

TABLE 12-06-A: Diagnostic Peaks of Common Binders/Resins in Automotive Paints
### TABLE 12-06-A: Diagnostic Peaks of Common Binders/Resins in Automotive Paints

<table>
<thead>
<tr>
<th>Binder/Resin</th>
<th>Coding</th>
<th>Key Peaks (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>single peak</td>
<td></td>
<td>1690</td>
</tr>
<tr>
<td>modified EPY</td>
<td></td>
<td>1730 1510 (asymmetric broadening)</td>
</tr>
<tr>
<td>water based</td>
<td></td>
<td>1690 770</td>
</tr>
<tr>
<td>Styrene</td>
<td>STY</td>
<td>1490 1450 760 700</td>
</tr>
<tr>
<td>Urea</td>
<td>REA</td>
<td>1655</td>
</tr>
</tbody>
</table>

### TABLE 12-06-B: Diagnostic Peaks of Common Pigments and Extenders in Automotive Paints

<table>
<thead>
<tr>
<th>Pigment/Extender</th>
<th>Coding</th>
<th>Key Peaks (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Carbonate</td>
<td>CAR CAC</td>
<td></td>
</tr>
<tr>
<td>Aragonite</td>
<td>CAR CAC ARA</td>
<td>1445 870 857 712</td>
</tr>
<tr>
<td>Calcite</td>
<td>CAR CAC CAL</td>
<td>1445 870 712</td>
</tr>
<tr>
<td>Chromate</td>
<td>CHR</td>
<td></td>
</tr>
<tr>
<td>Barium</td>
<td>CHR BCH</td>
<td>935 896 860</td>
</tr>
<tr>
<td>Potassium Zinc</td>
<td>CHR KZC</td>
<td>950 880 805</td>
</tr>
<tr>
<td>Strontium</td>
<td>CHR SCH</td>
<td>911 887 875 844</td>
</tr>
<tr>
<td>Oxide</td>
<td>OXI</td>
<td></td>
</tr>
<tr>
<td>Iron oxide</td>
<td>OXI FEO YEL</td>
<td>899 797 606</td>
</tr>
<tr>
<td>Silicon Oxide</td>
<td>OXI SIO</td>
<td></td>
</tr>
<tr>
<td>Cristobalite</td>
<td>OXI SIO CRI</td>
<td>1090 795 621</td>
</tr>
<tr>
<td>Opal, Diatomaceous silica</td>
<td>OXI SIO OPA</td>
<td>1099 795</td>
</tr>
<tr>
<td>Quartz</td>
<td>OXI SIO QUA</td>
<td>1081 798 779</td>
</tr>
<tr>
<td>Titanium Oxide</td>
<td>OXI TIO</td>
<td>600 (broad suppression)</td>
</tr>
<tr>
<td>Zinc Phosphate</td>
<td>PHO ZNP</td>
<td>1120 1080 950 1020 630</td>
</tr>
<tr>
<td>Silicate</td>
<td>SIL</td>
<td></td>
</tr>
<tr>
<td>Magnesium (Talc)</td>
<td>SIL MGS TAL</td>
<td>3676 1015 670</td>
</tr>
<tr>
<td>Aluminum (Kaolinite)</td>
<td>SIL ALS KAO</td>
<td>3696 1035 940 3620 1005 910</td>
</tr>
<tr>
<td>Barium Sulphate</td>
<td>SUL BAS</td>
<td>980 630</td>
</tr>
</tbody>
</table>

Diagnostic peaks below 600 cm\(^{-1}\) are not listed
More than one pigment/extender may be present
7 Limitations

Rayon and lyocell fibers are made from cellulose and will produce the same infrared spectra. These fibers shall be identified using microscopic examination techniques.

8 Literature References and Supporting Documentation


TE-12-07  PYROLYSIS GAS CHROMATOGRAPHY MASS SPECTROMETRY (PGCMS)

1  Scope

Pyrolysis gas chromatography mass spectrometry is used to compare and identify the pyrolytic products of samples. Heating the sample rapidly in an inert atmosphere will cause thermal degradation of the sample into volatile components, which may be separated in a gas chromatograph resulting in a pyrogram. The components may be identified using a mass selective detector, if desired. Imperative for successful PGC comparison is the reproducibility of the analytical procedure.

Pyrolysis gas chromatography mass spectrometry (PGCMS) is most often used in the comparison of paint samples. PGCMS provides a detailed pyrogram of the paint binder along with its additives and will typically provide improved discrimination between paint samples than infrared techniques. PGCMS may also be useful in the examination and comparison of fibers, plastics, tape, rubber, and other samples.

As pyrolysis gas chromatography mass spectrometry is a destructive technique, the sample size, condition, and preservation requirements dictate that the comparative tests shall be selected and applied in a reasonable sequence in order to maximize the discriminating power of the test results.

2  Related Chapters

Laboratory Equipment

Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS)

3  Safety

A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)

B. High temperatures and compressed gases are encountered with pyrolysis gas chromatography mass spectrometry. Precautions shall be taken to avoid burns.

4  Equipment and Materials

- Stereomicroscope
- Pyrolysis gas chromatograph mass spectrometer
- A chromatography column and conditions suitable for the application
- CDS Pyroprobe 5150 (or equivalent) with coil probe
- Quartz tube, quartz wool
- Scalpel, tweezers, probe
- Compressed gases (air, hydrogen, nitrogen or helium)
- Polymer reference set
- Polyethylene standard
5 Standards, Controls, and Calibration

A. The mass spectrometer shall be tuned on the day of use for individual samples and evaluated before case samples are run:
   1. The mass selective detector is tuned to ensure that the mass-to-charge ratios (m/z) are assigned correctly and to check for leaks.
   2. The tune values shall be within the range of accepted values as defined during the validation of the instrument or manufacturer recommended values in order to pass. Reference the local policy for acceptable range values.

B. A polyethylene standard will be run at least once every seven days the instrument is in use.
   1. If the pyrogram of the performance check of the standard is acceptable (see Laboratory Equipment), the Equipment Log will be marked pass and the pyrogram will be placed in the case record.
   2. If the pyrogram is not acceptable, perform a system check to locate and correct the problem. If the system check fails to produce an acceptable result, discontinue analysis, remove the instrument from service following CLS policy, advise the supervisor, and mark fail on the Equipment Log.
   3. Casework cannot proceed until the instrument can successfully complete the performance check. The results of the check will be placed in the case record and the pass indication noted on the Equipment Log.

C. A pyrogram of the blank probe will be run immediately prior to each sample, using all system components and full temperature-time profiles, to demonstrate there are no peaks from the previous sample.
   1. The blank pyrograms shall be placed in the case record.
   2. If the blank exhibits peaks from the previous sample, a second blank shall be obtained. If the second blank exhibits peaks from the previous sample, the probe shall be cleaned and the blank re-run until the pyrogram is acceptable.

6 Procedure

6.1 PGCMS Operating Conditions

The following are suggested parameters for PGCMS, but may be modified according to the sample type and the examiner’s preference. The column, pyrolysis conditions and program used shall be suitable to achieve the desired separation/identification for the application.

A. Column: The column used should be applicable to the type of separation desired for the application.

B. Pyroprobe: 20°C/msec ramp rate, 10 second burn time, 700°C pyrolysis temperature, interface at 200°C.

C. GC Program: 50°C or less initial temperature for 3 to 5 minutes, 5 to 15°C/minute ramp to 250°C (minimum) final temperature, hold final temperature for a minimum of 5 minutes.

D. MS Parameters: 0.00 min solvent delay, 29.0 low mass, 550.0 high mass, 150 threshold.
6.2 Sample Preparation

1. Select a sample of known and questioned items of approximately the same size. Analysis may be conducted with as little as 5-10 µg of sample.

2. Position the questioned sample within the quartz tube so that it is in the center area of the probe coils.

3. Place the probe into the pyrolysis interface. Allow sufficient time for the carrier gas pressure to stabilize.

4. Enter the sample identification information into the software.

6.3 Sample Analysis

1. Simultaneously start the pyrolysis of the sample and the gas chromatograph program to begin data collection.

2. Burn the quartz probe in air at the maximum temperature to remove residues, as necessary.

3. Ensure the pyrogram is labeled with case number, sample ID, date, and examiner initials.

4. Obtain, label, and print a blank pyrogram. Blank pyrograms shall be attenuated to no greater than the evidence sample.

5. Obtain, label, and print the pyrogram of the known sample (or other questioned samples). Print the pyrogram so the peaks are scaled similar to the other pyrograms in the case.

6. Compare the questioned sample pyrogram to the known sample pyrogram to determine if a positive association is found. The resulting patterns and relative peak heights of the components shall be considered.

7. The mass spectra of peaks of interest can be obtained and compared to mass spectra of peaks generated from a known sample or in literature.

8. All standard, blank, and evidence sample pyrograms shall be placed in the case file.

7 Interpretation

A. A positive association is noted when the questioned sample pyrogram exhibits a pattern of peaks with relative peak heights similar to the known sample pyrogram.

B. A negative association (exclusion) is when meaningful differences are observed in the pyrograms of the questioned and known samples.

C. The known and/or the questioned sample may be run in replicate to assess variations in the pyrograms and to ensure reproducibility.

D. A compound may be identified if the mass spectrum and/or retention time criteria are met. Refer to Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS) for criteria for mass spectrum identifications/matches.

E. A combination of diagnostic pyrolysis compounds (see Table 12-07-A) and comparison to pyrograms of known polymer reference standards, known paint binders, and pressure sensitive adhesive tape components is the best way to classify the paint binder type, polymer, and/or additive components of a sample.
<table>
<thead>
<tr>
<th>Binder/Polymer/Additive</th>
<th>Coding</th>
<th>Key Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylonitrile-Butadiene-Styrene</td>
<td>ABS</td>
<td>styrene, acrylonitrile, 1,3-butadiene</td>
</tr>
<tr>
<td>Acrylic Rubber</td>
<td>ACM</td>
<td>1-butene, n-butyl acrylate, 2-methoxy ethylacrylate, 2-methoxy ethanol, 1-butanol, ethyl acrylate, vinyl methyl ether</td>
</tr>
<tr>
<td>Alyld (Generic)</td>
<td>ALK</td>
<td>phthalic anhydride</td>
</tr>
<tr>
<td>Cellulose, Starch</td>
<td>CEL</td>
<td>levoglucosan, hydroxyacetaldehyde, pyruvic aldehyde, substituted furans</td>
</tr>
<tr>
<td>Chloroprene (Neoprene) Rubber</td>
<td>CR</td>
<td>hydrogen chloride, 1-chloro-4-(1-chlorovinyl) cyclohexene (chloroprene dimer), chloroprene</td>
</tr>
<tr>
<td>Epoxy</td>
<td>EPY</td>
<td>bisphenol A, p-isopropenyl phenol, p-isopropyl phenol, acetaldehyde</td>
</tr>
<tr>
<td>Natural Rubber</td>
<td>NR</td>
<td>isoprene, dipentene (isoprene dimer), 2,4-dimethyl-4-vinyl cyclohexene (isoprene dimer)</td>
</tr>
<tr>
<td>Plasticizers (Generic)</td>
<td></td>
<td>phthalates, adipates, sebacates</td>
</tr>
<tr>
<td>Polybutadiene</td>
<td>PBD</td>
<td>1,3-butadiene, 4-vinylcyclohexene (butadiene dimer), pentadiene</td>
</tr>
<tr>
<td>Polyethyl Acrylate</td>
<td>PEA</td>
<td>triethyl hex-5-ene-1,3,5-tricarboxylate (ethyl acrylate trimer), ethyl acrylate, ethanol</td>
</tr>
<tr>
<td>Polybutyl Acrylate</td>
<td>PBA</td>
<td>tributyl hex-5-ene-1,3,5-tricarboxylate (butyl acrylate trimer), 1-butene, n-butyl acrylate</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>PE</td>
<td>n-hydrocarbons (α,ω-diolefin, α-olefin, and n-alkane triplets)</td>
</tr>
<tr>
<td>Polyethylene Terephthalate</td>
<td>PET</td>
<td>2-(benzoyloxy)ethyl vinyl terephthalate, ethan-1,2-diyl divinyl diterephthalate, 4-(vinyl oxy carbonyl) benzoic acid, divinyl terephthalate, vinyl benzoate</td>
</tr>
<tr>
<td>Polymethyl Acrylate</td>
<td>PMA</td>
<td>trimethyl hex-5-ene-1,3,5-tricarboxylate (methyl acrylate trimer), methyl acrylate</td>
</tr>
<tr>
<td>Polymethyl Methacrylate</td>
<td>PMMA</td>
<td>methyl methacrylate</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>PP</td>
<td>2,4-dimethyl-1-heptene, propylene, methyl-branched n-hydrocarbons</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>STY</td>
<td>styrene, 5-hexene-1,3,5-triytribenzenes (styrene trimer), 3-butene-1,3-diyl dibenzene (styrene dimer)</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>PUR</td>
<td>cyclopentanone, butanediol, tetrahydrofuran</td>
</tr>
<tr>
<td>Polyvinyl Acetate</td>
<td>PVA</td>
<td>acetic acid, benzene, ethyl acetate, naphthalene, indene</td>
</tr>
<tr>
<td>Polyvinyl Alcohol</td>
<td>PVAH</td>
<td>acetaldehyde, crotonaldehyde, acetone, benzaldehyde</td>
</tr>
<tr>
<td>Polyvinyl Chloride</td>
<td>PVC</td>
<td>hydrogen chloride, benzene, naphthalene</td>
</tr>
<tr>
<td>Silicone</td>
<td>SIL</td>
<td>methyl-substituted cyclic siloxanes</td>
</tr>
<tr>
<td>Styrene-Butadiene Rubber, Styrene-Butadiene-Styrene Copolymer</td>
<td>SBR, SBS</td>
<td>styrene, 1,3-butadiene, 4-vinylcyclohexene (butadiene dimer)</td>
</tr>
<tr>
<td>Urethane Rubber</td>
<td>UR</td>
<td>cyclopentanone, diphenylmethane diisocyanate, 1,4,11,14-tetraoxaacecloicosane-5,10,15,20-tetraone</td>
</tr>
</tbody>
</table>

**Note:** Components are listed in order of relative peak intensity. Not all major and minor components are provided. Please note alkyds generally do not produce major diagnostic peaks without further derivatization of the sample prior to pyrolysis.
8 Literature References and Supporting Documentation


Manufacturer's operating manual for the CDS pyroprobe.


Wampler TP. Analytical Pyrolysis: An Overview with Forensic Applications. CDS Instruments.


TE-12-08 UNKNOWN SUBSTANCE EXAMINATION

1 Scope
Evidence submitted for analysis may consist of materials not routinely analyzed by the laboratory. The identification or characterization of these materials is accomplished by the examination of its physical and chemical properties and/or by the use of analytical instrumentation.

Some types of evidence submitted to the laboratory are not conducive to absolute identification, but can be compared to known materials. The comparisons can be cognitive, physical, chemical, microscopic, instrumental, or all of these.

2 Related Chapters
Chemical Screening Tests Unit
Miscellaneous Unit

3 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Biohazard precautions, as applicable

4 Equipment and Materials
- Stereomicroscope
- Polarized light microscope
- Instrumentation, as required
- Water and other solvents
- Chemicals and reagents as used

5 Standards, Controls, and Calibration
A. The color test reagents, with the exception of diphenylamine, will be made fresh.
B. On the day of use, reagents shall be quality tested against a known substance and a solvent blank shall be performed to demonstrate they react as expected. This shall be documented in the case record.
C. Be cautious when performing any destructive testing. There is often a very limited sample amount and many destructive tests may not provide useful information for a particular sample.

6 Procedure
Consideration of the quality and quantity of sample, the requested analysis and the case circumstances will determine the tests to perform.

6.1 Physical Examination
1. Document the appearance of the evidence, noting physical characteristics such as morphology, texture, color, etc.
2. Consider the need to determine weight and/or volume.
6.2 Solid Samples

A. Most solid samples will be crystalline and may be characterized and identified using microchemical methods. Non-crystalline amorphous solid substances will require other methods for analysis and identification.

1. Document the morphology of the unknown and determine whether the sample is crystalline or amorphous.
2. Perform **Microchemical Analysis** on crystalline substances, if necessary. Screening tests (see Chemical Screening Tests unit) may also be performed.
3. Document the optical properties of crystalline substances, i.e. refractive index, optic sign, birefringence.
4. Document the physical properties of amorphous solid materials, i.e. color, shape, hardness, elasticity, opacity. Observe the non-crystalline substance microscopically for the presence of any microscopic components.

B. Use instrumental analysis methods appropriate to the sample, if necessary.

1. **Fourier Transform Infrared Spectroscopy**
2. **X-ray Diffraction**
3. **Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS)**
4. **Scanning Electron Microscopy-Energy Dispersive Spectrometry**
5. **X-ray Fluorescence**

6.3 Liquid Samples

1. Determine whether the liquid sample is aqueous by performing miscibility tests with solvents such as water and hexane.
   a) Perform **Microchemical Analysis** to determine presence of ions, if necessary. Screening tests (see Chemical Screening Tests unit) may also be performed.
   b) Additional microchemical/color tests may be utilized if properly validated prior to use.
2. Appropriate solid phase microextraction (SPME) or liquid (solvent) extraction methods should be used for the analysis of mixtures as necessary. See also Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS).
3. Perform pH determination with pH paper.
4. If sufficient sample is available, it may be useful to evaporate the liquid and analyze the residue as solid sample.
5. Use instrumental analysis methods appropriate to the sample, as necessary.
   a) **Fourier Transform Infrared Spectroscopy**
   b) **Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS)**.

6.4 Volatile Samples

The volatile component(s) of a sample may be analyzed using solid phase microextraction (SPME) or the "dirty needle" technique. See also Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS).
6.5 Residues on Evidentiary Items

Evidence may be submitted with requests to find residues of unknown materials. Such unknown material residues can be solid substances or dried liquids, including OC spray and lubricants.

1. Analyze solid residues using the steps listed in Section 6.2.
2. Document and use appropriate extraction methods to extract residues as necessary.
3. Test unstained portions of the substrate using the same extraction methods used on the stained areas. See also Analysis of Substances by Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GCMS).

7 Interpretation

A. Identification is dependent on the quality of the test results. Identification can be achieved by comparison to data in reference libraries and/or to known samples. Unknown samples should be compared to known samples when known samples are available.

B. Identity of elements and/or compounds may often be suggested through a series of color tests, or confirmed by instrumental analysis. At times, it may be possible to develop a list of possible sources or uses of the identified components.

Example: The residue in the plastic bottle contained aluminum. The water wash of the residue had an acidic pH and contained chloride. Placing hydrochloric acid and aluminum foil in a sealed plastic bottle is a common method of creating an "acid bomb".

C. Though identification may not be achieved, useful information should be included in the report.

Example: The residue has a neutral pH and showed a negative presumptive test for the presence of chlorides, sulfates and nitrates. Therefore, we are unable to detect the presence of an acidic substance.

8 Literature References and Supporting Documentation


TE-12-09 X-RAY FLUORESCENCE (XRF)

1 Scope
X-ray fluorescence analysis is used to obtain elemental information of a sample based upon the energy of the X-rays emitted by the sample when bombarded by an X-ray beam.

Elements present in a sample can be identified by the characteristic X-rays that are emitted. Questioned and known samples can also be compared semi-quantitatively based upon spectral comparisons and elemental peak intensity ratios.

2 Related Documents
Safety Manual: Radiation Safety

3 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment
B. Protective eyewear shall be worn when filling the detector with liquid nitrogen.

4 Equipment and Materials
- X-ray fluorescence instrument
- Liquid Nitrogen
- Aluminum/copper standard (Al2024) and NIST Standard Reference Material (SRM) 1831 glass
- Sample holders/low element adhesive/sample support film

5 Standards, Controls, and Calibration
5.1 Energy Scale Calibration
A. Each day the instrument is used, the aluminum/copper standard is analyzed to demonstrate proper operation and energy calibration.
B. The aluminum/copper standard should be run at all of the time constant values that will be used for sample analysis. The aluminum peak should be at 1.486 keV and the copper peak at 8.04 keV.
C. If an automated run is performed, the energy calibration will only need to be completed on the day the automated run is started.
D. If the calibration fails to meet expected values, manual adjustments can be made and a re-calibration performed. If the calibration continues to fail to produce acceptable results, the instrument will be removed from service following CLS policy. Consultation with a service engineer may be needed.
E. The Equipment Log (LAB-405) will be marked pass or fail and the energy calibration summary placed in the instrument log.

5.2 Quality Control
A. The instrument will be performance checked on each day of use with the NIST Standard Reference Material (SRM) 1831 glass.
B. The LAB-405 shall be marked pass/fail and the data placed in the case record. If the performance check fails, remove the instrument from service. A service engineer may need to be consulted.
C. If the instrument is being used only for element identification, such as in an unknown substance case, then the following elements shall be identified in the NIST SRM 1831 sample (one point analyzed) to be considered passing: Na, Mg, Si, K, Ca, Fe, Zn, Sr, and Zr.

D. If the instrument is being used for semi-quantitative comparisons such as in a glass case, then the NIST SRM 1831 should be run at the following parameters:
   1. Voltage: 50 keV
   2. Current: 1000 µA
   3. 1200 Lsec
   4. Minimum of three points analyzed

E. If an automated sample run of ten (10) runs is performed, the NIST SRM 1831 sample should be included at the beginning and the end of the automated run.

F. The following element ratios will be calculated for each QC run and averaged together: Ca/Fe, Ca/Mg, Ca/K, Sr/Zr, Fe/Zr, Fe/Sr, and Fe/Zn. The performance check is considered passing when the elemental intensity ratios fall within the acceptable ranges that were developed during the validation process. Refer to the local policy for acceptable limits.

6 Procedure

6.1 Operating Conditions

1. Fill the detector Dewar with liquid nitrogen.
2. The time constant, current and voltage should be adjusted based upon the sample specimen being analyzed. Actual requirements may vary as determined by the specific analytical needs. Samples that are being compared should be run with the same settings. General guidelines are as follows:
   a) Beam current should be adjusted to achieve <50% deadtime
   b) Increased current provides higher count rates
   c) Increased voltage provides higher energy line excitations
   d) Lower time constants allow for higher throughput of counts
3. Allow the tube to stabilize for approximately 30 minutes.
4. Perform the aluminum/copper quality control check and SRM 1831 glass standard quality control check.

6.2 Sample preparation

1. Run samples directly on the stage if they are of sufficient thickness.
2. Raise small samples and thinner samples above the surface of the stage using a sample holder and/or supportive film to reduce X-ray scatter off of the surface of the stage.
3. Adhesive can be used to ensure that samples remain on the sample holder under vacuum.
4. Ensure samples used for comparisons are of similar size, shape, and thickness to each other.
6.3 Sample analysis

1. Evacuate the sample chamber if sample is to be run under vacuum.
2. Focus on the sample under low magnification. Switch to high magnification for analysis. Auto focus can be used to focus sample further.
3. Collect spectrum.
4. Examine the spectrum for peaks with characteristic energies and label the peaks.
5. Mark the spectrum with case number, initials, date, and sufficient information to identify the particular sample.

7 Interpretation

A. An element is identified when:
   1. A peak is significant, and
   2. The peak has the characteristic energy for that element.

B. Identification usually begins with high energy peaks and major peaks. Spectral artifacts such as sum peaks and escape peaks associated with the major peaks should be considered and marked.

C. Reference lines, or energies, may be obtained from several sources including energy slide rules, automatic element identification software, computer generated theoretical fit curves that can be superimposed on the spectrum, and published tables. Often these aids are used in a complementary fashion.

D. The presence of an element can be considered unequivocal only when a distinctive, unique set of lines is produced, or when a single peak occurs at an energy where it cannot be mistaken for another element or spectral artifact. Identification may not be possible if an element is present in low concentration or if the energy lines required for confirmation are overlapped with the lines of other elements.

E. If an automatic peak identification application is used, peak identification shall be confirmed visually by the analyst.

F. If samples are to be compared to each other, peak identification and spectral comparisons along with comparisons of peak intensity ratios can be used. Peak intensities correlate with the quantities of each element present. Elements used for peak intensity ratios should be close on the energy scale as long as reasonable peak resolution is obtained.

8 Limitations

Elements present below about 15-50 ppm may not be detected depending upon the element.

9 Literature References and Supporting Documentation


TE-12-10 MICROCHEMICAL ANALYSIS

1 Scope
Microchemical analysis encompasses the range of tests on substances that are carried out at a microscopic scale. Microchemical tests are relatively quick and easy to perform, and they give valuable information about the nature and identity of unknown materials. The tests require only small amounts of material, and can be performed directly on a microscope slide.

2 Related Chapters
Chemical Screening Tests unit
Unknown Substance Examination

3 Safety
   A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
   B. Biohazard precautions, as applicable

4 Equipment and Materials
   - Stereomicroscope
   - Polarized light microscope
   - Hot plate
   - Alcohol lamp
   - Deionized water
   - Acetone
   - Xylene
   - Chloroform
   - Acetic Acid
   - Hydrochloric Acid
   - Sulfuric Acid
   - Nitric Acid
   - Ammonium hydroxide
   - Sodium hydroxide
   - Squaric Acid
   - Ammonium dichromate
   - Cargille Refractive Index Liquids
   - Cargille Chemical Microscopy Reagent Set 1
   - Cargille Chemical Microscopy Reagent Set 2

5 Standards, Controls, and Calibration
   A. Verify and document that all reagents are working properly by testing a compound containing the ion of interest (positive control) and a compound lacking the ion of interest (negative control).
B. Test solvents for contamination by evaporating blank solvent on a microscope slide and observing any residue left behind.

6 Procedure

6.1 Stereomicroscopic Observation

1. Observe the morphological characteristics of the sample, such as particle size, crystal shapes, colors, and sample purity.
   a) Mixtures can be separated by particle picking.
   b) Crystals of similar appearance can be picked out of the sample, and microscopical and other tests can then be performed on single, relatively pure components.
   c) Chemical color tests may also be performed on minute samples on a microscope slide under the stereomicroscope.

2. Refer to the Chemical Screening Tests unit for color test procedures.

6.2 Solubility

1. Solubility and other chemical spot tests may be performed on a microscope slide.

2. A single grain should be tested in a drop of a solvent, such as water, acetone, xylene, and chloroform, to determine the chemical nature of the unknown.

3. Observe the behavior of the crystal at 100X magnification on the polarized light microscope. The same grain may be used in each solvent, once the solvent has evaporated.

4. Decant the liquid from undissolved sample with a glass rod, drawing the liquid away from the solid.

5. Use a filter paper to break the liquid channel.

6. Tests may then be run on the liquid sample, and further tests may be performed on the remaining solid.

6.3 Polarized Light Microscopy

1. Observe the crystallographic and optical properties of crystals using the polarized light microscope.

2. Determine whether crystals are optically isotropic, uniaxial, or biaxial.
   a) Observing anisotropic crystals at 400X with crossed polarizers, find a crystal exhibiting dull gray low order retardation as a good crystal for observing interference figures when viewed conoscopically.
   b) Use the interference figure to determine whether the crystal is uniaxial or biaxial.
   c) Also determine the optic sign using the 530 nm compensator.

3. If possible, determine the crystal form (cubic, hexagonal, tetragonal, orthorhombic, monoclinic, triclinic).
   a) To aid in identification of unknowns, recrystallization can be performed in the appropriate solvent.
   b) Well-formed crystals may be grown as the solvent evaporates.
4. Determine refractive indices of crystals using the Becke line method in Cargille Refractive Index liquids.
   a) **Cubic crystals will have only one refractive index.**
   b) **Choose a starting value of refractive index liquid, and then bracket the crystal with the refractive index liquids until the crystal refractive index is the same as the liquid.**

5. Uniaxial crystals will have two refractive indices, epsilon and omega.
   a) **Which one is greater in value depends on the optic sign of the crystal.**
   b) **Determine the value of epsilon and omega using the Becke line method in Cargille Refractive Index liquids.**
   c) **Calculate the birefringence of the crystal by subtracting the two values of refractive index.**

6. Biaxial crystals have three refractive indices.
   a) **Measure the maximum and minimum refractive indices using the Cargille Refractive Index liquids.**
   b) **Calculate the birefringence by subtracting the maximum and minimum refractive indices.**

7. Use the data obtained to determine from appropriate references the range of possible compounds that fit the measured optical properties. Once the list of possible substances has been determined, microchemical tests can be used to confirm the identity of the unknown.

### 6.4 Microchemistry

**Table 12-10-A** illustrates which of the methods described below to use in the testing of specific ions.

A. Microchemical tests involve chemical reactions that are performed on a microscope slide and observed on a polarized light microscope. Since most tests are performed without a cover slip, observations are made at 100X magnification.

1. Exercise care to avoid damage to the microscope objective with prolonged exposure to acid fumes.

2. Never leave slides on the microscope stage for prolonged periods of time.

B. The methods used refer to Chamot and Mason’s methods outlined in Handbook of Chemical Microscopy, Vol II. The methods are designed to test for free ions in solution. In some cases, the unknown is not directly soluble, so methods are described to bring them into solution.

C. The reaction products obtained are insoluble salts that crystallize under the conditions of the tests. The crystals that are formed by these reactions can be observed and compared to known results. Results are specific for the tested substance unless stated otherwise in the notes section of **Table 12-10-A**.

1. **Method I**
   a) **Dissolve the unknown in a drop of water (the test drop) on the microscope slide adjacent to a drop containing the reagent (the reagent drop).**
b) Draw the test drop to the reagent drop with a glass rod, creating a channel between the drops.

c) Observe the crystals formed.

2. Method II

a) Add reagent directly to the test drop, or add a small crystal of the unknown directly to a drop of the reagent.

3. Method III

a) Dry the test drop on the slide, then draw the reagent over the dry film with the glass rod.

b) Conversely, the reagent may be dried, and the test drop can be drawn across the dried reagent.

4. Method IX: this method is used to test for volatile components in the test drop.

a) Place a drop of the reagent on a cover slip or microscope slide.

b) Invert the cover slip above the test drop.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Reagent</th>
<th>Method</th>
<th>Notes: dilute test drop, moderately concentrated reagent</th>
<th>Results: colorless orthorhombic prisms terminated in pyramids; isomorphic with NH₄⁺, Rb⁺, Cs⁺, Ti⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>HClO₄</td>
<td>I</td>
<td>Notes: dilute test drop acidified w/dil. HCl</td>
<td>Results: deep yellow octahedra and cubes; isomorphic with NH₄⁺, Rb⁺, Cs⁺, Ti⁺</td>
</tr>
<tr>
<td>H₂PtCl₆</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squaric Acid</td>
<td>I</td>
<td></td>
<td>Results: 4- and 8-pointed stars and crosses</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>HClO₄</td>
<td>IX</td>
<td>See above for K⁺; to differentiate from K⁺, hang drop of reagent over test drop in dilute NaOH</td>
<td></td>
</tr>
<tr>
<td>H₂PtCl₆</td>
<td>IX</td>
<td></td>
<td>See above for K⁺; to differentiate from K⁺, hang drop of reagent over test drop in dilute NaOH</td>
<td></td>
</tr>
<tr>
<td>Squaric Acid</td>
<td>I</td>
<td></td>
<td>Results: black crosses and tablets</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>Uranyl Acetate</td>
<td>III</td>
<td>Notes: dry test drop, acidify reagent with acetic acid</td>
<td>Results: faint yellow tetrahedra, anisotropic</td>
</tr>
<tr>
<td>Squaric Acid</td>
<td>I</td>
<td></td>
<td>Results: short rods</td>
<td></td>
</tr>
<tr>
<td>Ag⁺</td>
<td>Acetic Acid</td>
<td>I</td>
<td>Results: pearly scales develop into long, thin plates</td>
<td></td>
</tr>
<tr>
<td>(NH₄)₂Cr₂O₇</td>
<td>II</td>
<td></td>
<td>Notes: acidify test drop with dilute HNO₃, add fragment of reagent to center of drop</td>
<td>Results: dark red pleochroic plates</td>
</tr>
<tr>
<td>Al⁺⁺⁺</td>
<td>NH₄ Molydate</td>
<td>II</td>
<td>Results: thin, colorless transparent plates</td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td>Reagent</td>
<td>Method</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Pb**</td>
<td>KI</td>
<td>I</td>
<td>Results: yellow hexagonal plates</td>
<td></td>
</tr>
<tr>
<td>Ca**</td>
<td>H₂SO₄</td>
<td>I</td>
<td>Notes: dilute reagent, PbSO₄ soluble in NaOH, CaSO₄ insoluble in NaOH Results: gypsum needles</td>
<td></td>
</tr>
<tr>
<td>Ba**</td>
<td>H₂SO₄</td>
<td>I</td>
<td>Results: amorphous appearing, digestion with HCl yields well-defined tabular grains; also insoluble in NaOH</td>
<td></td>
</tr>
<tr>
<td>Squaric Acid</td>
<td></td>
<td>I</td>
<td>Results: knife blades</td>
<td></td>
</tr>
<tr>
<td>Cu**</td>
<td>K₂Hg(SCN)₄</td>
<td>I or II</td>
<td>Notes: acidify with dilute HNO₃ Results: greenish-yellow mossy dendrites and boat shaped crystals</td>
<td></td>
</tr>
<tr>
<td>Zn**</td>
<td>K₂Hg(SCN)₄</td>
<td>I or II</td>
<td>Notes: acidify with dilute HNO₃ Results: white feathery crosses</td>
<td></td>
</tr>
<tr>
<td>Cd**</td>
<td>K₂Hg(SCN)₄</td>
<td>I or II</td>
<td>Notes: acidify with dilute HNO₃ Results: colorless orthorhombic prisms</td>
<td></td>
</tr>
<tr>
<td>Co**</td>
<td>K₂Hg(SCN)₄</td>
<td>I or II</td>
<td>Notes: acidify with dilute HNO₃ Results: deep-blue pleochroic orthorhombic prisms</td>
<td></td>
</tr>
<tr>
<td>Fe(III)</td>
<td>K(SCN)</td>
<td>II</td>
<td>Results: color test red Notes: Fe(II) no reaction with (SCN)⁻; to oxidize Fe(II) to Fe(III), treat test drop with conc HNO₃, evaporate to dryness, add HCl and evaporate, dissolve residue in water acidified with HCl</td>
<td></td>
</tr>
<tr>
<td>CrO₄⁻</td>
<td>AgNO₃</td>
<td>I or II</td>
<td>Results: amorphous dark red ppt with needles, platelets, and plates; recrystallize with Ag₂SO₄</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>AgNO₃</td>
<td>I</td>
<td>Results: white ppt</td>
<td></td>
</tr>
<tr>
<td>Br⁻</td>
<td>AgNO₃</td>
<td>I</td>
<td>Results: white ppt</td>
<td></td>
</tr>
<tr>
<td>I⁻</td>
<td>AgNO₃</td>
<td>I</td>
<td>Results: white ppt</td>
<td></td>
</tr>
<tr>
<td>SO₄⁻</td>
<td>AgNO₃</td>
<td>I</td>
<td>Notes: concentrated solution Results: colorless high-refractive rhomb-shaped tablets; in presence of CrO₄⁻ or Cr₂O₇⁻, Ag₂SO₄ crystals will be yellow to red and pleochroic; Ag₂SO₄ soluble in dilute HNO₃</td>
<td></td>
</tr>
<tr>
<td>Ba**</td>
<td>I</td>
<td></td>
<td>Results: exceedingly fine, insoluble in dilute HNO₃</td>
<td></td>
</tr>
<tr>
<td>PO₄⁻</td>
<td>AgNO₃</td>
<td>I</td>
<td>Results: light yellow ppt, tiny yellow dendritic 3-branch stars</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>Molybdate</td>
<td>I</td>
<td>Notes: reagent saturated and acidified with HNO₃, test drop should be neutral Results: yellow “lemon drops”</td>
<td></td>
</tr>
</tbody>
</table>

Printed copy is uncontrolled. Refer to electronic copy for current version.
### TABLE 12-10-A: Microchemical Test

<table>
<thead>
<tr>
<th>Ion</th>
<th>Reagent</th>
<th>Method</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>S⁻</td>
<td>Lead Acetate</td>
<td>IX</td>
<td>Notes: sample in HCl, reagent in hanging drop&lt;br&gt;Results: fine black or brown ppt</td>
</tr>
<tr>
<td>$\text{CO}_3^{2-}$</td>
<td>Lead Acetate</td>
<td>IX</td>
<td>Notes: sample in HCl, reagent in hanging drop&lt;br&gt;Results: x-shape crystals</td>
</tr>
<tr>
<td>$\text{ClO}_3^-$</td>
<td>Nitron Sulfate</td>
<td>I</td>
<td>Notes: reagent in acetic acid, both solutions dilute&lt;br&gt;Results: segmented needles that bundle radially or independently. These needles will be thicker than those formed from NO₃⁻</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Nitron Sulfate</td>
<td>I</td>
<td>Notes: reagent in acetic acid, both solutions dilute&lt;br&gt;Results: very thin birefringent needles that bundle radially</td>
</tr>
<tr>
<td>$\text{NO}_2^-$</td>
<td>Nitron Sulfate</td>
<td>I</td>
<td>Notes: reduce nitrate to NH₃ with metallic Al in NaOH; hanging drop of reagent&lt;br&gt;Results: yellow isotropic octahedra</td>
</tr>
<tr>
<td>Acetate</td>
<td>AgNO₃</td>
<td>I</td>
<td>Notes: reagent in acetic acid, both solutions dilute&lt;br&gt;Results: thin needles clumping into spheres, dendritic</td>
</tr>
<tr>
<td></td>
<td>AgNO₃</td>
<td>I</td>
<td>Results: fine needles, prisms greenish tint with reflected light</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td></td>
<td>Results: pearly scales develop into long, thin plates</td>
</tr>
</tbody>
</table>

### 7 Interpretation

A. Crystallographic and optical data obtained by polarized light microscopy are used to look up in reference books to determine the possible identities of the unknown crystal. If sufficient data is obtained about the unknown, a short list of possible identities will be obtained and the reference source is specified in the case record.

B. Microchemical tests are used to confirm or rule out the presence of ions from the short list of possible identities. When used in conjunction, the identity of the unknown may be determined by these methods.

C. When optical and crystallographic data are not obtained, microchemical tests, including solubility, spot tests, and crystal tests, can be used to determine the chemical nature of the unknown. The formation of the reaction product indicates the presence of the tested ion.

### 8 Limitations

These tests are not all confirmatory and may only be indicative of the presence of the tested ion. Further testing may be needed to confirm their presence.

### 9 Literature References and Supporting Documentation


TE-12-11 FLUORESCENCE MICROSCOPY

1 Scope
When exposed to certain wavelengths of light, some substances will emit light at a longer wavelength than that of the excitation wavelength. This phenomenon, known as fluorescence, can provide an added level of discrimination to microscopic examinations.

2 Safety
A. Universal Safety Precautions and use of appropriate Personal Protective Equipment (PPE)
B. Use a UV shield when operating the fluorescence microscope

3 Equipment and Materials
- Fluorescence Microscope
- Excitation and Barrier filters for varying wavelengths
- Microscope slides, coverslips
- Mounting Media (i.e. permount, glycerin, DI water, xylenes)

4 Standards, Controls, and Calibration
Before use, adjust the microscope to obtain Köhler illumination

5 Procedure
1. Mount samples in an appropriate mounting media.
   Note: If the mounting media selected fluoresces then it may be necessary to remount the samples in a more suitable media (i.e., glycerin, water, xylenes)
2. Turn on microscope and allow 10-15 minutes for lamp to stabilize.
3. Adjust the microscope for Köhler illumination.
4. Engage the sets of excitation and barrier filters and compare question sample to known sample.
   a) Document the presence or absence of fluorescence.
   b) If fluorescence is present, document the applicable observations:
      i. Excitation wavelength producing the maximum fluorescence
      ii. Color of fluorescence under various wavelengths
      iii. Depth of fluorescence penetration (if applicable)
      iv. Intensity of fluorescence

6 Interpretation
A. If no meaningful differences in the characteristics of the question sample and the known sample are detected, further testing is warranted before a conclusion can be determined.
B. If meaningful differences in the characteristics of the question sample and known sample are detected, then a conclusion is made that the question sample is not consistent with the known sample and therefore did not originate from the source of the known sample.
7 Literature References and Supporting Documentation


13 **FORMS**

**DIRECTORY OF FORMS**

<table>
<thead>
<tr>
<th>Document Name</th>
<th>FRN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Paint Examination</td>
<td>LAB-TE-03</td>
</tr>
<tr>
<td>2 Glass Examination</td>
<td>LAB-TE-04</td>
</tr>
<tr>
<td>3 Filament Examination</td>
<td>LAB-TE-05</td>
</tr>
<tr>
<td>4 Hair Examination</td>
<td>LAB-TE-06</td>
</tr>
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<td>5 Fiber Examination</td>
<td>LAB-TE-07</td>
</tr>
<tr>
<td>6 Trace Evidence Instrument Worksheet</td>
<td>LAB-TE-09</td>
</tr>
</tbody>
</table>
14 APPENDICES

APPENDIX 01 CATEGORIES OF ASSOCIATION

1 Scope

The following appendix is attached to Trace Evidence reports in order to provide information to customers regarding the use of categories of association.

Categories of Association Report Appendix

Texas Department of Public Safety - Trace Evidence Analysis

If no category of association is provided in the attached report, please disregard this appendix.

In the sub-disciplines of trace evidence, a statistical assessment of significance is not possible. The following categories of association are meant to provide context to the level of opinions reached in the report.

CATEGORY 1

Source Identity/Source Attribution - The compared samples exhibit characteristics demonstrating that the items were once part of the same object. Alternatively, the sample is positively identified.

CATEGORY 2

Associations of Class Characteristics

- Class characteristics are general characteristics that apply to a group of items. Associations of this category mean items could not be differentiated based on observed, measured, and/or chemical properties and therefore can not be eliminated as having come from the same source. There are varying degrees of associations within this category depending on the number of items a particular class characteristic can include. It should be noted that associations within this category cannot definitively establish that the items came from the same source.

CATEGORY 2A

Association with distinct characteristics - The items share distinct characteristic(s) that would not be expected to be encountered in the general population.

CATEGORY 2B

Association with conventional characteristics - The items share characteristics that have been manufactured or occur in nature and would be indistinguishable from the submitted evidence. Other items which share these properties may be available in the general population.

CATEGORY 2C

Association with limitations - The items share characteristics that are common amongst these kinds of natural or manufactured materials and would be indistinguishable from the submitted evidence. Limitations to this association could include variations due to condition of the evidence or limited characteristics present.

CATEGORY 3

Inconclusive - The observed, physical, and/or chemical properties do not provide enough information to associate or eliminate the compared samples.

CATEGORY 4

Dissimilar/Non-Association - Items are dissimilar in observed, physical, and/or chemical properties indicating they did not originate from the same source. However, the compared items share enough general characteristics that, due to reasonable variations in the source, the source itself cannot be associated or eliminated based on the specimen received.

CATEGORY 5

Elimination/Exclusion - The items are dissimilar in observed, physical, and/or chemical properties demonstrating they did not originate from the same source.