Laboratory Services
Trace Evidence

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1. Scope

1.1. This document describes the general approach to trace evidence examinations.

1.2. The Trace Section performs analyses on a wide variety of materials, some of which include fibers, hairs, paints, polymers, and tape.

1.3. This document is not meant to encompass all possible circumstances that may be encountered in forensic casework, nor is it a step-by-step process, as different types of trace evidence require different procedures. The selection of material(s) for analysis and the analytical scheme is determined by the examiner and is case-dependent.

2. Processing Physical Evidence

2.1. A general examination approach includes a visual examination of the item of evidence followed by a microscopic examination.

2.2. The evidence must be clearly documented in the technical record which can include photographs, sketches and/or written descriptions. See *ATF-LS-7.5 Technical records*

2.3. Each item should be examined separately (by time and/or space) unless items are packaged together. Ensure good laboratory practices are followed to minimize the potential of cross-transfer and/or contamination.

2.4. Different types of illumination (e.g., oblique lighting, lit magnifier, alternate light sources) can be used to detect trace evidence.

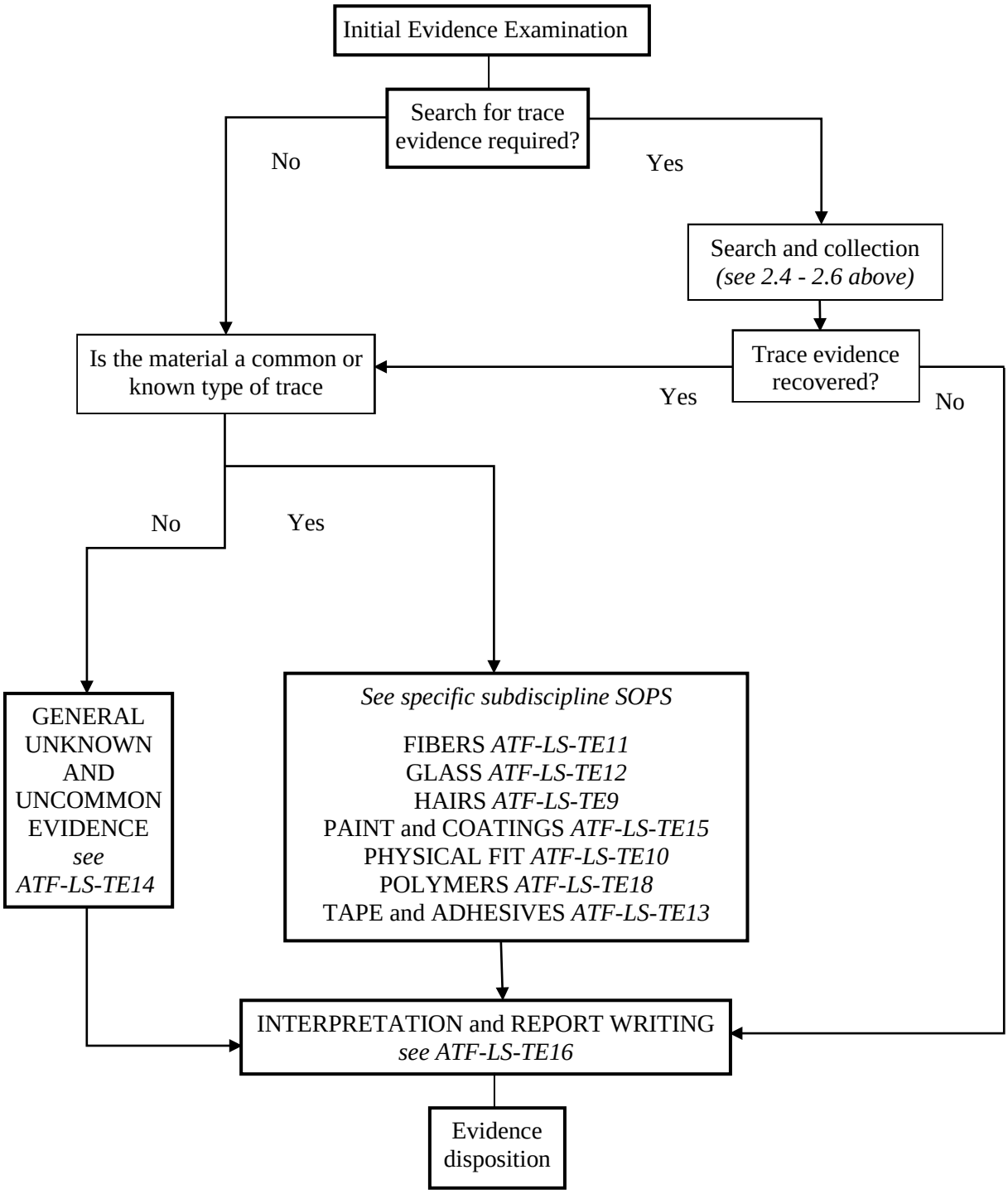
2.5. Collection techniques such as particle picking, scraping, brushing, tape lifting, etc., can be used if the visual and microscopical examinations warrant it.

2.6. Any materials of interest (for further examination and/or comparison) are separated and protected from alteration, contamination, or loss.

2.7. When a comparative examination is requested, the questioned item is evaluated to identify characteristics suitable for comparison prior to examination of the known item. In certain circumstances, it may be appropriate to perform a preliminary characterization of the known prior to the assessment of the unknown (e.g., determining fiber color of known prior to searching for fibers on a tape-lift).

3. General Evidence Examination (see flowchart)

General Trace Evidence Flow Chart



1. Scope

   1.1. This document describes the procedure for utilizing alternate light sources for examination. Examination of evidence using monochromatic light sources can assist in locating potential trace evidence, indicating the manufacturing process of glass, as well as comparing two or more samples.

   1.2. Fluorescence microscopy is utilized for detecting and characterizing fluorescing substances in materials such as pigments in paints and for comparing items such as fibers that have fluorescing dyes or optical brighteners. The procedure below is for the setup and use of the reflected light fluorescence attachment on the polarized light microscope (PLM) for mercury, xenon, and LED light sources.

   1.3. The use of an Ultraviolet (UV) viewing cabinet and hand-held alternate light sources are described below.

2. Instrumentation/Reagents

   2.1. Reflected Light Fluorescence Attachment

   2.2. Fluorescence Cubes

   2.3. Mounting media

   2.4. UV viewing cabinet

   2.5. UV hand-held lights

3. Safety Considerations

   3.1. Follow standard laboratory safety procedures and those recommended in manufacturer's instruction manuals.

   3.2. Gloves and safety glasses should be worn when handling the light source.

   3.3. Never look directly at the Ultraviolet (UV) light source.

   3.4. Allow the lamp housing and lamp to cool before changing a burned-out lamp.

4. Procedure for Analysis

   4.1. Fluorescence Microscopy

4.1.1. UV light source alignment and adjustment

4.1.1.1. The alignment and adjustment shall be conducted whenever the light source is changed or whenever necessary before an examination (e.g., image is partially obscured or unevenly illuminated).

4.1.1.2. Refer to manufacturer instructions manuals for specific instructions on the adjustment and alignment of the light source.

4.1.1.3. **Warning**: If using the mercury burner (lamp) the following precautions shall be followed:

4.1.1.3.1. If the burner does not ignite, turn the main switch off once, and then repeat after 5 or 10 seconds.

4.1.1.3.2. To avoid shortening the life of the burner, do not turn the burner off within 15 minutes of ignition.

4.1.1.3.3. After turning the burner off, it cannot be re-ignited until the mercury vapor cools and condenses to liquid. Wait for about 10 minutes before restarting the burner.

4.1.1.4. **Warning:** If using the X-cite LED, ensure that the source is kept on until the fan shuts off.

4.1.2. Reflected Light Fluorescence Observations

4.1.2.1. Close the vertical light path shutter and turn on the UV light source (See 4.1.2.5.1 if using the mercury burner).

4.1.2.2. Using the transmitted light source, focus on the specimen using the desired objective.

4.1.2.3. Bring a suitable cube into the light path and turn off the transmitted light source.

4.1.2.4. Open the vertical light shutter and refocus on the specimen, if necessary.

4.1.2.5. Adjust the brightness.

4.1.2.5.1. Mercury burner: Adjust the collector lens-focusing knob to where the brightness and evenness of the illumination in the field of view are at a maximum. Adjust the aperture diaphragm as needed.

4.1.2.5.2. LED: Adjust the brightness/intensity and aperture diaphragm as needed.

4.1.2.6. The intensity (e.g., none, low, moderate, high) and the color of the fluorescence shall be noted in the case record.

4.2. Other Alternate Light Sources

4.2.1. Viewing Box: Plug in the light box and use the rocker switches to view the sample under white light, shortwave UV (254 nm) and longwave UV (365 nm) light.

4.2.2. Hand-held light: Operate by pushing the red/black buttons or using the rocker switch on the back side of the light.

5. Quality Assurance and Controls

5.1. Fluorescence Microscopy

5.1.1. Proper alignment of the microscope is completed when the microscope is initially set up and is readjusted as needed during the examination.

5.1.2. A sample of known fluorescence shall be examined daily, prior to examining the case-related sample(s). The reference fiber F10.143 from the Microtrace Collection is used and checked under each filter. Only the filters that have a successful performance check will be utilized in casework. The details of the filters as well as the expected color are below for each fluorescent microscope in the laboratory. The results of the performance check shall be documented in the case record.

MICROSCOPE: Leica DM2700			
Turret position	**Filter description**	**Excitation region**	**Fluorescence**
2	Ultraviolet	360/20 (340 – 380 nm)	Blue
3	Blue	436/7 (432.5 – 439.5 nm)	Green/blue
4	Green Fluorescent Protein (GFP)	470/40 (450 – 490 nm)	Green
5	Green	537.5/45 (515 – 560 nm)	Red

MICROSCOPE: LEEDS COMPARISON			
Turret position (label)	**Filter description**	**Excitation region**	**Fluorescence**
3 (UW)	Ultraviolet	375/28 (361 - 389 nm)	Blue
4 (BVW)	Aqua	420/40 (400 – 440 nm)	Green/blue
5 (BW)	Blue	480/30 (465 – 495 nm)	Yellow
6 (GW)	Green	540/25 (527.5 – 552.5 nm)	Red

MICROSCOPE: Olympus BX60			
Turret label	**Filter description**	**Excitation region**	**Fluorescence**
WU	Ultraviolet	357.5/55 (330 – 385 nm)	Blue
●	Blue Violet Narrow	430/10 (420 – 440 nm)	Green
MF	Blue Violet Wide	420/20 (400 – 440 nm)	Green
WG	Green Wide	530/40 (510 – 550 nm)	Red
○/WB	Blue Wide	465/30 (450 – 480 nm)	Yellow

5.1.3. Microscopes should be cleaned and adjusted regularly by a manufacturer's service representative per the service contract agreement. The maintenance shall be documented (e.g., logbook).

5.2. Other Alternate Light Sources

5.2.1. A control can be checked if deemed necessary (e.g., comparison of scrim or glass). Expected results can be found in Table 1. The results of the performance check shall be documented in the case record when performed.

Table 1

Fluorescing scale (Reference #X28.1)	**Visible light**	**Short wave UV**	**Long wave UV**
NightSea scale – pink, yellow and green areas	Pink, yellow and green	Fluoresces dull pink/orange, yellow and green	Fluoresces bright pink, yellow and green
NightSea scale – black areas	Black	Does not Fluoresce	Does not Fluoresce

5.3. Fluorescence is a well-known and scientifically accepted method for the analysis and comparison of fluorescing materials in many types of trace evidence. Relevant examples of the broad nature of the method and related literature can be found in Section 6 (References).

6. References

ASTM E2228 Standard Guide for Microscopical Examination of Textile Fibers

Forensic Examination of Fibres, 3rd Edition. Edited by James Robertson, Claude Roux and Kenneth G. Wiggins. Pages 149, 172-175.

Leica DML Instruction Manual – Incident Light Chapter

Leica DM2700 M: Instructions for use

Leica DM2700 P: Supplement to the instructions for use for Leica DM2700 M

Leeds Forensic Systems, Inc. Optional Reflected Fluorescence Lighting Manual

Olympus BX-FLA Reflected Light Fluorescence Attachment Instruction Manual

1. Scope

    1.1. This method describes the procedure for basic microscopy setup on compound polarized light microscopes (PLM), but certain sections can also be used for brightfield (biological) microscopes. Also, the "Ocular Focus Adjustment" and the "Calculation of Ocular Scale Magnification Factors" sections could be used for stereomicroscopes and the "Ocular Focus Adjustment" can be used for the FTIR microscope.

    1.2. The proper setup of the microscope is important since the PLM is one of the most powerful analytical tools available to the forensic trace examiner. PLM allows an examiner to quickly characterize, identify, and compare particles/fibers encountered in case work by determining a variety of physical and optical properties which include size, morphology, surface texture, color, pleochroism, refractive index (indices), birefringence, sign of elongation, extinction, interference figure, optic sign, and crystal system.

2. Instrumentation/reagents

    2.1. Brightfield microscope

    2.2. Comparison microscope

    2.3. Polarizing light microscope (PLM)

    2.4. Stereomicroscope

    2.5. Phase contrast microscope

    2.6. Micrometer scale

3. Safety considerations

    3.1. Follow standard laboratory safety procedures.

4. Procedure or analysis

    4.1. Sample preparation

        4.1.1. Pre-cleaned slides can be used, or slides and cover slips can be cleaned with common solvents, such as methanol, ethanol, or glass cleaner; a dry Kimwipe can also be used.

4.1.2. Different mounting media can be used to allow the optical properties of a sample to be fully characterized.

4.1.2.1. Some mounting media are semi-permanent, such as Permount, or permanent, like Norland Optical. Note: Norland Optical needs UV light to set. Other mounting mediums are temporary, such as Cargille oils or glycerin mixture (with methanol or water).

4.1.2.2. The lot number and expiration date (if applicable) shall be documented in the examination record if it could affect analysis (e.g., determining refractive index of a fiber).

4.1.3. Refer to individual trace evidence sub-discipline procedures for further requirements regarding sample preparation.

4.2. Modified Köhler illumination/field diaphragm adjustment

4.2.1. Place a prepared slide on the microscope stage and turn on the microscope.

4.2.2. Adjust the light intensity to a comfortable viewing level and focus on a particle with the 10X objective or an objective of higher magnification.

4.2.3. Make sure the top condenser lens is in position and open the aperture diaphragm on the condenser.

4.2.4. Close down the field diaphragm.

4.2.5. Adjust the condenser height until the image of the field diaphragm (the edges of the diaphragm) is in sharp focus.

4.2.6. Reduce color fringes to a minimum with the aperture diaphragm.

4.2.7. Center the image of the field diaphragm by using the centering screws on the condenser.

4.2.8. Gradually widen the field diaphragm until it leaves the field of view. Adjust the aperture diaphragm as desired for the best resolution/contrast.

4.2.9. Readjust the light intensity to a comfortable viewing level.

4.3. Lamp adjustment

4.3.1. Refer to the manufacturer's manual for the procedure regarding the focusing and centering of the microscope lamp.

4.4. Stage centering procedure

4.4.1. Place a slide on the stage and focus on a particle with the 10X or higher magnification objective.

4.4.2. Locate a small particle and move it under the intersection of the crosslines.

4.4.3. Rotate the specimen 180 degrees, and if the particle does not stay under the crosslines, note the position.

4.4.4. Use the stage centering wrenches and move the stage so the particle is halfway to the intersection of the crosslines.

4.4.5. Move the slide so the particle is back under the intersection of the crosslines.

4.4.6. Rotate the stage, and the particle should stay under the crosslines. If the particle does not stay under the intersection of the crosslines, repeat steps until the stage is centered.

NOTE: Stage centering is not necessary on microscopes with a fixed stage.

4.5. Objective centering

4.5.1. Place a prepared slide on the stage and focus on a particle with the objective used to center the stage in the above procedure.

4.5.2. Locate a small particle and move it under the intersection of the crosslines.

4.5.3. Close the field diaphragm down and check that it is in good focus and centered.

4.5.4. Rotate the nosepiece to an objective having a different magnification and turn the stage 180 degrees monitoring the particle as above.

4.5.5. Adjust as was done when centering the stage, but this time use the centering wrenches in the nosepiece (<u>not</u> the stage centering screws) until the selected particle remains centered under the crosshairs when rotating the stage.

4.5.6. Follow the same procedure for each objective, if applicable.

4.6. Ocular Focus Adjustment

4.6.1. Verify the ocular with the crosslines and/or micrometer is in the right ocular tube and that the positioning pin on the ocular is inserted correctly in the ocular tube.

4.6.2. Block the light from the left ocular using a piece of paper or similar object.

4.6.3. Adjust the knurled ring on the right ocular and bring the crosslines and/or micrometer scale into good focus.

4.6.4. Focus on the specimen with the 40X objective. The specimen and the crosslines and/or micrometer should both be in sharp focus through the right ocular.

4.6.5. Block the light from the right ocular and view the specimen with the left ocular only.

4.6.6. Adjust the diopter adjustment ring on the left ocular to bring the particle into sharp focus.

4.6.7. Now the particle should be in good focus for both eyes.

4.7. Calculating the Ocular Scale Magnification Factor

4.7.1. The microscope's ocular scale is often used in a comparative manner, such as in comparing the number of ocular scale divisions in the width of two fibers. If a measurement is to be described in units other than ocular scale divisions, such as millimeters or micrometers, then a calibrated stage micrometer is used to calculate the ocular scale magnification factor. Once established, further performance checks of a microscope are not required unless the microscope has been moved or damaged.

4.7.2. If the stage micrometer becomes damaged, it shall be taken out of service and sent for a quality check by an external calibration lab. If all of the points on the stage micrometer are not certified/calibrated, then only the certified/calibrated points shall be used.

4.7.3. Usually for a microscope, the micrometer scale is 1 mm long divided into 100 divisions, so each division of the scale is 10 µm.

4.7.3.1. The micrometer scale is placed on the stage and brought into good focus.

4.7.3.2. Move the micrometer scale so it is parallel to the ocular scale but slightly off-set. The ocular scale shall also be in good focus (see "Ocular focus Adjustment" above).

4.7.3.3 Use as much of the length of the two scales to obtain the greatest accuracy. For example, for the 40X objective the division lines for both scales overlap in such a manner that 80 ocular scale divisions overlap 20 stage scale divisions. Since each stage scale division represents 10 µm, the length is 200 µm. 80 ocular scale divisions divided into 200 µm equals 2.5 µm per ocular scale division.

4.7.3.4. This process shall be conducted for each objective.

NOTE: When the ocular scale is calculated, it shall be recorded in the logbook.

4.8. Alignment of the polarizer and the analyzer with ocular crosslines

4.8.1. Set the rotating analyzer to the "zero" mark.

4.8.2. Place a straight synthetic fiber on the stage and bring the object into good focus.

4.8.3. Move the slide so the fiber is directly under the intersection on the crosslines.

4.8.4. Insert the analyzer (cross the polars) and rotate the polarizer in the condenser so the field of view is as black as possible.

4.8.5. Rotate the fiber to extinction.

4.8.6. Uncross the polars and check to see if the fiber is parallel with the "East-West" crossline or the "North-South" crossline.

4.8.7. If not, carefully loosen the head of the microscope and rotate the head until the fiber is parallel to one of the crosslines. Re-tighten the head.

4.8.8. Cross the polars and check that the fiber is at extinction when parallel to the crossline. If not, repeat the steps.

5. Quality assurance and controls

5.1. Proper alignment of the microscope is completed upon initial receipt of the microscope. The microscope is readjusted or optimized as needed during use. See 4.2 above for modified Köhler illumination.

5.2. A color/light balance check shall be performed on the comparison microscope prior to use in casework.

5.2.1. This is completed by checking the red, green, and blue reference fibers (F10.8, F10.126 and F10.195) and ensuring that a single fiber viewed on two slides appears similar in color and background.

5.2.2. Readjusting for Köhler illumination/modified Köhler illumination as well as changing the light intensity may assist with balancing the microscope.

5.2.3. Documentation of the check is recorded in the case record (e.g., “Color/light balance check completed”).

5.2.4. The comparison microscope will be taken out of service if the color/light does not appear similar with the reference fibers.

5.3. Dust can be detrimental to microscopes and optical quality. When a microscope is not in use, it should be protected (covered). Stereomicroscopes should have their oculars covered minimally while other microscopes should be covered in their entirety. Never leave a tube or objective port open so that dust can get to the internal surfaces. Through proper set-up, regular maintenance and adjustments, the quality of this method is maintained.

5.4. In instances where the microscope is not functioning properly (e.g., artifacts are seen in the field of view, the field of view is dark) and cannot be corrected by an examiner, a qualified service technician shall be called and/or the microscope will be removed from service.

5.5. Microscopes should be cleaned, lubricated, and maintained periodically by a qualified service technician. The maintenance shall be documented (e.g., Logbook).

5.6. The calibrated micrometer shall be taken out of service and sent out for a quality check if damage occurs.

6. References

Manufacturer’s Instrument Manual

“Polarized Light Microscopy" McCrone, McCrone, and Delly (2001 or previous editions) McCrone Research Institute, Chicago, IL 60616

McCrone W. C., Particle Characterization by PLM: Part I No Polars, The Microscope, Vol. 30, No. 3, 1982, p185-196

McCrone W. C., Particle Characterization by PLM: Part II Single Polar, The Microscope, Vol. 30, No. 4, 1982, p315-331

McCrone W. C., Particle Characterization by PLM: Part III Crossed Polars, The Microscope, Vol. 31, No. 2, 1983, p187-206

SWGMAT Fiber Guidelines https://www.asteetrace.org/subfiber

1. Scope

1.1. This method describes the procedure for use of the Microspectrophotometer (MSP). MSPs are used to measure color characteristics in transmission, reflectance, or fluorescence of microscopic samples in the ultraviolet, visible, and near infrared regions. MSP is especially helpful when trying to distinguish between dyed samples having a similar color. The sample area is viewed directly, which allows precise targeting of the region to be spectrally analyzed. MSP is non-destructive and often requires little sample preparation.

1.2. Reference(s) to applicable OSAC Registry documents

1.2.1. ASTM E2808 Standard Guide for Microspectrophotometry in Forensic Paint Examinations

2. Instrumentation/Reagents

2.1. Specifics regarding the MSP instrumentation (manufacturer and model) and operating conditions shall be included on the data or on a separate parameter sheet that will be maintained in the case record. The current version of the software will be documented in the logbook.

2.2. Mounting media for transmission and fluorescence such as Permount, Cargille oils, Entellen, or glycerol.

3. Safety Considerations

3.1. Keep lamp housings a safe distance away from flammable objects. Make sure lamp housings are cool prior to placing cover over instrument.

3.2. Follow standard laboratory safety procedures.

4. Procedure or Analysis

4.1. Alignment

4.1.1. When using the MSP, the microscope shall be properly aligned and adjusted prior to taking any measurements to assure optimum performance. Consult work instructions or manufacturer's manuals for alignment procedures.

4.2. Sample preparation

4.2.1. Sampling and preparation will vary depending on the type of sample, the light sources and filters utilized, and prior testing conducted on the sample. Many samples to be run on the MSP will be mounted on glass microscope slides in a mounting medium from previous microscopical examinations. Analyzing these samples is acceptable; however, several things shall be taken into consideration:

4.2.1.1. For transmission, the sample shall be thin / translucent enough to transmit light.

4.2.1.2. For fluorescence, consideration shall be taken regarding the mounting media. Many common mounting media fluoresce which may interfere with the sample's intrinsic fluorescence.

4.3. Dark and Reference Scans

4.3.1. A Dark Scan is a measurement of the instrument void of any light. A Reference Scan is a measurement of the light transmitting/absorbing effect of all the components except for the sample of interest. The instrument is directed to take both during "Collect Dark & Reference".

4.3.2. The Dark and Reference Scans are needed for all types of samples and new Dark and Reference Scans are needed at minimum when introducing a new slide or substrate. The instrument conditions (e.g., objective, aperture size) shall be the same for the Dark and Reference Scans as they are for the sample (excluding fluorescence).

4.3.3. Transmission and Reflectance: When the sample is mounted on a glass microscope slide, it should be noted that microscope slides, cover slips, and mounting media are not optical grade components; therefore, if moving outside of the field of view, new Dark and Reference Scans should be taken. Generally, for this type of sample, the reference measurement will be taken on a blank area of the slide. For reflectance, a reference measurement should normally be taken on the substrate of the sample itself, if possible, or on an item having a similar reflectivity to the sample.

4.3.4. Fluorescence: The collection of the Dark and Reference Scans do not need to be repeated for different filters as it is only using the Dark Scan; the Reference Scan is not relevant.

4.4. Sample collection

4.4.1. Whether collecting spectra in transmission, reflectance, or fluorescence, be sure that the appropriate light sources and filters are in use. Optimize the system when needed by running programs, such as Autoset Optimize, and by collecting a new Dark and Reference Scan as described above. The instrument conditions (e.g., objective, aperture size) shall be the same for the Dark and Reference Scan as they are for the sample (excluding fluorescence). The parameters used to collect the sample will vary depending on the properties of the sample. For specific instructions on collecting sample spectra, refer to the work instructions or manufacturer's manual.

4.5. Sampling

4.5.1. In most instances, multiple spectra will be collected of each sample to determine the range of variation. The number of spectra needed to determine this range will vary from one material to another (e.g., synthetic fiber dyes tend to be very uniform, so fewer spectra would be required to determine this variation than would be required for a natural fiber which generally absorbs dye in different amounts along the length of the fiber). The range of variation shall be considered when determining where to take the spectra from (e.g., sampling from areas that vary in properties such as color and shade).

4.5.2. For paint samples, E2808[1] recommends that at least five spectra are collected from non-effect paint films and a larger number for paints with effect pigments. The number is adjusted to capture the variation present within the sample. Small sample size or poor sample conditions may preclude this.

4.6. Display

4.6.1. There are many different and acceptable ways to display the spectra obtained from the MSP.

4.6.2. When comparing a questioned and a known sample, a display method shall be utilized that will allow the technical reviewer to see that the questioned sample falls within a range of variation seen in the known or that demonstrate the exclusionary differences. This may be done through mathematics (standard deviation and mean) or by simply displaying the questioned spectrum and the known spectra (spectral overlay), which is a recognized method for comparing data where the presence or absence of peaks, peak shapes, and relative intensities

[1] ASTM E2808 Standard Guide for Microspectrophotometry in Forensic Paint Analysis

are all considered. It shall be left to the discretion of the examiner to choose which display method is most appropriate for their case.

4.6.3. Transmission spectra can be displayed in % Transmission or Absorbance and Reflectance spectra can be displayed as % Reflectance or Absorbance. Absorbance data is linear regarding concentration, so this type of display may provide more information on relative concentrations.

4.7. Spectral Comparison and Interpretation

4.7.1. MSP spectral comparisons are conducted between spectra collected using similar sample preparation methods and similar instrumental parameters.

4.7.2. Comparisons include examination of peak shape, minima, maxima, inflection points, troughs, shoulders, relative peak intensities, and the curves or slopes between peaks.

4.7.3. When assessing differences between spectra, consider sample limitations (e.g., sample size, dirty samples, thickness) and instrument limitations (e.g., aperture).

4.7.4. Samples are considered distinguishable when exclusionary differences are observed. An exclusionary difference is defined as a difference in one or more characteristics between items that is sufficient to determine that the compared items did not originate from the same source, are not the same source, or do not share the same composition or classification[2]. Exclusionary differences in spectral comparisons: (1) are outside the variability of spectra originating from the same source; and (2) cannot be explained by considerations such as sample heterogeneity, contamination, different sample conditions, or different sample histories.

4.7.5. Samples are considered indistinguishable when no exclusionary differences are observed between compared spectral features.

5. Quality Assurance and Controls

5.1. Proper alignment of the microscope was completed when the instrument was installed. System alignment is checked prior to taking any measurements and is readjusted as needed during the examination.

[2] OSAC Preferred Terms. Available online [accessed October 2022] https://www.nist.gov/system/files/documents/2022/08/10/OSAC%20Preferred%20Terms_August%202022.pdf

5.2. Performance check

5.2.1. Performance checks shall be performed each day prior to use in casework. Consult work instructions or manufacturer's manuals on how to complete the performance checks. Performance checks for each lamp used will include the following:

5.2.1.1. Wavelength check utilizing Holmium Oxide and Didymium glass filters.

5.2.1.2. Photometric check utilizing ND 0.1, ND 0.5, and ND 1.0 neutral density filters

5.3. The calibration standards (glass and neutral density filters) are traceable to Standard Reference Materials issued by NIST. Filters shall be recertified upon the manufacturer's expiration date.

5.4. The alignment check, performance check, and calibration standard certificates are documented in the instrument logbook.

5.5. Validation

5.5.1. MSP is a well-known and scientifically accepted method for the identification, analysis, and comparison of many types of trace evidence. Relevant examples of the broad nature of the method and related literature can be found in Section 6 (References).

6. References

Operations Manual for the Microspectrophotometer

Biermann TW, Wiggins KG. "Colour Analysis of Fibres" in Forensic Examination of Fibres, 3rd ed., Robertson J, Roux C and Wiggins KG, ed(s), Taylor & Francis Group LLC: Boca Raton, FL, 2018.

Eyring MB, Visible Microscopical Spectrophotometry in the Forensic Sciences, Forensic Science Handbook, 2nd edition, Vol 1, Saferstein R, Ed., Prentice-Hall: Upper Saddle River, NJ 2002.

Stoecklein W. "The role of colour and microscopic techniques for the characterization of paint fragments" in Forensic Examination of Glass and Paint, Caddy B, Ed., Taylor and Francis: New York, NY 2001.

SWGMAT "Ultraviolet-Visible Spectroscopy of Textile Fibers Chapter" (2011 Update). Available online: https://www.asteetrace.org/static/images/pdf/03%20UV-VIS%20Spectroscopy%20of%20Textile%20Fibers%20Chapter%20%282011%20Update%29.pdf

1. Introduction

1.1 Pyrolysis is a technique used to break chemical bonds of molecules by the use of thermal energy only. Analytical pyrolysis is a technique to study molecules by observing their behavior during pyrolysis and the resulting molecular fragments.

1.2 Pyrolysis breaks large molecules in the pyrolysis chamber within a short period of time into smaller fragments, which are called pyrolysates. During pyrolysis, helium gas is constantly flowing through the pyrolysis chamber providing an inert atmosphere. This constant flow of helium carries the pyrolysates from the pyrolysis chamber into the GC column for separation and then the GC separated pyrolysates are sorted and detected by MS. A pyrogram (reconstructed total ion chromatogram) is acquired which represents the separated pyrolysates.

2. Instrumentation/Reagents

2.1 Gas Chromatograph: Capable of using capillary columns and being interfaced to a mass spectrometer.

2.2 Mass Selective Detector with EI Source: Capable of scanning between 20 and 600m/z with unit resolution or better, with continuous data output.

2.3 Computerized data station: Capable of storing chromatographic and mass spectral data from sample runs; Capable of performing, either through its operating system or by user programming, various data handling functions, including input and storage of sample data files, searching data files for selected compounds, and qualitative and semi-quantitative compound analysis.

2.4 Autosampler: Pyrolysis autosampler, accessories, and software

2.5 Carrier gas: Helium, 99.99% (high purity)

2.6 Sample holder: vertical quartz tube or alloyed metal cups

2.7 Commercially available polymers (e.g., polystyrene, polyethylene, Kraton, or other suitable polymer products).

2.8 Cleaning apparatus for sample holders (e.g., aluminum block, small butane torch, sample cup inspector)

2.9 Stereomicroscope and glass microscope slides

2.10 Scalpel with blades and other appropriate sampling tools

2.11 Analytical microbalance

3. Safety Considerations

3.1 All gas cylinders must be properly secured, and pressure regulators should be inspected whenever cylinders are replaced.

3.2 Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

3.3 Avoid direct contact with any of the heating elements associated with the pyrolyzer to include the heating coil and the furnace of the vertical micro-furnace pyrolyzer. These heating elements can be extremely hot and can cause burns.

4. Procedure or Analysis

4.1. Sample Preparation

4.1.1. The appropriate container will be used for analysis (e.g., vertical quartz tubes or an alloyed metal cup.)

4.1.2. Liquid samples

4.1.2.1. A liquid sample can be directly dispensed into the center of a metal alloy cup or vertical quartz tube. A suitable sample size is one that provides sufficient signal to identify the characteristic components.

4.1.3. Solid Samples

4.1.3.1. Sample size is sample dependent on instrument sensitivity and the chemical composition of the material; however, similar sample sizes should be used for all samples being compared. It is critical that all tools for sample preparation are clean; otherwise, contamination could lead to inaccurate results. The ideal positioning of a sample is in the center of the alloyed metal cup for the micro-furnace pyrolyzer or vertical quartz tube.

4.2 Data analysis and interpretation

4.2.1 The following will be used as a guide in determining the acceptability of the data.

4.2.2 Comparative Analysis - Comparison of an unknown sample to a known sample(s): Pattern comparisons are done using pattern recognition techniques and mass spectral identification techniques when deemed necessary. For ensuring the best results, it is very important to have samples of the same size and geometry. Pyrograms can be compared on screen, side-by-side, or using overlays. Documentation of pattern comparisons shall be included in the case jacket. Factors that should be considered include the peak shape, retention time, and the relative peak intensity.

4.2.2.1 The peak shape (symmetry, width, etc.) should display reasonable agreement between the samples.

4.2.2.2 The retention time and mass spectral data should have reasonable agreement with each other. The retention time of peaks being compared in two or more programs should be within $^{+}/_{-}$ 2% of each other.

4.2.2.3 The relative intensities of the peaks should display reasonable agreement between the comparison samples. Factors affecting peak intensities should be considered, which include sample size, heterogeneity of the sample, sampling technique, and the reproducibility of the pyrolysis process.

4.2.3 Identification analysis: Identification of a known or unknown sample shall be made by running a reference standard or in some circumstances, by comparing the sample to a standard reference library.

4.2.4 The presence of additional peaks should be further examined to assess if a possible source can be determined. Additional peaks could be inherent differences between samples, from extraneous material adhering to a sample, or system peaks, such as siloxanes.

4.2.4.1 If extraneous material is the likely source of the additional peaks, a new sample should be prepared and analyzed.

4.2.4.2 If it is suspected that the additional peaks are due to inherent differences, the heterogeneity of a sample can be assessed through the analysis of replicate samples.

4.2.5 Samples are considered distinguishable if one or more exclusionary difference is observed. The definition of an exclusionary difference is “a difference in a

feature or property between compared items (e.g., pyrograms) that is substantial enough to conclude that they did not originate from the same source".

4.2.6 Instrument operating parameters shall be included in the case record when the PyGC-MS is used in casework.

4.3 Limitations

4.3.1 PyGC-MS is a destructive analytical technique.

4.3.2 PyGC-MS is limited to identifying the organic materials present in a sample. No inorganic information can be obtained from this technique.

4.3.3 Sample size and/or condition may preclude examination by this technique.

4.3.4 The analysis of combined layers or items will hinder the ability to differentiate between the individual layers/items.

5. Quality Assurance and Controls

5.1. Tune and Performance Check

5.1.1. The instrument will be tuned within one week prior to casework analysis. The tune report shall be examined to ensure that the appropriate parameters are within their normal expected range specified by the manufacturer and initialed by the examiner performing the tune. Documentation that the values were verified as meeting manufacturer's specification will be maintained in the instrument logbook. The tune reports are maintained near the instrument. The tune date(s) will be recorded in the case file.

5.2. Quality Control

5.2.1. A suitable polymer standard (e.g., polystyrene, Kraton) will be run at the start and end of each casework related sequence. If a break in analysis occurs, an intermittent standard will be run for each break that occurs. The data shall be assessed each time the instrument is run to ensure operating performance, mass assignment, and overall good chromatography. A different suitable polymer standard will be run within at least 30 days of sample analysis to assess monthly operating performance, column selectivity, and continued integrity of the system (e.g., high-density polyethylene). Refer to the appropriate work instructions for monitored criteria (e.g., retention time, baseline separation), "pass/fail" criteria, and documentation requirements.

5.2.2. If the standard indicates poor chromatography, the error shall be corrected and documented in the logbook. Refer to instrument manual for suggested maintenance/troubleshooting techniques and/or contact the primary operator for assistance.

5.2.3. When maintenance is performed that affects chromatography, a quality control standard will be analyzed, and the appropriate monitored criteria will be recorded to ensure criteria are met. If changes outside the normal expected range occur in the tune and/or standards, the error shall be corrected and documented in the logbook. Refer to the instrument manual for suggested maintenance/troubleshooting techniques. All maintenance will be documented in the instrument logbook.

5.3. Material Control and System Blank

5.3.1. A material control (e.g., stainless steel cup, stainless steel cup with solvent, quartz tube) will be evaluated prior to casework samples to show that the material(s) used for sample preparation are free of contamination.

5.3.2. A system blank (e.g., stainless steel sample cup, quartz tube, no sample cup, or no quartz tube) will be run in between samples to demonstrate carryover is not occurring.

5.3.2.1. Carryover typically signifies that an overloaded sample was introduced into the system, leading to an atypical blank where extraneous peaks are present.

5.3.2.2. The presence of carryover may require running additional blanks to diminish the effects of an overloaded sample.

5.3.2.3. In addition to running additional blanks, adjusting the sample size to provide an adequate signal is advisable to help prevent the reoccurrence of carryover.

5.3.2 A blank demonstrates the lack of contamination of analytes of interest. A blank should not display any chromatographic peaks greater than the CO2 response. If extraneous peaks are present, an attempt will be made to determine the source of these peaks and will be documented in the case notes, otherwise, the affected exhibits will be reanalyzed.

5.3.3 Through regular quality control checks of known standards and upkeep of instrument logbooks the quality of the PyGC-MS method is assured.

6. References

6.1 ASTM International Standards

ASTM E1610 Standard Guide for Forensic Paint Analysis and Comparison
ASTM E3260 Standard Guide for Forensic Examination and Comparison of Pressure Sensitive Tapes
ASTM E3296 Standard Guide for Using Pyrolysis Gas Chromatography and Pyrolysis Gas Chromatography-Mass Spectrometry in Forensic Polymer Examinations

6.2 Instrument user, maintenance, and troubleshooting manual(s) for GC-MS, pyrolyzers, and associated accessories.

CDS Analytical, Inc., Pyrolysis Application Review

Frontier Laboratories LTC Operation Manual

FBI Performance Monitoring Protocol for the Pyrolysis-GC/MS

6.4 Other

Irwin, William J., Analytical Pyrolysis-A Comprehensive Guide, Chromatographic Sciences Series, Vol. 22, Marcel Dekker, Inc., New York, 1982.

Saferstein, R. "Forensic Analytical Pyrolysis", Proceedings of the International Symposium on the Analysis and Identification of Polymers 1984, pp 9-18.

Wampler, Thomas P, Applied Pyrolysis Handbook, Marcel Dekker, Inc., New York, 1995.

Wampler, T. P. and Levy, E. J., "Reproducibility in Pyrolysis, Recent Developments," Journal of Analytical and Applied Pyrolysis, 12, 1987, pp 75-82.

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1. Scope

    1.1. Raman spectroscopy is a non-destructive analytical technique used for identification of unknown chemicals. The Raman spectrum can provide enough characteristics to specifically identify a substance, and in some cases a mixture of substances. Reference spectra of thousands of materials are available for comparison and identification of unknowns.

2. Instrumentation/Reagents

    2.1. A Raman spectrometer equipped with a library of standards.

3. Safety Considerations

    3.1. All users should be properly trained on the safe operation of the instrument.

    3.2. Raman spectroscopy relies on the use of a laser. Laser-related safety considerations:

        3.2.1. The laser wavelengths used for Raman spectroscopy cause damage to eyes. The specific hazards are related to the wavelength of the laser and the instrument configuration. Refer to the user instructions for laser safety.

        3.2.2. The energy of the laser can cause localized heating of dark materials and some substrates (e.g. coffee filters). Avoid analysis of dark materials, or powders where dark flecks are present. Ensure the focal point of the laser is aligned with the analyte (e.g. not hitting paper under a sample).

    3.3. Potentially energetic materials, such as suspected explosives, should be analyzed with the following precautions:

        3.3.1. If available, use scan delay and move a safe distance from the instrument while the laser is on.

        3.3.2. Run vials without a cap.

        3.3.3. Isolate a small amount of material from the bulk where possible.

4. Procedure or Analysis

    4.1. Sample preparation will depend on the physical state of the sample (i.e. liquid, granular, solid).

4.2. See instrument work instructions for further guidance.

5. Quality Assurance and Controls

5.1. Performance Verification: The instrument must pass the performance test or manufacturer's self-test using a polystyrene standard within 31 days prior to use for casework. The results of the test will be documented in the instrument logbook.

6. References:

Bartick, E.G., Merrill, R.A., & Mount, K.H. (2001). Analysis of a Suspect Explosive Component: Hydrogen Peroxide in Hair Coloring Developer. Forensic Science Communications, 3(4).

Matthews, R.; Longworth, T.; Ong, K.; Zhu, L.; Brown, C.; Knopp, K. Testing of Ahura's Firstdefender Handheld Chemical Identifier Against Toxic Industrial Chemicals; Edgewood Chemical Biological Center, US Army Research, Development, and Engineering Command: Aberdeen Proving Ground, 2006.

Matthews, R.; Ong, K.; Brown, C.; Zhu, L.; Knopp, K. Evaluation of Ahura's First Defender Handheld Chemical Identifier; Edgewood Chemical Biological Center, US Army Research, Development, and Engineering Command: Aberdeen Proving Ground, 2006.

Moore, D., & Lee, K. (2007). Raman spectroscopy as a tool for long-term energetic material stability studies. Journal of Raman Spectroscopy, 38(9), 1221-1224.

1. Scope

1.1. The forensic hair examiner can analyze a questioned sample for the purpose of identifying it as a hair and determining whether it is animal or human. If the hair is from an animal, species identification may be attempted. If from a human, the somatic origin (body area) of the hair may be determined by general morphology. A human hair may also be associated to a particular ancestry group based on established models for each group. Forensic hair examiners trained in determining ancestral origin can differentiate between hairs of European ancestry, Asian ancestry, and African ancestry origin, all of which exhibit microscopic characteristics that distinguish one ancestral group from another. Also, hairs may be evaluated to determine if further microscopical comparisons and/or DNA analyses can be conducted.

2. Instrumentation/Reagents

2.1. Containers such as glassine envelopes, plastic bags or vials

2.2. Forceps

2.3. Sticky sided collecting materials such as tape or post-it notes

2.4. Glass microscope slides

2.5. Glass coverslips

2.6. Illuminated magnifier

2.7. Mounting medium

2.8. Stereomicroscope

2.9. Transmitted light microscope

2.10. Transparent securing substrates such as slides or sheet protectors

2.11. Vacuum, with clean filter attachments

2.12. Miscellaneous laboratory supplies or equipment

3. Safety Considerations

3.1. The examiner shall follow biohazard procedures and use universal precautions.

3.2. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

4. Procedure or Analysis

4.1. Recovery of hairs

4.1.1 For general processing guidelines, see *ATF-LS-TE Standard Approach for Examinations of Trace Evidence*.

4.1.2 Items from different individuals and different locations are separated in time and/or space. At no time should questioned items and known items be open at the same time in the same area for recovery of trace evidence.

4.1.3 Change gloves and clean tools between examining the evidence from the victim and the evidence from the suspect. Use separate laboratory coats during the collection of the known and questioned items.

4.1.4 Examine each item of evidence visually, with the aid of an illuminated magnifier, or low powered microscope.

4.1.5 If the item being examined contains hairs that are readily visible, collect them. As hairs are collected, they should be secured and/or preserved in an appropriate manner.

4.1.6 Care should be taken to avoid the loss of any hairs, especially when repositioning bulky items.

4.1.7 Adhesive tapes and/or other low tact adhesive mediums (e.g., post-it notes, lint rollers) may be used to recover hairs. The adhesive surface is placed on the item being examined and then pulled away.

4.1.8 Other collection methods may be used including scraping and vacuuming. If scraping is necessary, the item to be examined can be suspended above the examination surface and very gently scraped with a spatula. Scraping in a downward direction allows surface hairs to fall onto the examination surface for collection. Vacuuming can also be used to collect debris; however, it is not preferred because the debris recovered often represents far more than recent hair transfers. If vacuuming is necessary, separate filters should be used for each item/area.

4.1.9 When recovering hairs from tape present on submitted items, it is important to remember that exposed areas of the tape may contain environmental (scene) hairs that may be of no probative value, while hairs found under the adhesive or between tape layers may be extremely valuable. Other examinations (e.g., DNA, latent prints, other trace evidence) on these tape pieces are paramount and other disciplines may need to be consulted to determine the best examination sequence and proper evidence handling precautions. A latent print examiner should be consulted prior to separating multiple layers of tape to ensure that the separation will not affect their analysis. Hairs found in protected areas can be removed with clean forceps for examination. The exposed tape adhesive may be placed on a clean non-porous surface.

4.2 Hair examination and analysis

4.2.1 A reference collection of hairs may be useful to the examiner when determining if hairs are animal or human, or for identifying animal species, ancestry characteristics, or somatic origin.

4.2.2 Groups of questioned hairs cannot be assumed to be from the same individual, body area, or animal. Each hair will be examined independently from all others. Sample selection will be utilized when reporting probative microscopical hair examinations.

4.2.3 Observe the physical properties of the mounted hairs utilizing a transmitted light microscope having a magnification range of at least 100X to 400X. When possible, classify the hair as animal or human, identify species or ancestral characteristics, and identify somatic origin based on the characteristics noted.

4.2.4 The following microscopic characteristics are commonly used to characterize a hair as animal or human (Hair Characterization Level 1):

4.2.4.1 Human hairs

Medulla is generally amorphous in appearance and the width is generally less than one-third the overall diameter of the hair shaft.

Scales are not usually pronounced, and the structure is imbricate.

Pigment granules are usually distributed toward the cuticle (except for naturally red hair which is often distributed toward the medulla).

Generally, the color and pigmentation are consistent throughout the

length of the hair shaft.

The root is commonly club-shaped or stretched.

4.2.4.2 Animal hairs

Medulla is frequently continuous and structured. The width is usually greater than one-third the overall diameter of the hair shaft.

Scales are often pronounced, and the structure of the scales can be coronal, spinous, or imbricate.

Pigment granules are usually distributed toward the medulla. Radical changes in color along the length of the hair shaft, called banding, are common.

The shape of the root is highly variable.

Abundant ovoid bodies.

4.2.5 The following microscopic characteristics are commonly used to determine the ancestral origin of a human hair (Hair Characterization Level 2):

4.2.5.1 African Ancestry

Shaft diameter is moderate to fine with considerable variation (diameter range reported for head hairs is 60 – 90 μm)[1].

Pigment granules are densely distributed (hair shaft may be opaque) and arranged in prominent clumps or streaks.

Shaft has prominent twist and curl.

Cross-sectional shape is flattened.

4.2.5.2 Asian Ancestry (including Native American)

Shaft diameter is coarse and usually with little or no variation (diameter range reported for head hair is 90 - 120 μm)[1].

[1] Bisbing RE. The Forensic Identification and Association of Human Hair IN: Saferstein, R (Ed). Forensic Science Handbook, Vol.1, 2nd Edition, New Jersey: Prentice-Hall, 2002, pp. 390-428.

Pigment granules are densely distributed and often arranged in large patchy areas.

Medulla is prominent (often broad and continuous).

Cuticle is thick with typically clear cuticular margin.

Cross-sectional shape is round.

4.2.5.3 European Ancestry

Shaft diameter is moderate with minimal variation (diameter range reported for head hairs is 70 – 100 μm[1] with the mean diameter as 80 m[2]).

Pigment granules are sparse to moderately dense with fairly even distribution.

Cross-sectional shape is oval.

4.2.5.4 Mixed Ancestry

Hairs exhibiting characteristics common to more than one ancestral group.

4.2.6 The following microscopic characteristics are commonly used to determine the somatic origin of a human hair (Hair Characterization Level 2):

4.2.6.1 Head hairs

May be long with moderate shaft diameter variation.

Medulla absent to continuous and relatively narrow when compared to the structure of hairs from other body areas.

Cut, abraded, split, or broken tips.

May show artificial treatment or solar bleaching.

[2] Deedrick D, Koch S. Microscopy of Hair, Part 1: A Practical Guide and Manual for Human Hairs, Forensic Science Communications, Vol. 6, No. 1, 2004.

Soft texture, pliable.

4.2.6.2 Pubic hairs

Shaft diameter course with wide variations and buckling.

Medulla relatively broad and usually continuous when present.

Root frequently with a follicular tag.

Tip usually tapered, rounded, or abraded.

Stiff texture, wiry.

4.2.6.3 Limb hair

Diameter fine with little variation. Gross appearance of hair is arc-like in shape.

Medulla is discontinuous to trace with a granular appearance.

Tips usually taper and are often blunt and abraded. Scale ends are commonly rounded due to wear.

Soft texture

4.2.6.4 Axillary or underarm hairs

Resemble pubic hairs in general appearance, but less wiry.

Medullary appearance similar to limb hairs.

Diameter moderate and variable with less buckling than pubic hairs.

Tips long and fine, frequently with a bleached appearance.

4.2.6.5 Chest hairs

Shaft diameter moderate and variable.

Tip often darker in color, long and fine, arc-like.

Medulla may be granular.

Stiff texture.

4.2.6.6 Facial hairs

Diameter very coarse with irregular or triangular cross-sectional shape.

Medulla very broad and continuous; may be doubled.

Blunt or razor cut tips.

4.2.6.7 Eyebrow

Stubby, some diameter fluctuation, saber-like in appearance.

4.2.6.8 Eyelash

Short, stubby with little shaft diameter fluctuation, saber-like in appearance.

4.2.6.9 Transition hairs

Hairs in transitional areas of the body may include characteristics from two areas.

Trunk: A combination of features of limb and pubic hairs, a transitional hair.

Fringy: A combination of head and body hairs. Fine hairs often found in the nap of the neck or the hairline.

4.2.7 Screening hairs for other analyses

4.2.7.1 The suitability of human hairs for additional examinations, including microscopical comparison and DNA examinations, should be determined.

4.2.7.2 Suitable candidates for DNA analysis may be screened. If known head and/or pubic hairs are submitted and ancestry differences exist between known and unknown hairs, no further analysis may be necessary.

4.2.7.3 Certain somatic regions (e.g., head, pubic region) are generally considered suitable for microscopical comparisons though facial hairs also may be compared. The ATF Laboratories do not conduct hair comparisons; however, this analysis may be performed by another laboratory.

4.2.7.4 The growth stage of a hair is determined by the root, which can indicate whether a hair is suitable for nuclear DNA analysis.

4.2.7.4.1 Anagen (Actively growing phase): This root appears stretched and may have a distorted appearance. Pigment is usually present and cortical fusi are rarely seen. This root is soft and pliable, since the root has not been keratinized, making it suitable for nuclear DNA testing.

4.2.7.4.2 Telogen (Resting phase): This root appears as a keratinized bulb, lacking pigment, often including an abundance of cortical fusi. Telogen roots are not suitable for nuclear DNA testing unless tissue remains attached to the root. The mature root will naturally be sloughed from the head.

4.2.7.4.3 Catagen (Transitional phase): This root resembles a combination of a telogen and anagen root, starting to form a bulb but still having a stretched appearance. This hair still has soft keratin and is therefore still suitable for nuclear DNA testing. Tissue may be present around this root.

4.2.7.5 If root tissue is present on the human hair in question, or if the hair has an anagen or catagen root, the hair is suitable for nuclear DNA analysis. If no root tissue is present, hairs may be selected for mitochondrial DNA analysis. The ATF Laboratories do not conduct mitochondrial DNA examinations; however, this analysis may be performed by another laboratory.

4.2.7.6 Photographing the root and measuring the length of the hair prior to DNA analysis is recommended.

4.2.7.7 Hairs can be stored in a variety of ways including on slides with permanent mounting media or post-it notes.

4.2.7.8 Hairs selected for additional analysis shall be prepared by a qualified hair examiner. If permanently mounted, the hairs may be removed from the

slides by punching a hole in the coverslip using a scribe, placing a drop of xylene or other suitable solvent in the hole to loosen the mounting media, and removing the hair with tweezers. If using a non-permanent mounting media, the coverslip may simply be lifted, and the hair removed from the slide.

4.2.7.8.1 The hair and/or hair root may be cleaned in a solvent such as xylenes. The hair/hair root will be rinsed with sterilized water if it is to be forwarded to the Forensic Biology Section.

4.2.7.8.2 Roots shall be separated from the remaining hair as necessary and will be secured in a bullet tube for transfer to the Forensic Biology Section.

4.2.7.8.3 If the hairs are to be released to an external agency for analysis not performed at the ATF, the examiner should check with that agency for submission requirements.

4.2.7.9 New notes will be generated to document hair roots that are removed for nuclear DNA analysis after the Trace report is issued; these notes shall be included in the Trace technical record. A supplemental Trace report is not required because the new sub-exhibit will be reported by the Forensic Biology section. The new Trace notes will be technically reviewed by another qualified hair examiner. The technical and administrative reviews shall be documented on the case record review form indicating that no report has been issued.

5. Quality Assurance and Controls

5.1 Through proper training, competency testing, and proficiency testing of hair examiners as well as the use of high-quality microscopes, which are cleaned and maintained appropriately, the quality of this method is maintained.

5.2 Validation

The techniques described above for hair examination are well known and scientifically accepted in the forensic science community and in private industry. Relevant examples of related literature can be found in Section 6 (References).

6 References

6.1 OSAC Registry documents

E3316 Standard Guide for Forensic Examination of Hair by Microscopy

6.2 ASTM International Standards

E1459 Standard Guide for Physical Evidence Labeling and Related Documentation

E1492 Standard Practice for Receiving, Documenting, Storing and Retrieving Evidence in a Forensic Science Laboratory

6.3 Other

Bisbing R. The Forensic Identification and Association of Human Hair. In: The Forensic Science Handbook, Volume 1, 2nd edition, Saferstein R, Ed. Upper Saddle River, NJ: Pearson Education, 2002.

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Oien, CT. Forensic Hair Comparison: Background Information for Interpretation. Forensic Science Communications, Vol 1, No. 2, 2009.

Ogle R, Fox M. Atlas of Human Hair – Microscopic Characteristics. Boca Raton, FL: CRC Press. 1999.

Petraco N, Fraas C, Callery F, DeForest P. The Morphology and Evidential Significance of Human Hair Roots, J of For Sci, Vol 33 (1), pp 68-76, 1988.

Robertson J. Forensic Examination of Hair, London: Taylor and Francis, 1999.

1. Scope

1.1. To compare portions of pipes, tools, tapes, glass, fabrics, papers, and other items of evidence to determine whether those portions were once a part of or have been separated from, a particular source. This is done through a comparison of fractures and other surface features. If meaningful alignment of random characteristics can be established, an association can be made between two or more fragments/pieces and will demonstrate they were once joined together to form a single object.

2. Instrumentation/Reagents

2.1. Stereo and comparison microscope(s)

2.2. Micrometers, calipers, rulers

2.3. Casting media

2.4. Photographic equipment

3. Safety Considerations

3.1. Use appropriate personal protective equipment (glasses, gloves, lab coat).

3.2. Care shall be taken when handling exhibits with sharp edges.

4. Procedure or Analysis

4.1. Examine physical properties of item(s) to be compared (e.g., color, material type, dimensions, surface features). The questioned item shall be evaluated prior to the examination of the known item.

4.2. The separation method (e.g., cut, torn) can influence the features of a physical fit examination, however, specific damage assessment is outside the scope of this document.

4.3. Evaluate and compare class characteristics. When exclusionary differences are observed at any point during the examination, no further examinations are required.

4.4. Evaluate the shape of separation and check for any surface features that may be continuous on both sides of the separation. The elasticity of the object shall be taken into consideration especially in areas where the stretching due to separation may cause distortion in the physical fit.

4.5. If the item is of suitable thickness, examine the surface of the face of the separation to determine if the edge features correspond.

4.6. If the class characteristics of the separated pieces are compatible and if the pieces fit together in at least one of the following individualizing characteristics, it can be determined that the two items were at one time joined together to form a single continuous piece:

4.6.1. Along an irregular edge-to-edge border like a jigsaw puzzle matched over a reasonable length.

4.6.2. Continuous surface markings or internal features.

4.6.3. Three-dimensional fit

5. Quality Assurance and Controls

5.1. Performance checks and calibrations
Microscopes and/or micrometer/measuring devices shall be properly calibrated or adjusted when necessary, according to protocols for each instrument.

5.2. Validation
The techniques described above for the examination of physical fits are well known and scientifically accepted in the forensic science community and in private industry (e.g., fracture mechanics, engineering, and metallurgy). Relevant examples of related literature can be found in Section 6 (References).

5.3. Verifications

5.3.1. All probative physical fits (e.g., those that provide an association between a suspect and a scene or a suspect and a victim such as a roll of tape from a suspect's residence physically fits to a piece of tape on a pipe bomb) shall be verified by another qualified examiner (seconded).

5.3.2. Verifications shall be documented in the technical record in accordance with ATF-LS-7.7 Section 2.6 Casework Verification. The Physical Fit Verification Form can be used for such documentation.

6. References

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1. Scope

1.1. The forensic fiber examiner can conduct a variety of examinations on textiles for the purposes of characterizing and comparing types of fibers, fabric, cordage, and/or damage. In addition, they may evaluate and compare fabric impressions.

1.2. Fiber Examinations: Variations in morphological, physical features, optical properties and chemical composition can make fiber types distinctive. The forensic fiber examiner may be requested to analyze a questioned fiber to determine the fiber type as well as to attempt to determine the possible end use for that sample. When conducting comparisons, the examiner's goal is to assess the significance of any differences observed. The absence of any exclusionary differences between the questioned and known (Q and K) samples suggests that the items could have a common source of origin.

1.3. Fabric Damage: Textile items are occasionally submitted to the laboratory for examination to determine if and/or how the item has been damaged. The construction and composition of the textile are vital factors in assessing and understanding damage characteristics. Information as to the possible implement causing the damage and the manner in which it was caused may also be determined.

1.4. Fabric Impressions: Fabric impressions occur from the transfer of a fabric's construction pattern (i.e., weave, twill, stitching, seams) to the surface of another object. Impressions can be produced when the fabric leaves behind some material (i.e., blood, grease, dirt) on the receiving object, or when a fabric removes a material from the receiving object. A three-dimensional impression is produced when a fabric is pressed into the receiving object to the extent that it embeds into the material (e.g., impression in mud). The process of analyzing impressions is a step-by-step methodical approach that uses class and randomly acquired characteristics found in known and unknown impressions.

2. Instrumentation/Reagents

2.1. Camera or other imaging equipment

2.2. Containers such as glassine envelopes, plastic bags, or vials

2.3. Digital camera

2.4. Forceps

2.5. Glass microscope slides

2.6. Glass coverslips

2.7. Hot-Stage apparatus

2.8. Illuminated magnifier

2.9. Instrumentation

2.9.1. FTIR (*ATF-LS-E6*)

2.9.2. MSP (*ATF-LS-TE03*)

2.9.3. PyGC-MS (*ATF-LS-TE04*)

2.9.4. Raman (*ATF-LS-TE07*)

2.9.5. SEM-EDS (*ATF-LS-E3*)

2.10. Microscopes (*ATF-LS-TE01 / ATF-LS-TE02*)

2.10.1. Comparison microscope

2.10.2. Fluorescence microscope

2.10.3. Polarized light microscope

2.10.4. Stereomicroscope

2.11. Mounting media

2.12. Solvents/stains/microchemical reagents

2.13. Sticky-sided collecting materials, such as tape or Post-it Notes

2.14. High temperature silicone oil

2.15. Transparent securing substrates, such as sheet protectors

2.16. UV Light or alternative light source

3. Safety Considerations

3.1. The examiner shall follow biohazard procedures and use universal precautions.

3.2. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

4. Procedure or Analysis

4.1. Recovery of Fibers

4.1.1. For general processing guidelines, see *ATF-LS-TE00 Standard Approach for Examinations of Trace Evidence*.

4.1.2. Items from different individuals and different locations are separated in time and/or space. At no time should questioned items and known items be open at the same time in the same area for recovery of trace evidence.

4.1.3. Change gloves and clean tools between examining the evidence from the victim or scene and the evidence from the suspect. Use separate laboratory coats during the collection of the questioned and known items.

4.1.4. Examine each item of evidence visually, with the aid of an illuminated magnifier or low powered microscope.

4.1.5. If the item being examined contains foreign fibers that are readily visible, collect them. As foreign fibers are collected, they should be secured and/or preserved in an appropriate manner.

4.1.6. Care should be taken to avoid the loss of any foreign fibers, especially when repositioning bulky items.

4.1.7. Adhesive tapes and/or other low tact adhesive media (e.g., post-it notes, lint rollers) may be used to recover foreign fibers. The adhesive surface is placed on the item being examined and then pulled away.

4.1.8. Other collection methods may be used including scraping and vacuuming. If scraping is necessary, the item to be examined can be suspended above the examination surface and very gently scraped with a spatula. Scraping in a downward direction allows surface fibers to fall onto the examination surface for collection. Vacuuming can also be used to collect debris; however, it is not preferred because the debris recovered often represents far more than recent fiber transfers. If vacuuming is necessary, separate filters should be used for each item/area.

4.1.9. When recovering fibers from tape present on submitted items, it is important to remember that exposed areas of the tape may contain environmental (scene) fibers that may be of no probative value, while fibers found between the layers of tape may be extremely valuable. Other examinations (e.g., DNA, latent prints, other trace evidence) on these tape pieces are to be considered and other disciplines may need to be consulted to determine an appropriate examination sequence. A latent print examiner should be consulted prior to separating multiple layers of tape to determine the best way to proceed, prioritizing the preservation of evidence. Fibers found in the protected areas of the tape can be removed with clean forceps for examination. The exposed tape adhesive may be placed on a clean non-porous surface for transfer to a latent print examiner.

4.2. Characterization of fibers

4.2.1. A variety of techniques are available for the characterization of fibers. The specific technique(s) chosen will depend on the category and specification warranted. Physical features and/or optical properties can categorize fibers broadly as natural fibers and manufactured fibers. Each can be further broken down into sub-classifications.

4.2.1.1. Natural fibers are obtained from plants, animals, or mineral materials.

4.2.1.2. Manufactured fibers can be further described as regenerated, synthetic, or mineral based on the starting materials used to form the fibers. The Federal Trade Commission[1] has established generic names for manufactured fibers.

4.2.2. For screening purposes, a stereomicroscope is all that is warranted. For characterization, the fiber should minimally be mounted and viewed under a polarized light microscope.

4.2.3. Physical features such as crimp, length, color, luster, damage, and/or adhering debris can be viewed under the stereomicroscope and noted.

4.2.4. If the sample contains yarns, threads, or sections of fabric, construction should be recorded.

4.2.5. Different mounting media are available to the examiner. The type and refractive index is documented in the technical record.

[1] *Federal Trade Commission Rules and Regulations Under the Textile Products Identification Act,* Title 15, U.S. Code Section 70, et seq. 16 CFR 303.7.

4.2.6. A polarized light microscope shall be used to characterize the optical properties of the fibers.

4.2.7. Some features or properties that can be used to determine fiber type include:

4.2.7.1. Optical properties such as refractive index, birefringence, extinction properties (e.g., full, incomplete, undulating, or no extinction) cross-section, delustrant, and/or pigment.

4.2.7.2. Solubilities may be useful in distinguishing sub-class fibers (e.g., acetate, triacetate)

4.2.7.3. Twist and the presence or absence of lignin as determined by twist tests and dispersion staining (e.g., Herzberg stain, phloroglucinol stain) may be useful in distinguishing natural fibers.

4.2.7.4. Melting point range determined by hot stage microscopy can provide additional sub-class information for certain fibers (e.g., nylon, acetate).

4.2.7.4.1. Only a small length of fiber is necessary; however, thermal microscopy is a destructive technique.

4.2.7.4.2. The hot stage apparatus should be performance checked with a known melting point standard within 30 days of running casework samples. This will be documented in the technical record.

4.2.7.4.3. The melting point range is observed through a microscope. The melting point range (when the fiber starts to melt and has completely melted) is recorded and compared to known literature.

4.2.7.5. Chemical information obtained through FTIR and/or PGCMS can be used to support PLM results as well as determine the sub-class of certain manufactured fibers (e.g., nylon 6, nylon 6.6).

4.2.7.6. Elemental information obtained through SEM-EDS can be used to confirm certain mineral-based fibers (e.g., glass fibers).

4.2.8. Reference information (e.g., refractive indices, birefringence, melting point tables) as well as reference collections are available to the examiner.

4.3. Comparison of Fabric/Fibers

4.3.1. Selection of samples for analysis

4.3.1.1. Questioned fabric

4.3.1.1.1. Multiple pieces: Each recovered piece of fabric should be examined unless the number of pieces makes this procedure prohibitive. In that case, select sample(s) may be taken for further analysis. Because different/separate fabric pieces cannot be assumed to be from the same source, sample selection shall be utilized when reporting the examination of questioned samples.

4.3.1.1.2. Single large piece: When testing individual questioned pieces of fabric, certain tests may be conducted on smaller samples which are removed from a larger piece of fabric. In that instance, homogeneity is assumed. Accordingly, the results of those tests may be used to represent the larger piece (or pieces that have been physically fit together) as a whole.

4.3.1.2. Questioned fibers: When numerous fibers are present in the unknown sample, the examiner will evaluate the fibers on a case-by-case basis and must attempt to examine a representative sample of fibers. This is based on fiber type, color, diameter, and optical properties. Additional analysis can be performed on a select few, however the report will be clear as to what exactly was analyzed.

4.3.1.3. Known samples: When an entire known sample is submitted and is a textile, a piece of fabric and/or a selection of fibers, the examiner can only assume homogeneity to a certain extent. Knowledge of the manufacturing process for various fiber types, color, garment construction, upholstery, cordage/ropes, etc., aids in choosing an appropriate representative known. A representative sample[2] needs to consist of enough fibers to cover the range of different fiber types/colors present in the particular textile/fiber sample.

4.3.2. Fabric/textile comparison

[2] A representative sample is defined as *a part of the population selected for study that represents the variation in the whole.* Garfield FM. *Quality Assurance Principles.* Association of Official Analytical Chemists, Arlington, Virginia, 1991.

4.3.2.1. Perform a preliminary examination noting the size, shape, and condition (stains, patterns, damage) of both the known and the questioned samples.

4.3.2.2. Observe, document, and compare the construction of the fabric/textile including textile/fabric type (e.g., carpet, woven fabric) and/or specific type (e.g., level loop, jersey knit).

4.3.2.3. Physical Fit examination

4.3.2.3.1. The textile/fabric should be evaluated for a possible physical fit. See *ATF LS TE10 Examination of Physical Fits protocol*.

4.3.2.4. The textile/fabric should be broken down into its component yarns and fibers (see section 4.3.3 for fiber comparisons). Various features can be evaluated at the yarn level but note that not all of these are present on all samples. Some possible features to observe, document and compare are plies, twist, thickness of braid, coating, and length of tuft.

4.3.3. Fiber comparisons: There are many techniques that are available for the comparison of fibers. A combination of techniques that have the greatest potential for discrimination are used. Table 1 lists all the available techniques for fiber comparison with the shaded boxes representing techniques which are recommended. Depending on the fiber type, color and size, certain techniques may not be available or may not offer any additional information or discrimination power. For instance, MSP would be utilized on a blue fiber but not a gray fiber. Likewise, FTIR would be utilized on a nylon fiber but not on a cotton fiber.

Table 1. Techniques for the comparison of fibers.

Physical Features	Optical Properties	Microchemical Analysis	Color/Dye/Pigment Analysis	Instrumental Analysis
Stereomicroscopy	PLM	Solubility	Comparison Microscopy	FTIR (Manufactured Fibers)
Light Microscopy/ Comparison Microscopy	Light Microscopy/ Comparison Microscopy	Staining (Natural Fibers)	MSP	SEM-EDS/XRF
SEM	Fluorescence Microscopy			PyGC-MS
Melting Point				Raman
Physical Test (e.g., twist test, dispersion staining)				

4.3.4. If at any time during the comparative scheme of analysis an exclusionary difference is observed between the Q and the K samples, no further examinations need to be conducted and the samples can be reported as being dissimilar to one another.

4.4. Fabric Damage

4.4.1. The procedures for textile damage determination depend on case specifics as well as the evidence received. The construction and composition of the textile are important factors in assessing and understanding damage characteristics. In general, the steps to be taken are as follows:

4.4.1.1. Textile damage examinations can be complex and should be approached in context with case specific information. Therefore, the examiner should attempt to gain all the information that may account for the presence of any noted damage (e.g., scissor cuts created by first responders).

4.4.1.2. Conduct a visual examination of the textile determining the construction (e.g., plain weave, knit). Suspected damage should be approached from the largest to the smallest scale (i.e., fabric, yarn, and fiber).

4.4.1.3. Examine and document the damage in detail. Some characteristics that may be visible and documented include:

FABRIC: length, distortion, curl, shape, secondary cuts, steps, coloration
YARN: unraveling, isolated threads, planar array, ragged ends, steps, ruptured ends
FIBERS: splayed out, clean-cut ends, bulbous formation, melting

4.4.1.4. Perform simulation experiments if necessary. This should be done on an undamaged area of the submitted item if possible. Clearly mark any damage produced by the examiner.

4.4.1.5. Evaluate the visible characteristics of the damage, considering the limitations. Due to overlapping characteristics, it is not always possible to determine the cause of damage on a textile, nor may it always be possible to indicate the implement that caused the damage.

4.5. Fabric Impressions

Fabric impressions are not currently covered under the ANAB scope and therefore are reported without using the ANAB logo.

4.5.1. All examinations, relevant observations, and results shall be documented in the examination records and support conclusions reached. If at any time during the comparative scheme of analysis an exclusionary difference is observed between the Q and the K samples, no further examinations need to be conducted and the samples can be reported as being dissimilar to one another.

4.5.2. Detection/Collection/Processing: Impressions can be submitted to the laboratory in various forms (e.g., digital images, impressions on objects, lifts, casts). These impressions will be observed using oblique light, alternative light source, and/or digital, chemical, or physical enhancement. They may be captured using photography, scanning, lifting, and/or casting. They may also be processed digitally, physically, and/or chemically, to prepare for examination and/or optimize visibility. See also *ATF-LS-I1 Detection and Collection of Footwear and Tire Tracks* and *ATF-LS-I1 Appendix A - Processing*.

4.5.3. Suitability: The unknown impression needs to be assessed to determine whether it is suitable for comparison. Unsuitable impressions lack sufficient detail and will prevent meaningful comparisons with a known source. The quantity (how much of the impression is present) and quality (clarity) of detail are assessed. This assessment is dependent on several factors such as the substrate, interferences, and the presence or absence of scales. These factors may limit or qualify an examiner's conclusions.

4.5.4. Comparison: To compare a questioned impression to a known fabric, clear and detailed test impressions should be made with the known. The purpose of creating known test impressions is to record the characteristics of the fabric. Prior to making known impressions, the examiner should recognize and preserve other relevant physical evidence as well as document and photograph the original condition of the fabric. The case specifics will determine the number and types of impressions to be made.

4.5.4.1. The comparison can be a side-by-side comparison and/or a superimposed observation of the unknown impression with the known fabric impression.

4.5.4.1.1. Class characteristics such as specific design, weave, spacing, manufacturing characteristics and shape of the design, are evaluated and compared.

4.5.4.1.2. Randomly acquired characteristics (RACS) are evaluated according to their position, size, shape, orientation, and clarity. RACS shall be confirmed on the fabric itself when possible.

4.5.4.1.3. When sufficient RACS are present in the unknown impression and correspond with features on the known object, a Type I Inclusion can be made.

4.5.4.1.4. See *ATF-LS-TE16 Report Writing* for the interpretations and report wording for fabric impressions.

4.5.4.2. All comparisons are evaluated by a second qualified examiner (verifier). Verifications shall be documented in the technical record in accordance with *ATF LS 7.7 Section 2.6 Casework Verification.*

5. Quality Assurance and Controls

5.1. Quality is assured through the proper training and testing of examiners, the laboratory's technical review process, and the use of appropriate equipment that is maintained and performance checked.

5.2. The techniques described above for textile examinations are well known and scientifically accepted in the forensic community and private industry. Relevant examples of related literature can be found in Section 6 (References).

6. References

6.1. ANSI/ASB Standards

Best Practice Recommendation 021, Best Practices for the Preparation of Test Impressions from Footwear and Tires

Best Practice Recommendation 049, Best Practices for Lifting of Footwear and Tire Impressions

6.2. OSAC Registry Standards

ASTM E2224 Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy

ASTM E2225 Standard Guide for Forensic Examination of Fabrics and Cordage

ASTM E2228 Standard Guide for Microscopical Examination of Textile Fibers

6.3. ASTM International Standards

ASTM E1459 Standard Guide for Physical Evidence Labeling and Related Documentation

ASTM E1492 Standard Practice for Receiving, Documenting, Storing and Retrieving Evidence in a Forensic Science Laboratory

6.4. Scientific Working Group for Materials Analysis (SWGMAT) Documents found at htpp://www.asteetrace.org

Forensic Fiber Examination Guidelines

"Introduction to Fibers Chapter" (2011 Update)

"Microscopy Chapter" (2011 Update)

"UV-VIS Spectroscopy of Textile Fibers Chapter" (2011 Update)

6.5. Others

Gaudette, B.D. In Forensic Science Handbook; Saferstein, R., Ed.; Prentice Hall: Englewood Cliffs, N.J., 1988; Vol. II, Chapter 5

Gross, S. Fabric Damage: "Cuts, tears, burns and others." Presentation given at Advance Trace Evidence Symposium, June 2006, Ames, IA located on Trace FSL Shared Folder.

Nielsen, Mark, R., "Common Natural Fibers". Handout provided at a paper presentation at the Midwestern Association of Forensic Scientists annual meeting, October 1998 located on Trace FSL Shared Folder.

Palenik, Samuel, J., Microscopical Examination of Fibres. In: Forensic Examination of Fibres. 2nd ed., James Robertson and Michael Grieve, Philadelphia, 1999.

Petraco, N. and Kubic, T. Color Atlas and Manual of Microscopy for Criminalists, Chemists, and Conservators. CRC Press, Boca Raton, 2004.

Robertson J, Grieve M. Forensic Examination of Fibres 2nd Edition, Taylor & Francis, 1999.

Schubert, Glenn, "Fabric Impression Workshop" PowerPoint presentation from IPES located on Trace FSL Shared Folder.

1. Scope

1.1. Many different crimes involve the recovery of trace evidence from items of evidence which may contain glass. The forensic glass examiner may be requested to analyze a questioned sample to determine if the item is glass, and if so, what type of glass and/or the type of item the glass may have originated from.

1.2. In many instances, the forensic glass examiner may be requested to compare questioned and known (Q and K) glass samples based on their morphological characteristics, optical properties, and elemental compositions. The purpose for conducting a glass comparison is to ascertain whether a fragment of glass could have originated from a known source. If known and questioned glass samples are determined to possess the same morphological characteristics, optical properties, and elemental compositions it may be concluded that the questioned glass sample is consistent with having originated from the same source as the known glass sample or another glass with the same characteristics. Direction of force, sequence of impact, and glass source classification of certain glass types can also be determined.

1.3. The techniques described below for glass examination are well known and scientifically accepted in the forensic science community and in private industry.

1.4. Reference(s) to applicable OSAC Registry documents

1.4.1. *ASTM E2926-17 Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (μ-XRF) Spectrometry*

1.4.2. *ASTM E1967 Standard Test Method for the Automated Determination of Refractive Index of Glass Samples Using the Oil Immersion Method and a Phase Contrast Microscope*

2. Instrumentation/Reagents

2.1. Carbon tape/Mylar film

2.2. Clean paper

2.3. Cleaning solvents

2.4. Hot-stage microscope slides and cover slips

2.5. Immersion oils and corresponding reference glass

2.6. Instrumentation

2.6.1. Scanning electron microscope-energy dispersive spectroscopy (SEM-EDS) (*ATF-LS-E3*)

2.6.2. X-ray fluorescence (XRF) (*ATF-LS-E4*)

2.6.3. Glass Refractive Index Measurement (GRIM) (*See laboratory work instructions)*

2.7. Micrometer

2.8. Narrow band pass filters: Sodium D (589 nm); Hydrogen F (486 nm) and Hydrogen C (656 nm)

2.9. Spatulas, scalpels, tweezers and probes

2.10. Steel pulverizer or other device(s) to crush glass

2.11. Stereomicroscope (*ATF-LS-TE02*)

2.12. Tape or other adhesive device

2.13. Ultrasonic Cleaner

2.14. UV lamp, short and long wave

2.15. Vacuum cleaner and filters

3. Safety Considerations

3.1. The examiner shall follow the biohazard procedures and use universal precautions.

3.2. Sharps hazards are a particular concern when dealing with glass evidence and care shall be taken to minimize this danger which may include wearing PPE and handling glass pieces with suitable instruments.

3.3. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

4. Procedure or Analysis

4.1. Processing glass evidence

4.1.1. Examination area

4.1.1.1. The examiner's work surface must be cleaned prior to examining the evidence.

4.1.1.2. To the extent that packaging allows, examine each item separately. When necessary to prevent cross-transfer or contamination, known materials will be kept separate from questioned materials.

4.1.1.3. The examiner shall clean their tools between examining the evidence from the known and questioned sources. The questioned items and known items should not be open or uncovered at the same time in the same area.

4.1.2. Recovery of questioned glass particles:

4.1.2.1. The inside of each item's original container should be examined for the presence of questioned glass particles that may have been dislodged because of packaging and/or transit.

4.1.2.2. Items shall be visually examined for questioned glass. Additional collection methods such as scraping, vacuuming, and taping may also be conducted. If present, the inside of pockets and cuffs should be examined.

4.1.2.3. Clothing items from a single individual may be processed individually or together depending on the examination request. Shoes should be processed separate from clothing. Right and left shoes from a single individual may be processed together for trace evidence collections.

4.1.2.4. Particles recovered from debris may need to be cleaned prior to analysis.

4.2. Determination of glass and/or glass type

4.2.1. Glass particles can be recognized based on some or all of the following features:

4.2.1.1. Fracture: glass has irregular shaped broken edges (conchoidal fractures) with sharp edges.

4.2.1.2. Hardness: glass does not become indented when depressed with a metal probe.

4.2.1.3. Solubility: glass is not soluble in either water or organic solvents.

4.2.1.4. Isotropic: glass fragments are isotropic and will appear dark on a dark field during 360° rotation on the stage when viewed with crossed polars. Particles that show retardation colors (anisotropic particles) during rotation of the stage cannot be glass particles. However, it should be noted that toughened glass particles may show regions displaying very low birefringence due to stress.

4.2.1.5. Elemental composition: glass can be confirmed by its elemental composition.

4.2.2. Additional morphological characteristics can also be used to classify glass in different groups according to its end use:

4.2.2.1. Color, transparency (e.g., transparent green curved glass may indicate bottle glass).

4.2.2.2. Fluorescence using short (254 nm) and long (365 nm) wavelength light. Float glass can be easily identified using short-wave as a white/yellow/orange fluorescence can be seen on the side of a sheet of float glass that was in contact with molten tin during manufacturing.

4.2.2.3. Features such as surface coatings, markings, air bubbles, inclusions, laminates, etc. (e.g., headlight glass may have markings, laminated glass will have laminate between the two pieces of glass, mirror glass will have a reflective coating).

4.2.3. Elemental composition (XRF or SEM-EDS) can also be used to classify glass by its chemical composition.

4.2.3.1. Soda-lime-silicate glass is often sheet or container glass.

4.2.3.1.1 Published research[1] indicates that the Ca/Mg and Ca/Fe ratios will be higher in container glass than sheet glass; Mg and Fe

[1] Ryland S. Sheet or Container? Forensic Glass Comparisons with an Emphasis on Source Classification, *Journal of Forensic Sciences*, Vol 31, No. 4 Oct 1986, pp. 1314 – 1329.

are found in lower amounts in container glass as compared to sheet glass for end use purposes.

4.2.3.1.1 SRM 621 Soda-Lime container glass and SRM 1831 soda-lime sheet glass shall be analyzed along with case samples to confirm that the Ca/Mg and Ca/Fe ratios are consistent with published research.

4.2.3.2. Finding specific elements in glass may indicate end use. Boron is found in borosilicate glass which is often found in labware or cookware. Finding lead in glass could indicate a decorative glass.

4.3. Selection of samples for analysis

4.3.1. Questioned glass: A representative sample of the recovered glass should be taken for analysis as these questioned glass particles cannot be assumed to be from the same source (unless they can be physically fit together).

4.3.2. Known glass: Samples should be selected to encompass the variation expected for that glass type and therefore it may be left to the discretion of the examiner as to how many particles need to be selected for further comparison.

4.4. Analytical procedures for comparison of known and questioned glass samples:

4.4.1. Minimum criteria:

4.4.1.1. The minimum analytical scheme for glass includes morphological characterization, refractive index determination and trace elemental analysis. If at any time during the comparative scheme of analysis an exclusionary difference is observed between the Q and the K samples, no further examinations need to be conducted and the glass can be reported as being dissimilar to one another.

4.4.1.2. The questioned item is evaluated to identify morphological characteristics (e.g., color, shape) suitable for comparison prior to examination of the known glass. Any subsequent optical and elemental analysis of the unknown item shall be conducted prior to the known item.

4.4.2. Morphological characteristics: Depending on the size of the glass, some morphological characteristics cannot be determined.

4.4.2.1. Color, shape (curved, flat), surface features, texture, inclusions, and surface markings should be documented and compared.

4.4.2.2. Glass samples should be examined using both long and short wavelength UV and any noticeable fluorescence observations recorded. Certain constituents in glass may impart a particular type and/or degree of fluorescence. Glass samples may exhibit an overall fluorescence rather than only on one surface; therefore, even if the particle of glass has no flat surfaces, it should be examined under a UV light.

4.4.2.3. If two parallel manufactured surfaces are present, measure the thickness of the glass sample using a micrometer. For known samples, thickness measurements should be taken from several areas of that item to determine the range of thickness. While flat glass thicknesses are tightly controlled by modern manufacturing processes (not expected to vary by more than 0.15 mm/0.00591 inch for float glass and 0.25mm/0.00984 inch for other flat glasses)[2], older glass samples, curved glass, and container glass thicknesses can vary widely within the product itself.

4.4.2.4. Glass samples that exhibit high reflectivity but have a transparent manufactured surface may be low-E glass. These samples should be checked for surface continuity using a continuity tester. Low-E glass may conduct electrical current on one manufactured surface but not the other. If one surface conducts an electrical current, then the glass is most likely low-E glass.

4.4.2.5. Physical fit provides the only conclusive association between glass samples. Physical fit examinations will be performed in accordance with ATF Physical fit protocol (*ATF-LS-TE10*).

4.4.2.6. Record all pertinent visual observations concerning known and questioned glass samples.

4.4.3.Optical properties:

[2] Koons RD, et al. Forensic Glass Comparisons in Saferstein R., Ed, Forensic Science Handbook Vol 1, 2nd Edition, Prentice Hall, Upper Saddle River, New Jersey (2002).

4.4.3.1. Follow GRIM work instructions for instrument use, calibration, and sample prep.

4.4.3.2. Performance checks of the system must be performed using a separate reference glass of known refractive index, distinct from that used for the calibration. Acceptable performance criteria have been determined by the manufacturer for Locke reference glasses (within +/- 0.2° C). In-house instrument qualification also established acceptable performance criteria for Standard Reference Material NIST 1822 (within +/-0.0001 RI).

4.4.3.3. The refractive indices of the questioned and known glasses should be determined using the sodium D band pass filter. Additional refractive index determinations, using the hydrogen F and C band pass filters, may also be used. As a guideline[3] for known sources, a minimum of 20 refractive index measurements should be made when sampling a toughened float glass control, and a minimum of ten refractive index measurements should be made when sampling a non-toughened float glass control.

4.4.3.4. A minimum of three measurements of the questioned sample is recommended[4] for the comparison of refractive index of glass but case circumstances may warrant a lesser number.

4.4.3.5. Generally, two glass samples are considered indistinguishable if the average RI of the questioned glass sample falls within the minimum and maximum range of the known glass.

4.4.3.6. In situations where a minimal amount of glass is available for refractive index determinations, those glass samples that were mounted for refractive index determinations can be de-mounted, placed in a suitable container, and retained with the evidence or left on the slide, sealed with tape and retained with the evidence.

4.4.4. Elemental Analysis (XRF / SEM-EDS):

4.4.4.1. Instrument calibration and performance checks.

[3] ASTM E1967-19 Standard Test Method for the Automated Determination of Refractive Index of Glass Samples Using the Oil Immersion Method and a Phase Contrast Microscope.

4.4.4.1.1. Follow protocols for required calibration and performance checks (*ATF-LS-E3* and *ATF-LS-E4*).

4.4.4.1.2. Additional performance checks have been established during in-house instrument qualification for the XRF for glass analysis:

The performance of the X-ray source is checked using the calibration standard and ensuring that the maximum counts is within 10% of the established parameters determined.

NIST SRM 1831 is analyzed and must be within the established parameters determined.

4.4.4.2. The pieces of glass may be cleaned in a solvent and sonicated to remove any material adhering to the surface.

4.4.4.3. Samples can be placed on carbon tape and the carbon tape can be affixed to an appropriate substrate. Other appropriate mounting materials (e.g., Mylar® film) may also be used.

4.4.4.4. The known and questioned samples should be similar in size and positioned and/or analyzed in a way to reduce incident angles.

4.4.4.5. The glass fragments for K and Q are analyzed using the same parameters. Typical parameters for glass are aimed at obtaining over 5000K counts with allowing a higher voltage for heavier elements. Typical parameters for glass are minimum 40 kV, 300 A and 1000 live seconds.

4.4.4.6. Comparisons of elemental spectra from the K and Q samples are performed on a qualitative basis. Collect multiple spectra to ensure that the questioned glass fragments and known glass source(s) are adequately characterized. When practical, analyze a minimum of three measurements on each questioned specimen examined and nine measurements on known glass sources.

4.4.5. XRF Spectral comparisons

4.4.5.1. Reproducible differences in detected elements between samples demonstrate that they have exclusionary differences. These comparisons can be conducted on the spectra.

4.4.5.2. If peak identification does not discriminate between samples, further spectral comparisons are conducted.

4.5.5.2.1 Visual comparison: Reproducible differences in spectral shapes and relative peak heights between samples is documented by spectral overlays.

4.5.5.2.2 Peak intensity ratio comparison: Reproducible differences between samples in peak intensity ratios can demonstrate that the samples have exclusionary differences. The modified $\pm$ 3s interval is used for the current polycapillary optic/ Silicon Drift Detector (SDD) system at the ATF.

For each elemental ratio, calculate the mean (m) and the standard deviation (s) of the known measurements. NOTE: RSD is the relative standard deviation.

Calculate two different intervals ($\pm$3s and 3% RSD_{min}):

- $\pm$3s = mean $\pm$3(RSD)
- 3% RSD_{min} = mean $\pm$ (3*3%) = mean $\pm$ 9%

Using the larger interval, compare the mean for the questioned sample to the interval for the known sample.

If, for one or more elements, the average ratio in the questioned sample does not fall within the interval for the known sample, it can be determined that the samples are not from the same source.

The following elemental ratios will typically be used, when possible: Ca/Mg, Ca/Ti, Ca/Fe, Sr/Zr, Fe/Zr, and Ca/K. Additional elemental ratios can also be included (e.g., Ti/Fe, Mn/Fe).

NOTE: The SEM-EDS is a screening tool for glass examinations and can readily be used to discriminate glass. However, in comparative examinations if elemental composition by XRF is not performed or available and the exhibits are not discriminated by other techniques, a limited association is typically reported.

4.5. DIRECTION OF FORCE: This procedure is used to determine the direction of force for non-tempered window glass and other non-tempered flat glass items.

4.5.1. Minimum criteria:

4.5.1.1. An adequate amount of the total glass fragments from a broken pane or the entire window frame must be submitted in order to sufficiently reconstruct that object, identify a point(s) of impact and make an appropriate determination with regard to direction-of-force.

4.5.1.2. The window and/or fragments should be marked according to their orientation (inside vs. outside) in order to make direction-of-force determinations.

4.5.1.3. Direction of force determinations cannot be conducted on tempered glass samples or samples that have been broken by heat.

4.5.2. Analytical Procedures for Direction-of-Force:

4.5.2.1. Lay the fragments out in a consistent orientation based on float surface fluorescence, paint residue, surface debris or other characteristics.

4.5.2.2. Reconstruct the broken item as completely as possible.

4.5.2.3. Determine the point(s)-of-impact and attempt to locate the radial cracks associated with each impact point.

4.5.2.4. Examine for the presence of stress lines (Wallner lines/ridges) that are present on the fractured surface of the radial cracks. These stress lines (ridges) will be at right angles on the fractured surface edge opposite the direction of force for radial cracks. 4R rule – Ridges on Radial cracks are at Right angles at the Rear (side opposite of the force). Observe the stress lines from at least two radial cracks that emanate from a single point near the impact. The 4R rule is unreliable on tempered glass (toughened glass), laminated glass and small glass panes held tightly in the frame.

4.6. SEQUENCE OF IMPACT: This procedure is used to determine the sequence in which multiple impacts have occurred in a broken glass pane.

4.6.1. Minimum criteria:

4.6.1.1. This determination can only be made on non-tempered glass sources. Even so, the pane must have held together long enough while the item

was being broken, for the pattern to develop. Typically, only laminated or wire reinforced panes will do so.

4.6.2. Analytical Procedures for Sequence of Impact:

4.6.2.1. If necessary, reconstruct the glass using the fragments submitted.

4.6.2.2. Determine points-of-impact and identify radial cracks.

4.6.2.3. If multiple points-of-impact can be identified, examine the cracks formed by each impact and attempt to determine the relationship between these impacts. Cracks formed by a second impact will terminate at cracks that were formed by the original impact.

4.6.2.4. Using these observations, determine the sequence of these impacts.

4.7. For interpretation and results of glass comparisons as well as report wording, see *ATF-LS-TE-16*

5. Quality Assurance and Controls

5.1. Through proper training and testing of glass examiners as well as through the use of high-quality equipment, which is appropriately cleaned, maintained, and quality checked (e.g. calibrated, performance checked) the quality of this method is assured.

6. References

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1. Scope

1.1. When tape is received as evidence, a multi-disciplinary approach must be considered. Latent print examiners, trace evidence analysts, and scientists from any other discipline involved should work together to devise a scheme of analysis so as to glean as much information as possible for all disciplines. The initial examiner may examine the tape for trace evidence, or the tape could be transferred to a trace evidence examiner for examination and recovery of any trace evidence on the tape. Prior to latent print processing, a sample of tape may be removed or preserved for possible future tape examinations. An attempt should be made to take the sample from a portion of the tape which will be least likely to contain fingerprints. Care should be taken when processing the ends of the tape to minimize damage to the torn ends, preserving the ends for a possible physical fit. If tape needs to be cut from an item, any cuts made by an examiner should be made and/or labeled in such a way as to be easily recognized (e.g., zigzag cut, exaggerated angle cut).

1.2. Quite frequently, glue/adhesive/sealant/filler material (hereafter simply referred to as "adhesive") will not be inventoried as a separate item but will be a component of the exhibit being examined (e.g., part of the tape, device component). Adhesives should be examined for the inclusion of trace evidence on or embedded in the adhesive.

1.3. Reference(s) to applicable OSAC Registry documents

1.3.1. ASTM E3085 Standard Guide for Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations
1.3.2. ASTM E3260 Standard Guide for Forensic Examination and Comparison of Pressure Sensitive Tapes
1.3.3. ASTM E3233 Standard Practice for Forensic Tape Analysis Training Program

2. Instrumentation/Reagents

2.1. Tweezers, scalpel, and other appropriate tools

2.2. UV light

2.3. Appropriate solvents (chloroform, toluene, etc.)

2.4. Micrometer and other measuring devices

2.5. Microscopes

2.5.1. Polarized light microscope

2.5.2. Stereomicroscope

2.6. Glass microscope slides and coverslips

2.7. Mounting media

2.8. Camera or other Imaging Equipment

2.9. Instrumentation

2.9.1. FTIR (*ATF-LS-E6*)

2.9.2. MSP (*ATF-LS-TE03*)

2.9.3. Pyrolysis GC-MS or High Temperature GC-MS (*ATF-LS-TE04*, *ATF-LS-FD2*)

2.9.4. XRD (*ATF-LS-E5*)

2.9.5. XRF or SEM-EDS (*ATF-LS-E4*, *ATF-LS-E3*)

3. Safety Considerations

3.1. The examiner shall follow all the biohazard procedures and use universal safety precautions.

3.2. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

4. Procedure or Analysis

4.1. Minimum standards and controls

4.1.1. The examiner shall clean the examination area and/or change the examination paper between questioned and known samples and when it seems appropriate.

4.1.2. The examiner shall clean their tools between samples.

4.1.3. If at any time during the comparative scheme of analysis an exclusionary difference is observed between the Q and the K samples, no further examinations

need to be conducted and the tapes or adhesives can be reported as being dissimilar to one another.

4.2. Physical Fit for tapes and adhesives

4.2.1. If a physical fit is made, no further chemical analysis is required. Refer to Examination of Physical Fits protocol (*ATF LS TE10*).

4.3. Sample selection/Representative sample

4.3.1. Sample selection

4.3.1.1. Each recovered piece of questioned tape or adhesive should be examined unless the number of pieces makes this procedure prohibitive. In that case, select sample(s) may be taken for further analysis. Because different/separate tape or adhesive pieces cannot be assumed to be from the same source, sample selection shall be utilized when reporting the examination of questioned samples.

4.3.2. Representative sample

4.3.2.1. Questioned sample: When testing individual questioned pieces of tape or clumps of adhesive, certain tests may be conducted on smaller samples which are removed from that larger piece or clump. In that instance, homogeneity is assumed. Accordingly, the results of those tests may be used to represent the larger piece (or pieces that have been physically fit together) as a whole.

4.3.2.2. Known sample: It can be assumed that the known tape from a roll or known adhesive from a tube is homogeneous and therefore a representative sample from the known can be utilized and reported.

4.4. Chemical Analysis for tapes and adhesives

4.4.1. Purpose

4.4.1.1. Chemical analyses are performed on tapes and adhesives to aid in the identification of the source and/or for comparison between tapes and adhesives.

4.4.2. Analytical Procedure - Tape

The analytical scheme will vary depending on the type of tape and if it is a characterization or a comparison.

4.4.2.1. Characterization: The minimum analysis for the characterization of tape includes:

Visual and/or microscopic examination to determine type of tape (e.g., duct, electrical).

If any chemical information is reported, instrumental analysis is required (e.g., reporting polyvinyl chloride).

4.4.2.2. Comparison: The minimum analytical scheme for comparison of tapes includes physical characterization and separate analysis of each major component (backing, adhesive, and reinforcing material, if present). This analysis must include the use of at least two instrumental techniques (one for organic and one for elemental analysis) for the backings and adhesives. The questioned item is evaluated to identify physical characteristics (e.g., color, layer structure, dimensions) suitable for comparison prior to examination of the known tape. Any subsequent chemical and elemental analysis of the unknown item shall be conducted prior to the known item.

Duct tape (i.e., poly-coated cloth tape)

- Physical characteristics that may be evaluated include:
  - Color (backing and adhesive)
  - Width
  - Thickness
  - Backing texture
  - Scrim count (yarns per square inch)
  - Adhesive by UV light
  - Cross section of backing and adhesive
  - PLM of adhesive

- Analysis of reinforcing material including construction and composition. Examine the fibers per protocol for the Examination, Analysis and Comparison of Textiles (*ATF LS TE11*)

- Instrumental analysis techniques for backing and adhesive:
  - FTIR
  - Raman
  - PGCMS

- § SEM-EDS
- § XRF
- § XRD
- § MSP (backing only)

Electrical tape (i.e., vinyl tape)

- ◦ Physical characteristics that may be evaluated include:
  - § Color of backing and adhesive
  - § Width
  - § Thickness
  - § Backing texture
  - § Cross section of backing and adhesive
  - § PLM of adhesive

- ◦ Instrumental analysis techniques for backing and adhesive:
  - § FTIR
  - § Raman
  - § PGCMS
  - § SEM-EDS
  - § XRF
  - § MSP (backing)

Other tapes (i.e., packaging tape, masking tape, filament tape, office tape)

- ◦ Physical characteristics as previously described

- ◦ Chemical analysis of backing and adhesive as previously described

- ◦ Fibers or fabric analysis including construction and composition. Examine the fibers per protocol for the Examination, Analysis and Comparison of Textiles (*ATF LS TE11)*

- ◦ Clear packaging tapes should be examined by PLM and the following can be determined and/or compared:
  - § Extinction angle relative to the machine edge
  - § Retardation colors of the tapes
  - § Monoaxially oriented polypropylene (MOPP) or biaxial oriented polypropylene (BOPP) films
  - § If the films are BOPP, determine the BOPP angles

4.4.3. Analytical Procedure – Adhesives

The analytical scheme will vary depending on the type of adhesive and if it is a characterization or a comparison.

4.4.3.1. Characterization: The minimum analysis for the characterization of an adhesive includes:

Visual and/or microscopic examination to report that an adhesive is present.

If any chemical information is reported, instrumental analysis is required (e.g., reporting styrene butadiene, silicone, epoxy)

4.4.3.2. Comparison: The minimum analysis for the comparison of adhesives includes physical characterization and instrumental analysis to include the use of at least two instrumental techniques (one for organic and one for elemental analysis). The questioned item is evaluated to identify physical characteristics (e.g., color, opacity, texture) suitable for comparison prior to examination of the known adhesive. Any subsequent chemical and elemental analysis of the unknown item shall be conducted prior to the known item.

If dried adhesives are to be compared with liquid materials from a tube or bottle, a known comparison standard sample shall be prepared by mixing the sample, if necessary, applying a portion of the sample to a clean glass slide and allowing the sample to thoroughly dry/cure. In some instances (such as with moisture cure adhesives) the adhesive may need to be placed in a moist environment and may need to be heated to facilitate complete curing. Ensure that the sample is protected from any dust or contamination while it is being cured.

Physical characteristics that may be evaluated include:

- Color
- Texture
- Solubility
- Elasticity
- Porosity
- Opacity
- UV
- PLM

Instrumental analysis techniques:

- § FTIR
- § Raman
- § PGCMS
- § SEM-EDS
- § XRF
- § XRD

5. Quality Assurance and Controls

5.1. Through proper training and competency testing of examiners, and through the use of high-quality equipment, which is appropriately cleaned, maintained and quality checked (e.g., calibrated, performance checked), the quality of this method is assured.

5.2. Because tapes and adhesives are mass produced, a questioned tape or adhesive can never be positively identified back to a specific source unless a physical fit is confirmed.

5.3. Follow each instrument protocol regarding performance checks and the use of appropriate standards, controls and blanks.

5.4. Validation

5.4.1. The techniques described above for tapes and adhesive examination are well known and scientifically accepted in the forensic community and in private industry. Relevant examples of related literature can be found in Section 6 (References).

6. References

6.1. ASTM International Standards

E2809 Standard Guide for Using Scanning Electron Microscopy/X-ray Spectrometry in Forensic Polymer Examinations
E3296 Standard Guide for Using Pyrolysis Gas Chromatography and Pyrolysis Gas Chromatography-Mass Spectrometry in Forensic Polymer Examinations

6.2. Scientific Working Group for Materials (SWGMAT) Documents available at: www.asteetrace.org

SWGMAT Guideline for Assessing Physical Characteristics in Forensic Tape Examination

SWGMAT Guideline for Using Light Microscopy in Forensic Tape Examinations

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1. Scope

1.1. Any substance or item that may be taken away from a crime scene or left at a crime scene by the suspect or victim may become important evidence. For this reason, a plethora of different types of substances may become evidence in a case. These types of materials may include but are in no way limited to items such as building materials including wood, leather, metal, matches, and household goods such as cleaning products or food items. As a part of the investigation, the trace evidence examiner may be asked on occasion to characterize or compare these items. It is impossible to design a single analytical scheme that can analyze all substances. Due to this fact, the examiner must assess the items on a case-by-case basis and determine an appropriate analysis scheme using common methods, laboratory equipment, and known reference materials or standards suitable for the characterization and/or comparison of the submitted items.

2. Instrumentation/Reagents

2.1. Due to the wide variety of substances that may be encountered, the following is a list including some of the equipment and/or materials which may commonly be used:

2.1.1. Microscopes (*ATF-LS-TE01 / ATF-LS-TE02*)

2.1.1.1. Polarized light microscope

2.1.1.2. Stereomicroscope

2.1.1.3. Comparison microscope

2.1.1.4. Fluorescence microscope

2.1.2. Instruments

2.1.2.1. FTIR (*ATF-LS-E6*)

2.1.2.2. GC-MS or PyGC-MS (*ATF-LS-E9 / ATF-LS-TE04*)

2.1.2.3. Microspectrophotometer (MSP) (*ATF-LS-TE03*)

2.1.2.4. Raman (*ATF-LS-TE07*)

2.1.2.5. SEM-EDS (*ATF-LS-E3*)

2.1.2.6. XRF (*ATF-LS-E4*)

2.1.2.7. XRD (*ATF-LS-E5*)

2.1.3. Hot-stage Microscopy

2.1.4. Miscellaneous solvents and/or chemicals

2.1.5. Glass microscope slides, cover slips, mounting media

2.1.6. Litmus paper

3. Safety Considerations

3.1. The examiner shall follow biohazard procedures and use universal precautions.

3.2. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

4. Procedures

4.1. General Information

4.1.1. When attempting to characterize general unknown substances, contact with the investigating officer prior to any analyses may provide useful information about items related to the victim, suspect, or crime scene. This could prove useful in narrowing down potential sources or the possible identity of the general unknown in question. When a particular substance is suspected or known to the examiner as a possible source/identity of the unknown item of evidence, it may prove useful to call the manufacturer of the consumer product for information about product processing, ingredients, and packaging. Internet searches are also a good source of information.

4.2. Visual Examination

4.2.1. Visual examination of the submitted item is often the first step in characterization or comparison of general unknowns or uncommon evidence items. Low power magnification may be used when applicable. This may be the only step necessary to identify some evidence items. Any significant physical characteristics such as size, color, texture, shape, or odor should be noted.

4.2.2. If the specimen is a liquid, check for sediments, suspensions, and any liquid interface. Foaming upon shaking may indicate soap or detergent.

4.3. Procedure

4.3.1. Due to the wide range of samples encountered in this type of case work, the type of analyses conducted on the specimen will be determined on a case-by-case basis. Using the case history, the type of known sample submitted as a guide if available, and the observations made during the visual examination; the examiner should decide which analytical methods are appropriate. The following are just a few of the common laboratory methods that may be used:

4.3.1.1. Microscopy

4.3.1.1.1. Microscopical examinations may lead to identification of the unknown substance and may be the only method necessary for comparison of some uncommon evidence items. General morphology as well as observation of the substance under controlled lighting conditions will aid in the characterization and comparison. The nature and type of material will dictate what sample preparation is needed as well as what microscope(s) will be utilized. Starch, leather, wood, paper, and plant material are just a few of the substances which can be identified and compared using stereomicroscopy and polarized light microscopy (See *TE02 Set-up and Use of the Microscope*).

4.3.1.2. pH

4.3.1.2.1. Test pH of a liquid sample and if possible compare it to pH of a control. If pH is unusual, the examiner may test for acids or bases, such as hydrochloric acid and sodium hydroxide (See Appendix I).

4.3.1.3. Volatile and halogenated compounds

4.3.1.3.1. If unusual odors are present, consult with a fire debris examiner for the best way to proceed. Some halogenated compounds can be detected by spot tests (See Appendix I).

4.3.1.4. Toxic metals

4.3.1.4.1. Toxic metals can be detected by using the Reinsch test. This test can be applied directly to body fluids, tissue slurries, food, and drink.

Mercury, arsenic, silver, bismuth, and antimony can be detected with this test (See Appendix I).

4.3.1.5. Inorganic substances

4.3.1.5.1. Water extractions are sometimes needed to test for inorganic substances. Silver nitrate and barium chloride are good reagents for general testing of samples for cyanide, arsenic, and numerous anions. Silver nitrate, barium chloride, and other reagents are described in Appendix I.

4.3.1.6. Acids/bases

4.3.1.6.1. Acidic/basic organic extractions can be tested for the presence of organic substances on the GC-MS. The extraction may include clean up steps to eliminate unwanted compounds, e.g., fats.

4.3.1.7. Instrumentation

4.3.1.7.1. Some solid samples may be analyzed and compared on a variety of laboratory instruments such as the FTIR, SEM-EDS, XRF, XRD, MSP, or PyGC-MS (See individual instrument protocols listed above).

4.3.1.8. Specific analysis

4.3.1.8.1. Microchemical Tests (see Appendix I)

4.3.1.8.1.1. Chemical spot tests (color tests) can be used to indicate the presence of functional groups within the sample. Generally, the functional group is indicated to be present through the formation of a colored complex, a precipitate, or the release of a gas. Microscopical crystal tests can be used on organic and inorganic compounds to characterize and possibly identify them by their crystal shape. Once a crystal has been characterized, its identity may be confirmed utilizing other instrumental techniques. The term microchemistry will be applied to both chemical spot tests and crystal tests.

4.3.1.8.2. Bank dyes and lachrymators (see Appendix II)

4.3.1.8.2.1. Evidence can be examined for the presence of bank dyes and/or lachrymators. Alternate lighting can assist with finding stains that may be extracted and analyzed on the PyGCMS.

4.3.1.8.3. Paper match examinations (see Appendix III)

4.3.1.8.3.1. If a physical fit is not made or possible between a paper match to a book of matches, additional physical and chemical analysis can be completed. Match heads and/or match stems can be examined for physical characteristics including color, porosity, shape, wax line, measurements of width, length, and thickness. In addition, the torn fibers can be compared as well as microscopical comparison of inert ingredients in the match head. Further chemical analysis can be completed by SEM-EDS, XRF, XRD and/or MSP.

4.3.1.8.4. Wood examinations (see Appendix IV)

4.3.1.8.4.1. The most reliable approach of characterizing wood is based on its microscopic features. A low power microscopical examination (10-30X) of prepared wood samples can be used to identify wood as soft or hard wood, or if enough sample is present, classify it to genus or species. The later classifications will require thin sectioning of the wood sample for examination via high power microscope (100-400X).

4.3.1.8.4.2. NOTE: Wood exams are not currently covered under the ANAB scope and therefore are reported without using the ANAB logo.

5. Quality Assurance and Controls

5.1. Appropriate controls, blanks and reference materials should be used for each test. Controls or standards are often not submitted with evidence. A similar store-bought item may prove useful as a reference.

5.2. Refer to individual instrument protocols for appropriate instrument blanks, controls, calibrations / performance checks, and adjustments.

5.3. Microscopes, micrometers / measuring devices, and all scientific equipment should be properly calibrated or performance checked according to the protocols for each instrument (see *ATF-LS-E21 – Maintenance and Performance of Measuring Devices*)

5.4. The techniques described above and in the appendices for examination of general unknowns and uncommon evidence are well known and scientifically accepted in the forensic community and private industry. Relevant examples of related literature can be found in Section 6 (References).

5.5. For comparisons, the questioned item is evaluated to identify physical characteristics (e.g., color, layer structure, dimensions) suitable for comparison prior to examination of the known sample. Any subsequent chemical and elemental analysis of the unknown item shall be conducted prior to the known item. In certain circumstances, the known may need to be screened to determine a suitable analysis scheme for unusual evidence.

6. References

6.1 General unknown

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APPENDIX I--Frequently Used Micro Chemical Tests

1. It should be noted that slight variations in the formulations of each of these reagents may be acceptable. Regardless, all chemical reagents should be tested on a known sample prior to each use in order to test the reliability of the reagent. When a reagent is made, the bottle shall be labeled with the name of the reagent and the date it was made or lot number at a minimum. Records shall be kept as to who made the reagent and that it was tested for reliability. The list below is not all inclusive, but any reagents or tests used in the laboratory shall be well documented in literature and generally accepted in the scientific community.

2. General Tests

2.1 10% HCl--acidify test sample with drops of dilute HCl. Gas evolution indicates bicarbonates, carbonates, cyanides, hypochlorites (bleach), nitrates or nitrites. Use caution as cyanide gas is very poisonous.

2.2 5% $AgNO_3$--precipitates many ions. Most precipitates are white.

2.3 5% $BaCl_2$--precipitates many ions. Most precipitates are white.

2.3.1 Precipitated by $AgNO_3$ and insoluble in HNO_3:

Iodide, I^-	Sulfide, S^{-2}
Bromide, Br^-	Cyanide, CN^-
Chloride, Cl^-	Thiocyanate, SCN^-
Hypochlorite, ClO^-	

2.3.2 Precipitated by $AgNO_3$ and soluble in HNO_3:

Cyanates, CNO^-	Boric acid, H_3BO_3
Carbonic acid, H_3CO_3	Iodic acid
Oxalic acid, $C_2H_2O_4$	

2.3.3 Precipitated by $AgNO_3$ and $BaCl_2$; soluble in HNO_3:

Sulfites, SO_3^{-2}	Thiosulfates, $S_2O_3^{-2}$
Arsenite, As^{+3}, As_2O_3	Arsenate, As^{+5} , AsO_4^{-3}
*Phosphate, PO_4^{-3} yellow w/ $AgNO_3$	Chromic acid
Carbonate, CO_3^{-2}	
Bicarbonate, HCO_3^- cream w/ $AgNO_3$	

*Silver nitrate does not precipitate phosphoric acid due to acidic medium.

2.3.4 Precipitated by $BaCl_2$ and insoluble in HNO_3:

-Sulfate, SO_4^{-2} (high concentrations of sulfate can cause crystal formation with silver nitrate)

-Fluoride, F-

1.1 1% Diphenylamine/Concentrated Sulfuric Acid (fresh) -- blue color develops with the presence of the following oxidizers: chloride, bromide, iodide, chlorates, nitrates, nitrites, hypochlorite, bromate, iodate, permanganate, Fe^{+3}, Sb^{+5}, and peroxides. An immediate and permanent blue/purple indicates NO_3^-. A similar color is obtained with relatively concentrated solutions of $FeCl_3$. Immediate blue colors are produced by ClO_3^- and NO_{2-} but color from the latter fades rapidly and in about 1 minute is yellow green. At low levels, color development may occur after standing a short time. Similar reactions may also be observed with chloride, bromide, iodide, hypochlorite, bromate, iodate, permanganate, Fe^{+3}, Sb^{+5}, and peroxides.

1.2 Fujiwara Test--indicates presence of chloral hydrate, trichloroacetic acid, chloroform, bromoform, iodoform, and other compounds with at least two halogen atoms attached to one carbon. *Procedure*: to 1 mL of sample, add 1 mL 5N NaOH and 1 mL pyridine. Heat for two minutes in boiling water. Red or pink color in pyridine layer is positive.

1.3 Reinsch Test--indications for mercury, silver, arsenic, antimony and bismuth. *Procedure*: Add 3 mL conc. HCl to 15 mL sample. Immerse a copper wire that has been cleaned with concentrated HNO_3 in sample and heat gently (80-90^o) for 1 hour. Examine copper for discoloration every fifteen minutes. A silvery deposit is given by mercury and silver. A black deposit is given by bismuth and arsenic. A purple deposit is given by antimony.

1.4 5% Brucine Sulfate in H_2SO_4--orange to red color indicates nitrates, nitrites, or chlorates.

1.5 Sugar test--to a drop of sample or solid sample add 1 drop of 15% 1-naphthol in ethanol (EtOH) and then 3-4 drops of concentrated. sulfuric acid. If sucrose or fructose is present, a blue to purple color will appear; if glucose or maltose is present, a pink-red color will develop.

1.6 Metals by Ammonium Sulfide--to a drop of liquid sample acidified with 5% HCl, add a drop of aqueous $(NH_4)_2S$. Perform tests in hood. Many **metal ions** give colored precipitates:

1.6.1 Black precipitate: indicates Hg, Pb, Ag, Bi, Cu, Co, Ni, or Fe. With addition of concentrated HCl: Bi dissolves; Pb turns grey; Fe turns rust colored or dissolves to orange solution.

Yellow precipitate and solution indicates Cd.
Dark brown precipitate indicates Sn.
Reddish-brown precipitate indicates Pt.
Peach precipitate and solution indicates Mn.
Orange precipitate indicates Sb.
Milky white precipitate indicates Zn. ZnS is soluble in excess $(NH_4)_2S$.

3 Specific Tests

3.3 Ethchlorvynol--add crystals of diphenylamine to an alcoholic solution of the sample; slowly trickle in concentrated H_2SO_4. Red color positive.

3.4 Thiocyanate (nitroprusside)--add drop of 5% ferric chloride. Red color is positive.

3.5 Cyanide--add two drops of concentrated H_2SO_4 to 2-3 drops sample in test tube. Cover top of tube with a cover slip with a hanging drop of $AgNO_3$; warm at 80° C for 4-5 min. Search hanging drop for crystals of AgCN--tiny, highly refractive, short rods or sheaves of slender needles. Rod's RI's $n_{\wedge} = 1.685$ and $n_{||} >> 1.685$.

3.6 Arsenates--red precipitate with $AgNO_3$. Add a drop of 5% $AgNO_3$ to a drop of sample. View crystals with microscope.

3.7 Arsenites--yellow precipitate with $AgNO_3$. Best if ammoniacal $AgNO_3$ is used. Add concentrated ammonium hydroxide to 5% $AgNO_3$ until precipitate dissolves upon mixing. Add drop of this reagent to drop of sample. View crystals with microscope.

3.8 Oxalic acid, oxalate salts--to the acid or acid solution of the salt add drop of 10% ferrous sulfate. Yellow precipitate positive.

3.9 Lithium ion--add sample drop to glass slide and heat to dryness to remove any possible ammonium salts. Add drop of 15% hexamethylenetetramine (hexamine) to dried residue. Transfer this drop to another glass slide in two separate drops. To one drop add a crystal of $K_3Fe(CN)_6$ (potassium ferricyanide); to the other a crystal of $K_4Fe(CN)_6$ (potassium ferrocyanide). The ferricyanide yields yellow octahedra that appear birefringent due to high strain within the crystal; ferrocyanide yields short rods and radial clusters of rods. To help form the ferrocyanide crystals, push crust at edge of drop back into the middle and scratch slide with a glass rod. *Negative samples* of the ferricyanide also yield stars and yellow octahedra; however, these crystals are of *very low* birefringence.

3.10 Bleach containing Hypochlorite -- pH should be basic. Test with hanging drop of 5% silver nitrate by acidification with 5% HNO_3. Wash and dry precipitate in reagent drop with distilled water and dissolve precipitate with drop of 50% ammonium hydroxide. Add

coverslip and use PLM to look for formation of highly refractive cubic crystals of silver chloride along edge of coverslip. This indicates the presence of chloride ion from evolution of Cl_2 from the test drop. Crystals are then confirmed as AgCl via X-ray analysis.

3.11 Iodine Solution - Place a small amount of material on a microscope slide and cover with a cover slip. Add I_2 reagent and allow it to flow under the coverslip. Examine utilizing PLM. Starch grains and gelatinized starch particles stain purple/blue to red/brown. Color produced depends on the amylase content.

3.12 10% Povidone-Iodine (Betadine) Solution - Examine utilizing PLM. Starch grains and gelatinized starch particles stain purple/blue to red/brown. Color produced depends on the amylase content. Advantage of this test over the Iodine Solution is that the "Maltese" cross can be observed after the starch grains pick up the stain.

3.13 Fehling's Test for Reducing and Non-Reducing Sugars – A material to be tested is gently heated to a boil in a drop or two of Fehling's solution. If a reducing sugar (e.g. lactose, maltose, etc.) is present, the solution will turn yellow/orange. For a non-reducing sugar, the solution will stay blue. To test for a non-reducing sugar (e.g. sucrose), warm the material to be tested in dilute HCl and then add the Fehling's solution. The solution will turn yellow/orange if a non-reducing sugar was originally present.

3.14 Selleger's Stain and Graff "C" for cellulose fibers – Add stain to paper fibers which have been disintegrated and dispersed on a microscope slide. Cellulose fibers will stain different colors depending on pulp make-up and previous chemical treatment.

3.15 Ammonia or Ammonium Ion -- precipitate using hanging drop of 10% platinum chloride by volatilizing ammonium ion to ammonia by adding 10% sodium hydroxide to test sample. To test for presence of ammonia gas (anhydrous ammonia) place drop of reagent on glass slide and place slide in airtight container with specimen. Allow it to sit an appropriate amount of time (overnight if necessary) to allow for the formation of octahedral crystals indicative of the ammonium ion reaction product. The resulting crystals formed can be rinsed with distilled water, dried, and analyzed via IR spectroscopy.

3.16 Ethylene Glycol—See Section 4.3.1.3.1 Procedure above and/or consult a fire debris examiner.

3.17 Hydrogen Peroxide -- Use two tests.

3.17.4 Reduction test: Place one drop of 1.0% potassium ferricyanide/0.5% ferric chloride in spot well. Add test drop(s). Prussian blue coloration indicates hydrogen peroxide. Very dilute solutions may give a green coloration.

3.17.5 Oxidation test: Soak filter paper with 0.5% lead acetate. Hold over open bottle of 24% ammonium sulfide. Paper will become brown due to formation of PbS. Allow paper to dry. Spot paper with drop of sample. A white coloration indicates hydrogen peroxide. If only one of the tests is positive something other than hydrogen peroxide is indicated.

APPENDIX II—Bank dyes and lachrymators

1. Identification of bank dyes

1.1 Examination of stained items

1.1.1 Note areas with bright red stains. Record physical properties applicable to the stain such as location on substrate, dimensions, pattern, etc. in the examination record.

1.1.1.1 NOTE: MAAQ is a fine powder and is easily distributed. Take care not to contaminate unstained areas during handling of evidence so as to maintain an area available for a comparison sample.

1.1.2 Remove a section of the evidence containing the stain. If available, remove a section of an unstained area as a comparison sample. If removal is not possible, swab the stained and unstained areas with separate swabs wet with methanol.

1.1.2.1 NOTE: Do not sample all of the stain if it is not necessary; a 1-inch by 1-inch”x1” heavily stained area should be more than enough for analysis. If a garment has been laundered prior to submission, a comparison sample will not be possible, and the sample taken will have to be larger.

1.1.3 Wash the two sections or swabs with approximately 5 mL methanol into separate evaporating dishes. If using swabs, extract one unused swab as a control. Filter if necessary. Evaporate the washings down to a volume suitable for analysis (approximately 1 mL or until solution is bright red).

1.1.4 In a separate area, make up separate dilute reference samples.

1.1.4.1 Dilute the reference MAAQ in methanol, concentrated just enough to attain a bright red color.

1.1.4.2 Dilute (approximately 1% w/v) solutions of CN and/or CS reference materials in methanol.

1.1.5 Run a sample each of the unknown, the comparison sample (if available), the control (if needed), and the reference on GC-MS.

1.2 Examination of bleached items

1.2.1 Examine evidence. Note areas with faint red stains, bleached areas, or areas with damage or holes. Record physical properties applicable to the stain or damage such as location on substrate, dimensions, pattern, etc. in the examination record.

1.2.2 If possible, remove a section of the evidence containing the stain or damage. If removal is not possible, swab the area with swabs wet with methanol.

1.2.2.1 NOTE: A comparison sample will probably not be available with bleached items

1.2.3 Wash the sample with methanol into a beaker. Filter the washing into an evaporating dish. Evaporate the washing down to a volume suitable for analysis (approximately 1 mL).

1.2.4 In a separate area, make up a dilute solution of chlorinated MAAQ derivatives:

1.2.4.1 Place 5 mg of MAAQ into an Erlenmeyer flask with 5 mL methanol

1.2.4.2 Add 20 mg of $FeCl_3 \cdot 6H_2O$ to the solution

1.2.4.3 Add 700 µL of 7% sodium hypochlorite solution

1.2.4.4 Allow the precipitate to settle, filter off the supernatant

1.2.4.5 Set the precipitate to dry

1.2.4.6 Place a small amount of the precipitate in methanol for analysis

1.2.5 In a separate area, make up a dilute solution of reference MAAQ in methanol, concentrated just enough to attain a bright red color.

1.2.6 Run the unknown, the control (if needed), and the MAAQ and Cl-MAAQs references on PyGC-MS.

2. Identification of lachrymators

2.1. Examination of a substrate

2.1.1. Examine the evidence and note the presence of any stains. Employ alternative lighting as necessary. Record physical properties applicable to the stain such as location on substrate, dimensions, pattern, color, etc.

2.1.2. If possible, remove a small section of the evidence containing the stain as well as an unstained area as a comparison. If removal is not possible, swab the stained and unstained areas with separate swabs wet with methanol.

2.1.2.1. NOTE: Do not sample all of the stain if it is not necessary; a 1-inch by 1-inch"x1" heavily stained area should be more than enough for analysis. If a garment has been laundered prior to submission, a comparison sample will not be possible, and the sample taken will have to be larger.

2.1.3 Wash the sections or swabs with ~ 5 mL methanol in two separate evaporating dishes. If using swabs, extract one unused swab as a control. Filter if necessary.

2.1.4 Evaporate the washings down to a volume suitable for analysis - approximately 1 mL or until solution is dark orange.

2.1.5 In a separate area, prepare dilute (approximately 1% w/v) solutions of CN, CS, and/or capsaicin reference materials in methanol.

2.1.6 Run the unknown, the comparison sample (if available), the control (if needed), and the reference materials on PyGC-MS.

2.2 Examination of a canister

2.2.1 Examine canister, noting brand, ingredients and volume/mass of contents according to label. If available, note serial number.

2.2.2 Record physical properties applicable to the sample such as solution morphology, color, etc.

2.2.3 Using a pipette, place one drop of the dispensed liquid into an auto-sampler vial. Fill the vial to the 1.5 mL mark with methanol, seal. If canister is empty, rinse interior with a minimal amount of methanol and place rinse into an auto-sampler vial.

2.2.4 In a separate area, prepare dilute (approximately 1% w/v) solutions of CN, CS, and/or capsaicin reference materials in methanol.

2.2.5 Run the, the unknown, the comparison sample (if available), the control (if needed), and the reference materials on PyGC-MS.

APPENDIX III – Paper Matches

1. Matchbooks are produced from paperboard which is finished and treated with an anti-afterglow solution. The paperboard rolls are cut into long strips called combs. These combs are then dipped into a wax, dried, and then dipped into the match-head solution and dried again. The head is mainly composed of potassium chlorate (oxidizer), sulfur (fuel) and glue with some inert ingredients. The standard match book will contain two combs of 10 stems, a total of 20 matches.

2. Physical characteristics

2.1 Initially, the examination and comparison of matches is made by visual inspection including utilization of a stereo binocular microscope. Some features may only provide class characteristics, whereas others may provide distinctive characteristics. These features are as follows:

2.1.1 Match Head

2.1.1.1 The match head color, porosity, shape, and size should be noted. Even burned heads may reveal this information.

2.1.2 Stem color

2.1.2.1 The front facing surface layer of the match stem frequently has a distinctly different color as compared to the underlying match stem body due to pigmentation and/or dying. Even the front surface of brown/tan stem matches can have a slightly different appearance than the interior of the match body. The use of a simple longwave UV lamp or alternate light source may also be employed during the examination of match stems which may provide additional comparative information.

2.1.3 Wax line

2.1.3.1 The wax on the match stem can normally be seen as a slight darker discoloration on the upper portion of the match stem. The depth of the wax line on the match stems can vary between books and within a book of matches.

2.1.4 Stem width

2.1.4.1 The width of matches usually falls into two groups; ones that have a width of approximately 3.3 mm and ones that have a width of

approximately 2.7 mm. The approximately 2.7 mm (specification is 0.0108 inches) width was a patented dimension and matches exhibiting this width was only manufactured by D. D. Bean & Sons (3). However, it must be noted that this does not mean that the matchbook will have "D. D. Bean & Sons" markings on the match cover since D. D. Bean & Sons produces matches with this dimension for other companies and other companies produce matches other than 2.7 mm for D.D. Bean & Sons.

2.1.5 Stem length and thickness

2.1.5.1 The match stem length, when placed at the cardboard base of the matchbook should correspond to the length of the known unburned matches in the matchbook. If the match is burned, a portion of the head must still be present to conduct an accurate comparison. The match thickness does not vary much and cannot be related to a particular manufacturer.

2.1.6 Base stem cut/indent

2.1.6.1 Some matchbooks may be cut or have an indentation at the base of the match stem to aid in removal of the match from the matchbook. The cut/indent may be consistent on every match or vary within a book.

2.1.7 Cut edge abnormalities

2.1.7.1 Cut edge abnormalities appear along the vertical edge of the match body as small irregular cuts or tears. These imperfections are due to a cutting blade becoming dull over time and are another potential point of comparison to an adjacent match in a book.

2.1.8 Crosscut and torn fibers

2.1.8.1 Crosscut and torn fibers may provide distinctive characteristics that can associate a match to a particular matchbook. Crosscut (horizontal) and torn (vertical) fibers are noted as darker colored fibers contrasted against the more lightly colored fibers. Crosscut (horizontal) and torn (vertical) fibers are recognized under low magnification utilizing a stereomicroscope. The horizontal fibers are fibers which cross individual match stems and have been cut during the manufacturing process. Vertical fibers are the contrasting fibers which run from the base into the match stem and are torn in two when the match is

removed from the book. Torn fibers are less useful when attempting to make a positive association since the tearing action of the match from the cardboard base may distort any comparison. The vertical fibers may not be torn in two but could be completely pulled from the base or stem when the match is removed. One can increase the contrast between the fibers in the match stems by use of stains, but it should be noted that the use of stains may permanently alter the color of the match stems. One simple way to increase the contrast between fibers is to place a droplet of an 80:20 deionized water: ethanol (EtOH) on the match stems, allow it to set for a moment, and then wick off any excess liquid.

2.1.9 Inclusions

2.1.9.1 Foreign matter inclusions are common artifacts in match stems and may be cut in two when adjacent stems are cut by the blade.

3. Analytical techniques

3.1 PLM

3.1.1 Some of the common inert ingredients that may be present in the match head that can be quickly characterized by PLM include quartz (irregular grains, $\omega = 1.544$ and $\varepsilon = 1.553$), glass fragments (irregular chips, $n \sim 1.52$), diatoms ($n \sim 1.44$ with very fine structure), and wollastonite (fibrous, $\alpha \sim 1.62$, $\beta \sim 1.63$, $\gamma \sim 1.64$). Pigments and starch grains may also be noted during a PLM examination. The presence or absence of any constituent may provide quick differentiation. Also, it helps if one removes the water-soluble components with warm water using micro extraction techniques. Pigments and inclusions in stems can also be characterized by PLM.

3.1.2 Potential analysis of fibers in the match paperboard by differential staining has been reported (Dixon) however this is a destructive method. Refer to references, specifically ASTM D1030, for further detail for the following stains:

3.1.2.1 Herzberg and Selleger's stains

3.1.2.2 Graff "C" stain

3.1.2.3 Green and Yorston stain

3.2 SEM-EDS

3.2.1 SEM-EDS can provide bulk elemental information and can also be employed to characterize and identify particulate material, pigments and inclusions as well as can confirm the constituents characterized by PLM.

3.3 XRF

3.3.1 Samples should be analyzed using a 40 KeV excitation energy to allow heavier elements such as strontium (Sr) and zirconium (Zr) to be detected. Spectra of the match heads and for stems can be completed.

3.4 XRD

3.4.1 XRD can provide identification of crystalline components of match heads as well as can confirm some constituents characterized by PLM.

3.5 MSP

3.5.1 Transmission or reflectance can be utilized. Red match heads have been shown to be differentiated by this technique.

APPENDIX IV - Wood

Wood exams are not currently covered under the ANAB scope and therefore are reported without using the ANAB logo.

1. In order to microscopically identify wood as a softwood or a hardwood, thin transparent sections can be cut and mounted from "bulk" samples. Characteristics found within these sections are used to identify a sample of wood as hardwood or softwood. Determine if the piece of wood is large enough for stereomicroscopic examination and thin sectioning. If not, only a microscopical examination of wood fibers can be performed.

2. Wood fibers

   2.1 If only wood fibers are to be examined, a stain such as Safrinin may be used and the sample can be mounted in an appropriate mounting medium. Examine using a high-powered microscope. Look for microscopical characteristics, if present, that will allow classification of fibers as hard or soft wood; and, if appropriate, mechanically or chemically pulped. Some characteristic features may be present to determine a more specific classification.

   2.1.1 Softwoods are also known as Gymnosperms or conifers.

   2.1.1.1 There are microscopic features that may be observed in mounted cross-sections that are characteristic of softwoods.

   2.1.1.2 Cross section – Resin canals, rays, and tracheids.

   2.1.1.3 Radial section – Bordered pits, tracheids.

   2.1.1.4 Tangential section – Ray (fusiform with resin canal and uniseriate) and radial walls of vertical tracheids.

   2.1.2 Hardwoods are also known as dicots or broad-leaved.

   2.1.2.1 There are microscopic features observed in mounted cross-sections that are characteristic of hardwoods.

   2.1.2.2 Cross section – Rays and vessels.

2.1.2.3 Radial section – Squared ended parenchyma, vessel elements, and rays.

2.1.2.4 Tangential section – Boat-shaped rays, vessel elements, and square ended parenchyma.

3. Wood fragments

3.1 For larger wood fragments, razor cuts are made on the whetted wood to obtain either a clean cross-sectional surface for stereoscopic examinations, or thin sections for high power microscopic examinations. Thin cuts from the cross, radial, and tangential sections are made, if possible.

x - Cross section – a section cut perpendicular to the grain

t - Tangential section – a section cut along the grain that is more or less parallel to the growth layer

r - Radial section – a section cut along the grain that is perpendicular to the grain direction

3.2 Cross-sectional surfaces are examined via low power microscopy and keyed according to Hoadley, Trimpe (MAFS) key and/or other suitable keys. Document the source of the key(s) used in case records. Comparison to standard wood blocks can be helpful.

3.3 Thin sections may be treated with a stain such as Safranin and mounted in an appropriate mounting medium between slide and cover slip. The preparation may be heated to remove air bubbles. Examine sections via high power microscope. Samples are characterized according to Hoadley, Trimpe (MAFS) key and/or other suitable keys. Document the source of the key(s) used in case records. Comparison to the thin section standards can be helpful.

4. Report results to the appropriate category of characterization.

1. Scope

1.1. Many different types of crimes may involve situations where a paint or coating is transferred, where paint is sprayed or applied to an object which is submitted as evidence, or when comparing coatings on manufactured items. In these cases, the examiner is commonly asked to compare questioned and known (Q and K) coatings or paints based on their physical and chemical compositions. In conducting those comparisons, the examiner's goal is to assess the significance of any differences observed. The absence of exclusionary differences between the Q and K samples suggests that the coatings or paints could have had a common source. The examiner may also analyze a questioned coating or paint to attempt to determine its end use.

1.2. Coatings are defined as *a liquid, liquefiable, or mastic composition that is converted by evaporation, cross-linking, or cooling to a solid or semisolid protective, decorative, or functional adherent layer after application*[1]. Coatings include, but are not limited to, paints, varnishes, sealers, and stains. Paint is defined in general as *a pigmented coating*[1].

1.3. The properties of the questioned and/or known samples may include physical (e.g., color, layer structure, surface features, fluorescence), microscopical (e.g., layer structure) and chemical properties. Chemical composition may be determined and compared by micro-solubility/micro-chemical tests, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy – energy dispersive spectroscopy (SEM-EDS), X-ray diffraction (XRD), X-ray fluorescence (XRF) and pyrolysis gas chromatography – mass spectrometry (PyGC-MS).

2. Instrumentation/Reagents

2.1. Scraping utensils

2.2. Tweezers, scalpel, and other appropriate tools

2.3. Clean paper

2.4. Evidence containers for repackaging trace evidence (e.g., plastic petri dishes, glassine envelopes)

2.5. Biohazard safety equipment (if necessary)

2.6. Vacuum and vacuum filters

[1] ASTM D16 Standard Terminology for Paint, Related Coatings, Materials, and Applications

2.7. Spot plates

2.8. Microscope slides

2.9. Temporary or permanent mounting media

2.10. Appropriate solvents and micro-chemical test reagents (acetone, chloroform, etc.,)

2.11. Microtome and embedding media

2.12. Microscopes (*ATF-LS-TE01* / *ATF-LS-TE02*)

2.12.1. Polarized light microscope

2.12.2. Stereomicroscope

2.12.3. Comparison microscope

2.12.4. Fluorescence microscope

2.13. Camera or other Imaging Equipment

2.14. Instrumentation

2.14.1. FTIR (*ATF-LS-E6*)

2.14.2. MSP (*ATF-LS-TE03*)

2.14.3. Pyrolysis GC-MS or High Temperature GC-MS (*ATF-LS-TE04 / ATF-LS-FD2*)

2.14.4. Raman (*ATF-LS-TE07*)

2.14.5. XRD (*ATF-LS-E5*)

2.14.6. XRF (*ATF-LS-E4*)

2.14.7. SEM-EDS (*ATF-LS-E3*)

3. Safety Considerations

3.1. The examiner shall follow all biohazard procedures and use universal precautions.

3.2. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

3.3. Precautions need to be taken when using sharp objects.

4. Procedure or Analysis

4.1. Processing for paints and coatings

4.1.1. Examination area

4.1.1.1. The examiner's work surface must be cleaned prior to examining the evidence.

4.1.1.2. At no time should questioned items and known items be open at the same time in the same area for recovery of trace evidence.

4.1.1.3. Change gloves and clean tools between examining the evidence from questioned items and known items.

4.1.2. Recovery of trace evidence

4.1.2.1. See general processing guidelines, *ATF-LS-TE Standard Approach for Examinations of Trace Evidence*.

4.1.2.2. Items shall be visually examined for trace evidence or with the aid of an illuminated magnifier or low powered microscope.

4.1.2.3. Additional collection methods, such as scraping or vacuuming, may also be conducted. Taping is not recommended as the adhesive may interfere with additional examinations of the coatings or paint.

4.2. Physical Fit for paints and coatings

4.2.1. Refer to Examination of Physical Fits protocol (*ATF-LS-TE10*)

4.2.2. If a physical fit is determined between probative evidence items (e.g., Q and K items), no further chemical analysis is required.

4.3. Sample selection/Representative sample

4.3.1. Sample selection

4.3.1.1. If several questioned samples are recovered from the same exhibit, they can be examined using stereomicroscopy, polarizing light microscopy, and/or fluorescence microscopy to determine whether the samples are consistent in appearance (color, layering, and microscopic characteristics) to one another. Additional analyses (e.g., micro-chemical tests, FTIR, SEM-EDS, MSP, PyGC-MS) can then be performed on a select number of the questioned samples.

4.3.2. Representative sample

4.3.2.1. Questioned sample:

4.3.2.1.1. Multiple pieces: Select a representative sample of each visually different type of coating or paint from the questioned item for further analysis.

4.3.2.1.2. Single piece: When testing a questioned item, certain tests may be conducted on a portion of the item that was removed from a larger piece or clump. In that instance, homogeneity is assumed; however, the variation within the sample will need to be evaluated. Accordingly, the results of those tests may be used to represent the larger piece (or pieces that have been physically fit together) as a whole.

4.3.2.2. Known sample: Some items (e.g., automotive paint on a single panel) can be assumed to be homogeneous while other items (e.g., architectural paint) will need to be evaluated to determine the variation within the sample.

4.4. Characterization of paints and coatings

4.4.1. The analytical scheme for characterization will vary depending on the type of material (e.g., clear coat, multilayered paint sample), the circumstances of the case, and the examinations requested by the customer.

4.4.2. The analysis for the characterization of coatings and paint includes:

4.4.2.1. Visual and/or microscopical examination to describe or indicate the physical properties of the sample (e.g., clear coating).

4.4.2.1.1. Some physical characteristics of paint may indicate its end.

Small circular droplets can indicate spray paint.

Brittle chip with specific sequenced layers (e.g., clear, color, dull color) can indicate automotive paint.

Malleable chip with multiple-colored layers can indicate architectural paint.

Yellow or white material with reflective beads can indicate road paint.

4.4.2.2. If any chemical information is reported, instrumental analysis is required (e.g., clear *acrylic* coating).

4.5. Comparison of paints and coatings

4.5.1. The questioned item is evaluated to identify physical features (e.g., color, layer structure) suitable for comparison prior to examination of the known. Any subsequent chemical and elemental analysis of the unknown item shall be conducted prior to the known item.

4.5.2. If at any time during the comparative scheme of analysis an exclusionary difference is observed between the Q and the K samples, no further examinations need to be conducted and the samples can be reported as being dissimilar to one another (Exclusion).

4.5.3. If samples have been subjected to different conditions (e.g., age, weathering, burning) caution should be used when interpreting differences and additional testing may be needed to confirm an exclusion.

4.5.4. There are many techniques that are available for the comparison of paints and coatings. Use a combination of techniques that have the greatest potential for discrimination. Table 1 lists the available techniques for paint and coating comparisons with the shaded boxes representing techniques which are recommended. Depending on the color and size, certain techniques may not be available or may not offer any additional information or discrimination power. For instance, MSP would be utilized on a blue paint layer but not a gray paint layer.

Table 1. Techniques for the comparison of coatings and paint.

Physical Features	Optical Properties	Microchemical Analysis	Color/Dye/Pigment Analysis	Instrumental Analysis
Stereomicroscopy	PLM	Solubility	Comparison Microscopy	FTIR
Light Microscopy/ Comparison Microscopy	Light Microscopy/ Comparison Microscopy		MSP	SEM-EDS/XRF
SEM	Fluorescence Microscopy			PyGC-MS
Melting Point				Raman

4.5.5. Microscopical Examinations

4.5.5.1. The sample is examined under the microscope. The physical properties of each sample are noted.

4.5.5.2. The layer sequence, color, texture, thickness of the layers, pigment morphology (for paint), and any unusual features should be noted. Pigment types can be identified in paint samples.

4.5.5.3. If multiple layers are suspected, a thin peel, cross section, or bevel cut should be prepared to visualize the layers. If a cross section is made, it may be hand sectioned or mounted in an appropriate mounting medium and sectioned using a microtome for subsequent analysis. Comparison of the layers may require a comparison microscope.

4.5.6. Solvent / Microchemical Tests

4.5.6.1. Solvent or microchemical tests can be used for coatings and paint; however, much of this information can be obtained from instrumental analysis. If utilizing instrumentation, solvent or microchemical tests are not typically warranted.

4.5.6.2. Solvents and chemical reagents are prepared and documented according to generally accepted formulas. Chemicals shall be checked on known

samples or in some manner that assures they are working properly, and these checks shall be documented in the technical record.

4.5.6.3. Place the sample on a microscope slide or spot plate.

4.5.6.4. Apply the reagent or solvent and observe as it comes into contact with the sample. Any resulting reaction should be recorded. Note the effect of each reagent on the individual layers for the Q and K sample. Use of a stereomicroscope will aid in the observation of any reaction.

4.5.7. Instrumental Analysis

4.5.7.1. Follow instrument protocols and work instructions for required performance checks and appropriate parameters.

4.5.7.2. When comparing samples, the same analytical techniques and parameters should be used for both the Q and K samples.

4.5.7.3. Analysis using some instrumentation may not be appropriate or possible due to the condition, size and/or type of sample.

4.5.7.4. Generally, when sample size is limited, destructive testing is performed after all non-destructive testing is complete.

5. Quality Assurance and Controls

5.1. Reference collections of known coatings, including paints and pigments, are available, as well as reference data from the instruments. When using a known reference sample for analysis, include the unique reference number in the technical record.

5.2. Quality is assured through the proper training and testing of examiners, the laboratory's technical review process, and the use of appropriate equipment that is maintained and performance checked.

5.3. The techniques described above for coating and paint examinations are well known and scientifically accepted in the forensic community and private industry. Relevant examples of related literature can be found in Section 6 (References).

6. References

6.1. ASTM International Standards

ASTM D16 Standard Terminology for Paint, Related Coatings, Materials, and Applications

ASTM D5380 Standard Test Method for Identification of Crystalline Pigments and Extenders in Paint by X-Ray Diffraction Analysis

ASTM E3295 Standard Guide for Using Micro X-Ray Fluorescence (μ-XRF) in Forensic Polymer Examinations

6.2. Applicable OSAC Registry documents

ASTM E1610 Standard Guide for Forensic Paint Analysis and Comparison

ASTM E2809 Standard Guide for Using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy in Forensic Polymer Examinations

ASTM E2937 Standard Guide for Using Infrared Spectroscopy in Forensic Paint Examinations

ASTM E3234 Standard Practice for a Forensic Paint Analysis Training Program

ASTM E3296 Standard Guide for Using Pyrolysis Gas Chromatography and Pyrolysis Gas Chromatography – Mass Spectrometry in Forensic Polymer Examinations

6.3. Other

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1. Scope

1.1. This document describes the information that is included in trace evidence laboratory reports regarding the interpretation and reporting of the overall conclusion(s) for trace evidence examinations.

1.1.1 This document provides guidance on the interpretation of examinations and example report wording for conclusions based on the most common types of trace examinations but can be applied to other trace materials. This document does not contain all possible examples, case circumstances, or all types of evidence that can be examined.

1.1.2 Reports should use wording similar to that described below, but alterations may be needed for specific cases. Alterations could include using a table, bullet points, or different wording. A report will include the justification for the conclusion reached and, where applicable, any limitations or caveats. If an applicable example is not listed below, one should be used that is most similar to the exams being performed or most appropriate.

2. General

When writing a report, the examiner shall accurately reflect the findings of the examination, providing interpretations where appropriate, and clearly communicate those findings to the reader, including any additional significance or limitations. For comparisons, a qualitative approach to communicate the significance of an association or exclusion is used and based on a) the foundational validity of the scientific methods used for the comparison of the items, b) discrimination capabilities of the analytical protocol, and c) existing knowledge of how discriminating the compared characteristics are based on survey studies, reference collections, industry, or manufacturing knowledge, and/or databases. This approach focuses primarily on fibers, paint, glass, and tape but can be applied to other trace materials. A review article[1] provides a thorough bibliography that also serves as the body of work supporting the approach presented throughout this procedure for the interpretation of trace comparisons.

3. Types of analysis

3.1. Characterization Analysis

The following descriptions are meant to provide context to the conclusions used in the reporting of trace material characterization examinations. Every category of

[1] Trejos T, Koch S, Mehltretter A. Scientific foundations and current state of trace evidence – a review. *Forensic Chemistry*, 18, May 2020.

characterization may not be applicable in every case nor for every material. When no analysis has been completed, wording such as "apparent" is often used.

3.1.1 Categories of characterization

3.1.1.1. **Identification** – The analytical data provides reliable information to specify a particular chemical or product or contains specific characteristics that compare to a known standard or reference collection material which ensures its identification (e.g., acrylic fiber, human hair, OEM paint). Wording such as "identified", "were determined", and "was deemed" is often used in this context.

3.1.1.2. **Classification** – When a known standard or a reference collection material is not available; when a sample is grouped into a category based on shared traits or characteristics; or when the evidence lacks quantity, quality, and/or detail to support an identification of a specific chemical, product, or species. (e.g., European head hair, spray paint, type of fabric damage). Wording such as "consistent with" and "characteristic of" is often used in this context.

3.1.1.3. **Indication** – The analytical data suggests a particular type of material but does not support a classification or identification. (e.g., *indication of acrylic modification* in paint sample with peak at ~ 1160 cm^{-1} on FTIR)

3.1.2 Hair non-comparison examinations:

3.1.2.1 If human hairs are reported, the following storage statement must be included in the report.

The human hair(s) in Exhibit X may contain biological evidence subject to specific storage and preservation requirements. Please reference the current version of ATF O 3400.1 to review the storage and preservation requirements of this evidence for the purposes of possible future DNA analysis.

3.1.2.2 Wording for suitability for microscopical comparisons of hair

When the somatic origin of head, facial, or pubic region has been determined, it can be reported that the hair(s) "are" suitable for microscopical comparison.

If somatic origin has not been determined, it can be reported that the hair(s) "may' be suitable for microscopical comparison.

3.1.2.3 An ancestry/body origin disclaimer with the classification will be added to an Appendix at the end of a report when ancestry and/or body determination is reported.

All ancestral and body area conclusions are based on the microscopic characteristics observed and their consistency with characteristics expected for a particular ancestral group or body area. How an individual identifies their ancestry may not correlate with the determinations made here.
Hairs are typically classified as follows.

Ancestry:
African
European *(including individuals of Middle Eastern descent)*
Asian *(including Native Americans)*
Mixed *(exhibits characteristics of more than one ancestral group)*
Undetermined

Note: Hairs from individuals of Latin American origin may exhibit microscopic characteristics of one or more ancestral groups listed above.

Body Origin:
Head
Pubic
Body *(more specific classification may be made)*
Undetermined

3.2. Comparison Analysis

A trace evidence comparison is conducted to determine whether the compared samples can be discriminated based on their physical and chemical properties. This allows the examiner to evaluate if the samples could or could not have shared a common source. A variety of factors influence the significance of comparative findings, such as the type of material being compared, the discriminating capability of the analytical techniques utilized, or the presence of highly discriminating characteristics. The interpretation scale is used to provide context to this significance.

Some materials may not to be suitable for a meaningful comparison (e.g., white cotton fibers) or the result of the comparison is inconclusive as to whether the samples can be included or excluded as having a common origin due to a limiting factor (e.g., constraints of sample size, condition of the evidence, limitation or results of the test(s)).

3.2.1 Interpretation Scale

The following italicized wording is included as an appendix in the report of comparative exams with the exception of when only an "exclusion" conclusion is reached. In this instance, only the "exclusion" wording is necessary in the report.

The following descriptions are meant to provide context to the opinions reached in this report. Not every type of conclusion may be applicable in every case or for every material type.

Type I Inclusion: Source Identification *– Source Identification is the highest degree of association between items. This association provides the strongest support that the items originated from the same source as opposed to different sources. Source Identification, which includes a physical fit, is reached when the items display physical features that correspond/re-align in a manner that is not expected to be replicated.*

Type II Inclusion: Inclusion with Highly Discriminating Characteristics *– This is the highest degree of association that can be determined in the absence of a Source Identification. This type of association provides strong support that the items originated from the same source as opposed to different sources. The items correspond in all measured physical properties, chemical composition and/or microscopic characteristics and share highly discriminating characteristic(s) that would rarely be expected to occur in the relevant types of materials examined.*

Type III Inclusion: Inclusion with Discriminating Characteristics *– This type of association provides support that the items originated from the same source as opposed to different sources. The items correspond in all measured physical properties, chemical composition and/or microscopic characteristics; however, other items have been manufactured or could occur in nature that would also be indistinguishable from the examined materials.*

Type IV Inclusion: Inclusion with Limitations *– This type of association provides limited support that the items originated from the same source as opposed to different sources. Therefore, the possibility that the items came from the same source cannot be eliminated. As compared to the categories above, this type of association has decreased evidential value due to limiting factors such as the items are more commonly*

encountered, a limited analytical scheme was conducted, or minor variations were observed in the data.

***Inconclusive** – No conclusion could be reached regarding an inclusion or an exclusion between the items.*

***Exclusion with Limitations** – This conclusion provides support that the items originated from different sources as opposed to the same source due to observed differences; however, an Exclusion conclusion was not reached due to limiting factors such as possible natural or manufactured source variations, damage or contamination that cannot be removed or avoided.*

***Exclusion** – The items display differences that support that the two items did not originate from the same source.*

4. Interpretation of evidence
The following are examples of how the interpretation scale is applied to specific types of evidence.

4.1 Fabric damage

Type I Inclusion: Source Identification
Not applicable to fabric damage examinations. See Fibers for fabric comparisons.

Type II Inclusion: Inclusion with highly discriminating characteristics
It is unlikely this association could be used for fabric damage examinations

Type III Inclusion: Inclusion with discriminating characteristics
Damage consistent with an implement that has measurable class characteristics
- Pinking shears or dimensions of hole correspond with knife

Type IV Inclusion: Inclusion with limitations
Lack of distinguishing features in the damage produced by these implements and the inability to distinguish the cuts made from one type of implement from another
- Common scissors

Inconclusive
Damage due to a lack of features recorded or changes to the damage after it was made (melting, unraveling, stretching, etc.)

Exclusion with Limitations
The questioned item exhibits some dissimilarities to the known item but lacks sufficient quality or detail for an absolute exclusion to be made

Exclusion
Damage is different from that produced with an implement. For instance, damage on the shirt is consistent with coming from a knife and a comparison implement is a scissor

The mechanism which caused the damage could also be different (e.g., a cut vs a tear)

4.2. Fabric impressions

Type I Inclusion: Source Identification

Items share a combination of class characteristics and randomly acquired characteristics that demonstrates the questioned impression(s) were made by the known item

Type II Inclusion: Inclusion with highly discriminating characteristics

Some distinctive characteristics

- Officer's badge, a ragged hole, a patch

Type III Inclusion: Inclusion with discriminating characteristics

Items correspond in all class characteristics.

Common construction (twill pattern) with seams, unusual construction patterns, airbag singe patterns designating driver or passenger side airbag

Type IV Inclusion: Inclusion with limitations

Limiting factors in the impression or photograph

- Scale is not present or 1:1

Common construction (twill pattern in blue jeans)

Inconclusive

Interference from substrate
Limited sample size
Lack of characteristics

Exclusion with Limitations

The questioned impression exhibits some dissimilarities to the known exhibit but lacks sufficient quality or detail.

Exclusion

Differences in pattern or spacing

4.3. Fibers

Type I Inclusion: Source Identification

Not applicable to single fibers, only applicable to fabric. The items exhibit physical features that demonstrate they were once part of the same object.

Type II Inclusion: Inclusion with highly discriminating characteristics

Questioned and known fibers have corresponding surface contamination or post-manufacturing marks such as staining or corresponding damage

Conditions that limit the possible sources of the fibers (e.g., fibers found in a vehicle with a limited number of passengers wearing known garments and only correspond to one garment)

Type III Inclusion: Inclusion with discriminating characteristics

Samples correspond in all chemical and physical characteristics with typical analytical scheme
- Questioned colored nylon fiber corresponding to a known carpet

Type IV Inclusion: Inclusion with limitations

Commonly observed fiber/fabric type (white cotton, blue denim cotton)
Limited characteristics to differentiate among fibers (colorless polyester or undyed natural fiber such as linen)
Limited amount of sample to adequately assess heterogeneity of the source
Minor explainable or demonstrable variation due to established causes such as damage (e.g., impact), alteration (e.g., heat or chemical exposure), or known contamination (e.g., biological fluids)
Circumstances where a narrow range of fiber type and color are likely to be encountered (e.g., same type of uniform worn by multiple people), which could lead to a random association
Reduced analytical scheme, damaged fibers, or limited sample size

Inconclusive

Some difference which may be due to questioned fiber being too damaged for full analysis, or
The questioned fiber possesses similar characteristics to the known sample but also exhibits some differences such as:
- Post-depositional changes
- Known sample not being truly representative

Exclusion with Limitations

Change or damage that could be from exposure to heat, chemicals or environmental effects
Limited amount of known sample of a suspected source that is highly variable (e.g., trunk liner)
Manufacturing variation/irregularities

Exclusion

Different in microscopical, physical, and/or chemical properties

4.4. Glass

Type I Inclusion: Source Identification

The items exhibit physical features that demonstrate they were once part of the same object

Type II Inclusion: Inclusion with highly discriminating characteristics

Samples correspond in all chemical and physical characteristics and include characteristics atypical of glass
- Association of glass fragments characterized by refractive index (RI) and elemental analysis using micro-xray fluorescence (μXRF) when Sr, Zr, (at

Signal to Noise Ratio (SNR) >10) or an element that is less commonly or rarely detected in glass by XRF (as determined by E2926) is used in element intensity ratio comparisons

Type III Inclusion: Inclusion with discriminating characteristics

Samples correspond in all chemical and physical characteristics with typical analytical scheme

- Association of glass fragments characterized by RI and elemental analysis using μXRF when elements equal to or greater than 37 (Rb) are below the limit of quantitation

Type IV Inclusion: Inclusion with limitations

Samples correspond in all chemical and physical characteristics, but there is a limitation

- Reduced analytical scheme (e.g., only RI or RI and elemental analysis by SEM-EDS)
- Limited sample or sample condition that prevents adequate characterization.

Inconclusive

Samples exhibit both similarities and differences such that no meaningful conclusion can be reached

The questioned glass is insufficient to do most examinations (e.g., physical/optical examinations can identify the sample as glass, but the sample is too small for other comparison methods).

Exclusion with Limitations

- Not applicable to glass

Exclusion

Physical, chemical, or optical exclusionary differences between the compared glasses.

4.5. Paint

Type I Inclusion: Source Identification

The items exhibit physical features that demonstrate they were once part of the same object

Type II Inclusion: Inclusion with highly discriminating characteristics

Samples correspond in all chemical and physical characteristics and include characteristics atypical of most paint systems

- Multiple layer automotive paint with OEM and non-OEM layers
  - § OEM system with at least one aftermarket basecoat or primer layer above the original clear coat
  - § OEM system with 2 or more factory repairs (i.e., three or more total basecoat/clearcoat sequences)
  - § Automotive paint systems with architectural paint present
- Architectural paint with two or more different color layers

Type III Inclusion: Inclusion with discriminating characteristics

Samples correspond in all chemical and physical characteristics with typical analytical scheme

- Multiple layered OEM paints
- Non-automotive single-layered colored paint where there is knowledge of substantial discrimination power (e.g., red architectural paint) or product distribution information that reduces the potential sources
- Two or more white architectural layers of different chemistries

Type IV Inclusion: Inclusion with limitations

Samples correspond in all chemical and physical characteristics, but there is a limitation

- Lacking necessary analysis in typical analytical scheme
- Smears
- Single-layer automotive paint (e.g., clear coat or unremarkable base coat such as a white color acrylic melamine binder system with primarily titanium dioxide as an extender pigment)
- Single-layer paint having limited discrimination studies or product manufacturing distribution information

Inconclusive

Samples exhibit both similarities and differences such that no meaningful conclusion can be reached

- Sample with known contamination has a dissimilar elemental profile
- Questioned sample of spray paint can have differing pigment to binder ratios that cannot be reproduced from the small amount of paint left in the known spray can due to mixing differences
- Clear coat cross-transfer in which both known vehicles have indistinguishable clear coat chemistries

Exclusion with Limitations

Limited application to paint comparisons

- One sample of paint in a comparison has an extra layer (e.g., anti-chip layer). No analytical differences were observed in the corresponding layers. The vehicle is no longer available to collect an additional known sample

Exclusion

Compared paints are different in physical and/or chemical properties

4.6. Tape

Type I Inclusion: Source Identification

The items exhibit physical features that demonstrate they were once part of the same object

Type II Inclusion: Inclusion with highly discriminating characteristics

Unusual or distinctive features (e.g., not enough for a physical fit but has a defect or two) and corresponds in all other physical and chemical characteristics
Post-manufacturing marks such as damage, writing, or paint overspray

Type III Inclusion: Inclusion with discriminating characteristics

Samples correspond in all chemical and physical characteristics with typical analytical scheme

Type IV Inclusion: Inclusion with limitations

Limited analytical scheme due to sample size
Backing or adhesive only
Minor explainable or demonstrable variation in one of the comparison samples due to established causes such as:

- Sample heterogeneity of the scrim
- Known contamination of the sample(s)
- Having a sample of insufficient size to adequately assess the homogeneity of the entity from which it was derived

Inconclusive

No conclusion can be reached between the questioned and known samples
The question item is too damaged, degraded, or contaminated to conduct most examinations.

Exclusion with Limitations

A conclusion not commonly used for tape exams

Exclusion

Compared tapes are different in physical and/or chemical properties

4.7 Other

Type I Inclusion: Source Identification

The items exhibit physical features that demonstrate they were once part of the same object

Type II Inclusion: Inclusion with highly discriminating characteristics

Samples correspond in all examined chemical and physical characteristics and have additional unusual features not expected for that type of material

- Example: Adhesive with similar extraneous material attached to it.

Two items physically fit together, but potentially lack enough detail or quality to state that no other item could also fit.

- Example: A match from a matchbook with no surrounding matches and indistinct tear that contains one single colored fiber traversing the tear.

Items that are too damaged to physically fit back together, but their breaks align.

Type III Inclusion: Inclusion with discriminating characteristics

Samples correspond in all examined chemical and physical characteristics
Match sticks or glitter

- Samples correspond in physical size, microscopic characteristics, and chemical composition (FTIR, elemental)

Coating

- A coating that has more than one layer

Type IV Inclusion: Inclusion with limitations

Samples correspond in all chemical and physical characteristics, but there is a limitation such as:

- Lack of features to compare
- Lack of discrimination studies or manufacturing knowledge
- Common material (e.g., hot melt glue, PVC pipe)

Only physical measurements taken (limited analytical scheme)

Single clear coating

Matchstick with only a portion remaining (e.g., head is not present for analysis)

Inconclusive

Samples exhibit similarities and differences such that no conclusion can be reached

Too little sample is available for comparison (known or questioned)

Exclusion with Limitations

Dissimilarities between known and questioned, but insufficient for a definitive exclusion due to limiting factors such as:

- Apparent contamination that cannot be removed or avoided in analysis
- Indications of change or damage that could be from exposure to heat

Exclusion

Different in physical, optical, and/or chemical properties

5. Test Methods and Methods of Analysis

5.1 The following techniques will be listed in reports, if used during the examination process:

Fourier transform infrared spectroscopy (FTIR)
Glass refractive index measurement (GRIM)
Pyrolysis gas chromatography-mass spectrometry (PGC-MS)
Microscopical examination
Microspectrophotometry (MSP)
Physical and chemical examinations
Raman spectroscopy
Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS)
Spot tests
Visual examination
X-ray diffraction (XRD)
X-ray fluorescence spectroscopy (XRF)

6. Activity level considerations

Activity level is an aspect of comparative examinations that considers factors such as transfer mechanisms and persistence. Factors include the presence of the evidence in a particular location, quantity and/or condition. Activity level can affect the significance of an association and may warrant additional statements within the report.

6.1. Activity level factors can include but are not limited to:

Large number of items (e.g., fibers) transferred
Location of evidence (e.g., underneath victim's fingernails)
Multiple associations
Cross transfer
Conditions that limit the possible source (e.g., numerous fibers embedded in an interior surface of a vehicle with a known number of passengers)
Reasonable explanation for a transfer of fibers (e.g., two individuals living together)
Condition of evidence that indicates an activity (e.g., spray paint droplets indicate the paint was applied wet to the surface, stretched hair root may indicate it was forcibly removed)
Evidence embedded rather than loosely adhered

6.2. Examples of activity level statements can be added to the report:

Number of pieces:
The large number of fibers recovered from Exhibit 1 indicates direct contact occurred with a textile.

The large number of glass fragments recovered from Exhibit 1 indicates a recent exposure to broken glass.

Physical characteristics:
The fibers embedded in the damaged area of the suspect vehicle indicate that the vehicle has been in forceful contact with a fabric-clad item.

Considering the presence of damaged fibers on the suspect fender consistent with the victim's jacket and pants, there is an indication of contact between the victim and the suspect vehicle.

Although the fibers were blue denim cotton, which are commonly found in the environment, finding blue denim cotton fibers embedded in the damaged area of the vehicle lends more significance to their evidentiary value.

A clump of hairs, similar in color, found in the victim's hand all contained stretched roots and adhering tissue/root sheath which are indicative of the hairs having been forcibly removed.

The physical characteristics of the paint on Exhibit 1 establishes that the paint was sprayed on when applied.

It should be noted that glass fragments can only originate from broken objects and not intact ones.

Multiple or cross transfers:
Based on the results, four (4) distinct associations are reported. These findings provide stronger support for a common source than a single association alone and therefore, the overall significance of the reported results is increased due to the multiple associations.

Numerous fibers were found on the victim's shirt that could not be distinguished from the fibers comprising the known shirt from the subject. Numerous fibers were found on the subject's shirt that could not be distinguished from the fibers comprising the known shirt from the victim. This cross transfer provides stronger support for contact having occurred between the two shirts than either transfer alone and reduces the chance that the fibers were all deposited by coincidence.

7. Report wording examples
The following examples are meant to give guidance with regards to report wording; however, exact wording should be left to the discretion of the examiner.

7.1. Type I Inclusion: Source Identification

Fiber (Fabric) wording examples:
*The torn edge of the questioned fabric piece physically fits and aligns with the torn edges of the known shirt fabric. This provides the strongest support that the fabric piece originated from and was at one time part of the shirt, as opposed to it originating from another torn fabric source (**Type I Inclusion**).*

*The Exhibit 1 and Exhibit 2 pieces of fabric corresponded in edge contour including yarn length characteristics that corresponded across the tear. This demonstrates that these two pieces of fabric were once part of a single unit (**Type I Inclusion**).*

Fabric impression wording example:
The fabric impression from Exhibit 2 corresponded in construction, weave, and the presence of multiple holes to the known fabric from the Exhibit 1 jeans. Therefore, the

*Exhibit 1 jeans are the source of the fabric impression in Exhibit 2 (**Type I Inclusion**). This conclusion was reached because these distinct characteristics would not be expected to be repeated in another source.*

Glass wording example:
*A physical/fracture fit was observed based on corresponding random characteristics on the broken edges of the Exhibit 1 piece of glass and the broken edges of Exhibit 2, the known source. Therefore, this correspondence demonstrates that the two pieces of glass were once part of the same glass object (**Type I Inclusion**).*

Paint wording example:
*Examination and comparison of Exhibits 1 and 2 revealed corresponding fracture contours, surface configurations, and layer structures of the two paint chips. This demonstrates that the items originated from the same damaged source (**Type I Inclusion**).*

Tape wording examples:
*Unknown duct tape fragments from the scene (Exhibits 1, 8 and 9) were visually and microscopically compared to the end of the roll of grey duct tape in Exhibit 4 and to each other. Multiple physical fits were made between the pieces of tape in Exhibits 1, 8 and 9 as well as a physical fit was made between one piece of duct tape in Exhibit 8 to the end of the roll of tape in Exhibit 4. Therefore, all of these pieces of tape including the roll of tape were at one time a single unit (**Type I Inclusion**).*

*Based on distinct features of the torn edge on one end of the Exhibit 1 piece of tape and the end of the roll in Exhibit 2, Exhibit 1 was observed to physically correspond with the end of Exhibit 2. This provides the strongest support that Exhibit 1 originated from and was at one time a part of Exhibit 2 as opposed to it originating from another used roll (**Type I Inclusion**).*

Other wording example:
*The Exhibit 1 and Exhibit 2 plastic fragments corresponded in edge contour and had surface scratches that corresponded across the break. This demonstrates that these two pieces of plastic were once part of a single unit (**Type I Inclusion**). This conclusion was reached because these alignments fit together in a manner that is not expected to be repeated in another source.*

7.2. Type II Inclusion: Inclusion with highly discriminating characteristics

Fiber wording example:
The Exhibit 1 fibers were compared to the known fibers in Exhibit 2 and found to correspond in color and type (insert color and type here), microscopic characteristics, cross-section (insert shape), fluorescence, chemical composition. In addition, both

*Exhibits 1 and 2 corresponded in distinctive characteristics (list distinctive characteristics, e.g., damage indicative of singeing). Therefore, the Exhibit 1 fibers came from Exhibit 2 or another source having the same highly discriminating characteristics (**Type II Inclusion**). This type of association was reached because both Exhibit 1 and Exhibit 2 display characteristics atypical of the relevant population of this evidence type. (Further description of the highly discriminating characteristics present are added here).*

Fabric impression wording example:
*The impression from Exhibit 1 corresponded in construction and weave to the known shirt in Exhibit 2. In addition, there was an outline of a badge and some distinctive characteristics found in both the Exhibit 1 impression and the known shirt/badge in Exhibit 2. Therefore, the Exhibit 1 impression could have been made by the Exhibit 2 known shirt or another source having the same highly discriminating characteristics (**Type II Inclusion**).*

Glass wording example:
*The Exhibit 1 known glass fragments and the Exhibit 2 questioned glass fragment are clear, colorless glass that show characteristics of tempered glass. Comparison of Exhibits 1 and 2 by visual and microscopical techniques, refractive index, and elemental analysis by μ-X-ray fluorescence determined that they could not be differentiated based on their physical characteristics, their optical characteristics, or their elemental composition. These combined methods have shown to be highly discriminating between glass sources. Therefore, the questioned glass originated from the window as represented by the known sample or another source of broken glass indistinguishable in the measured properties (**Type II Inclusion**). This type of association was reached because coincidental associations of glass originating from different sources could occur but are expected to be highly unusual, specifically with the elemental composition in both samples. It should be noted that glass fragments can only originate from broken objects and not intact ones.*

Paint wording example:
*Exhibit 1 was examined microscopically and contained a nine-layered paint chip that corresponded in color and layer structure (list layers), chemical composition, and elemental composition to the known paint in Exhibit 2. Therefore, the Exhibit 1 paint came from the same source as the Exhibit 2 known paint sample or another source having the same highly discriminating characteristics (**Type II Inclusion**). This type of association was reached because both Exhibit 1 and Exhibit 2 display characteristics atypical of original equipment manufacturer (OEM) paints [or atypical of the relevant population of this evidence type]. (Further description of the highly discriminating characteristics present are added here)*

Tape wording example:
*The tape in Exhibit 1 corresponded in construction, general appearance (color [list color], size, etc.) chemical composition, and elemental composition to the known tape roll in Exhibit 2. [Further description of the highly discriminating characteristics present shall be added.] Therefore, the Exhibit 1 tape could have come from the same source as the Exhibit 2 known tape roll or another source having the same highly discriminating characteristics (**Type II Inclusion**).*

Other wording examples:
*The match from Exhibit 1 corresponded in general physical characteristics and the break edges align [has highly discriminating characteristics] with the matchbook in Exhibit 2. This indicates that Exhibit 1 and Exhibit 2 were likely part of a single unit, however, due to [damage of the evidence, lacking detail, or other limitation] an identification is not possible (**Type II Inclusion**).*

7.3. Type III Inclusion: Inclusion with discriminating characteristics

Fiber wording example*:*
*The Exhibit 1 fibers were compared to the known fibers in Exhibit 2 and found to correspond in color and type (insert color and type here), and microscopic characteristics. In addition, one fiber from within Exhibit 1 and Exhibit 2 were further analyzed and found to be similar in cross-section (insert shape) and chemical composition. Therefore, Exhibit 1 could have come from Exhibit 2 or another source with the same characteristics (**Type III Inclusion**). This type of conclusion was reached because other textiles containing fibers made to the same specifications (type, color, microscopic characteristics, etc.) would also be indistinguishable from these fibers). The techniques utilized in this comparative analysis can readily distinguish different fibers.*

Fabric damage wording example:
*The damage in the Exhibit 1 shirt was examined under the stereomicroscope and determined to be cut. Sample cuts, made by the Exhibit 2 pinking shears, were consistent in physical appearance (angles, lengths of angle segments, etc.) with the damage found in Exhibit 1. Therefore, the damage in Exhibit 1 could have been made by the Exhibit 2 pinking shears or another source with the same characteristics (**Type III Inclusion**). This conclusion was reached because other pinking shears with the same measurements could leave indistinguishable characteristics.*

Fabric impression wording example:
*The Exhibit 1 impression corresponded in construction and weave pattern as well as seam lines to the known pair of pants in Exhibit 2. Therefore, the known pants in Exhibit 2 could be the source of the impression in Exhibit 1 (**Type III Inclusion**). This type of conclusion*

was reached because other items (can list item type) may have been manufactured to the same specifications that would be indistinguishable from the submitted evidence.

Glass wording example:
*The glass fragments received as Exhibit 1 (known) and the glass fragment received as Exhibit 2 (questioned) are clear, colorless glass that show characteristics of flat glass. Comparison of Exhibits 1 and 2 by visual and microscopical techniques, refractive index and elemental composition by μ-X-ray fluorescence determined that they could not be differentiated based on their physical and optical characteristics and their elemental composition. These combined methods have shown to be discriminating between glass sources. Therefore, the questioned glass originated from the windshield (Exhibit 1) submitted as a known sample or another source of broken glass indistinguishable in the measured properties (**Type III Inclusion**). This type of association was reached because the techniques utilized in this comparative analysis can typically distinguish most glass products. It should be noted that glass fragments can only originate from broken objects and not intact ones.*

Paint wording example:
*The paint chip in Exhibit 1 was examined microscopically and corresponded in color and layer structure (list layers), chemical composition, and elemental composition to the known paint in Exhibit 2. Therefore, the Exhibit 1 paint could have come from the same source as Exhibit 2 or another source with the same characteristics (**Type III Inclusion**). This type of conclusion was reached because paints are mass-produced, and other paints manufactured to the same specifications as Exhibit 2 would also be indistinguishable from this paint. The techniques utilized in this comparative analysis can typically distinguish most paint products.*

Tape wording example:
*The piece of tape in Exhibit 1 corresponded in construction, general appearance (color (list color), size, etc.) chemical composition, and elemental composition to the known tape roll in Exhibit 2. Therefore, Exhibit 1 could have come from the same source as Exhibit 2 or another source with the same characteristics (**Type III Inclusion**). This type of conclusion was reached because other rolls of tape produced at the same manufacturing plant and with the same specifications would also be indistinguishable. Due to differences between tape products, the analytical techniques used in the analysis of these items allow for a high degree of discrimination.*

Tape wording example:
Ten pieces of white duct tape found at the scene (Exhibits 5, 6, 7 and 8) were compared to known white duct tape from Exhibit 32 (suspect's residence). No physical fits were made between any of the unknown white tape and the known roll of white tape. The unknown pieces of white duct tape in Exhibits 5, 6, 7 and 8 were similar to the known white duct

*tape in Exhibit 32 in construction and physical characteristics (color of backing, color of adhesive, scrim weave and width when available). In addition, one piece within Exhibit 6 and the known tape in Exhibit 32 were further analyzed. These tapes were also found to be similar in microscopic characteristics of adhesive and scrim fibers, scrim construction, chemical composition and elemental composition. Therefore, the unknown white duct tape analyzed from the scene (Exhibit 6) could have come from the known white duct tape in Exhibit 32 or another source having the same characteristics (**Type III Inclusion**). This type of conclusion was reached because other rolls of tape produced at the same manufacturing plant and with the same specifications would also be indistinguishable. Due to differences between tape products, the analytical techniques used in the analysis of these items allow for a high degree of discrimination.*

Other wording example:
*The matchsticks in Exhibits 1 and 2 from the scene were similar in color of shaft, size (length and width) and visual color of residue/match head material when visible. In addition, two matchsticks from Exhibit 1 and four matchsticks from Exhibit 2 were further analyzed and also found to be similar in microscopic characteristics of the wooden shaft, chemical composition, and elemental composition of the green residue/match head material. Therefore, the matchsticks in Exhibits 1 and 2 could have a common source however, other matchsticks may have been manufactured to the same specifications that would be indistinguishable from the submitted evidence (**Type III Inclusion**).*

7.4. Type IV Inclusion: Inclusion with limitations

Fiber wording example:
*The Exhibit 1 fibers were compared to the known fibers in Exhibit 2 and found to correspond in color and type (insert color and type) and chemical composition. Therefore, Exhibit 1 could have come from the same source as Exhibit 2 or another textile with the same characteristics (**Type IV Inclusion**). This conclusion is limited because this type of fiber [or list fiber type e.g., blue cotton] is ubiquitous and may have limited forensic value.*

Fiber wording example:
*The Exhibit 1 fibers were compared to the known fibers in Exhibit 2 and found to correspond in (insert characteristics here). Therefore, Exhibit 1 could have come from the same source as Exhibit 2, however this association is limited due to [the sample size/the condition of the sample/being commonly encountered/a lack of characteristics available for comparison] (**Type IV Inclusion**). Other items may have fibers manufactured to the same specifications that would be indistinguishable from the submitted evidence.*

Fabric damage wording example:
Exhibits 1 and 2 were examined with a stereomicroscope for fabric damage. Fabric damage was observed in Exhibits 1 and 2, which was determined to be consistent with

*being cut. In addition, the fabric damage exhibited characteristics that indicated the damage was caused by scissors. The Exhibit 3 scissors could be a possible source of this damage (**Type IV Inclusion**). This conclusion is limited because most straightedge scissors would be included as a possible source of the damage noted.*

Fabric impression wording example:
*The known shirt in Exhibit 2 corresponded in construction and weave pattern to the partial fabric impression in Exhibit 1. Therefore Exhibit 2 could be the source of the Exhibit 1 impression, however this association is limited due to the commonality of the fabric construction [or other limitation] in the Exhibit 1 impression (**Type IV Inclusion**). This type of conclusion was reached because other items (can list item type) may have been manufactured to the same specifications that would be indistinguishable from the submitted evidence.*

Glass wording example:
*The glass fragment received as Exhibit 1 (questioned glass) and the glass fragments received as Exhibit 2 (known) are indistinguishable by refractive index and elemental analysis with SEM-EDS. Therefore, the questioned glass originated from the known window or another source of glass with the same refractive index and elemental composition (**Type IV Inclusion**). This type of association was reached due to the limited number of characteristics available for comparison between the known and questioned sample. In glass specimens where only refractive index and SEM-EDS data can be measured, the chance of finding coincidental associations is significantly greater. SEM-EDS is limited to the detection of major and minor elements but not suitable for detection of trace elements. More discriminating techniques could not be applied due to the limited size of the questioned sample.*

Paint wording example:
*The paint chip in Exhibit 1 was examined microscopically and corresponded (insert characteristics here) to the known paint in Exhibit 2. Therefore, Exhibit 1 could have come from the same source as Exhibit 2, however this association is limited due to [the sample size/the condition of the sample/being commonly encountered/a lack of characteristics available for comparison] (**Type IV Inclusion**). Other items [cars/tools/etc.] may have paint [paint systems] manufactured to the same specifications that would be indistinguishable from the submitted evidence.*

Tape wording example:
The tape from Exhibit 2 corresponded in (insert characteristics here) to the known tape roll from Exhibit 1. Therefore, Exhibits 1 and 2 could have come from a common source; however, this association is limited due to [the sample size/ the condition of the sample/being commonly encountered/a lack of characteristics available for comparison]

*(**Type IV Inclusion**). (Optional) Other items [tape] may have been manufactured to the same specifications that would be indistinguishable from the submitted evidence.*

Other wording examples:
*The matchstick in Exhibit 1 was compared to the matchsticks in Exhibits 2 and 3. The matchsticks were similar in shaft color, physical dimensions and microscopic characteristics. Therefore, the matchsticks in Exhibits 1, 2 and 3 cannot be eliminated as having a common source (**Type IV Inclusion**). This examination was limited due to the missing heads of the matches which can provide additional discrimination.*

*The Exhibit 1 lacquer recovered from the pipe was compared to the known lacquer sample from the suspect's garage (Exhibit 2). The samples were consistent in color (clear), chemical composition and elemental composition. Therefore, Exhibit 2 cannot be eliminated as a possible source of the questioned lacquer (**Type IV Inclusion**). This type of association was reached due to the limited characteristics available for comparison in clear lacquer. Other items may contain lacquer manufactured to the same specifications that would be indistinguishable from the submitted evidence.*

7.5. Inconclusive

Fiber wording example:
*The Exhibit 1 blue nylon fibers were compared to the blue nylon fibers from the Exhibit 2 known carpet and found to correspond in microscopic characteristics. However, slight differences were noted in the color between the Exhibits 1 and 2 fibers. Because of the questioned fibers' exposure to the elements, no conclusion can be reached regarding an association or exclusion between the questioned and known fibers (**Inconclusive**).*

Fabric damage wording example:
*The Exhibit 1 shirt contained damage that was determined to be consistent with a cut. In addition, there was additional damage of stretching. The Exhibit 2 knife was evaluated to determine if it could be the source of the damage on Exhibit 1. Due to the additional damage on the shirt, no conclusion can be reached regarding whether or not the Exhibit 2 knife could have made the cut in the Exhibit 1 shirt. (**Inconclusive**).*

Fabric impression wording example:
*The photographs on the Exhibit 1 CD contained images of a fabric impression, however the substrate that the impression was on interfered with the pattern. Therefore, no conclusion can be reached as to the source of the questioned impression (**Inconclusive**).*

Glass wording example:
Although there are some similarities between the Exhibit 1 questioned glass and the Exhibit 2 container, the fragment size of Exhibit 1 does not allow for the complete

*comparison of optical or chemical properties. Therefore, no opinion can be reached regarding an association or exclusion between items (**Inconclusive**).*

Paint wording example:
*The paint in Exhibit 1 was examined microscopically and corresponded in color and layer structure (list layers) and chemical composition to the Exhibit 2 known sample. However, Exhibits 1 and 2 differed slightly in elemental composition which could be due to the heterogeneity of paint or because the samples are from different sources. Therefore, no conclusion can be reached regarding the inclusion or elimination of these paint samples (**Inconclusive**). [Include any relevant limitations to this examination that may have contributed to the inconclusive conclusion]*

Tape wording example:
*The tape from Exhibit 1 corresponded in general appearance and chemical composition to the known tape in Exhibit 2; however, the elemental composition of the adhesive was slightly different from the known due to possible contamination. Therefore, no conclusion can be reached regarding the association of these tape samples (**Inconclusive**). [Include any relevant limitations to this examination that may have contributed to the inconclusive conclusion]*

7.6. Exclusion with Limitations

Fiber wording example:
*Questioned fibers were compared to fibers composing the samples from the known trunk liner using (insert methods here). The questioned fibers were different in (insert characteristics here) to the fiber samples from the known trunk liner, indicating the items did not originate from the same source; however, possible reasons for this difference include that the source is highly variable, and the fiber samples provided may not be representative. Therefore, this difference is insufficient for an exclusion conclusion (**Exclusion with Limitations**).*

Fabric damage wording example:
*The holes in Exhibit 1 were examined with a stereomicroscope and determined to be consistent with a cut. The holes were all less than 1 centimeter in length. The knives in Exhibits 2 and 3 were compared to the damage in Exhibit 1. The single edge blade in Exhibit 2 could be a source of the holes in Exhibit 1 (**Type III Inclusion**). The single edge blade in Exhibit 3 was wider and all of the sample cuts produced larger holes. However, due to the possibility only the tip of Exhibit 3 made the cuts, an exclusion conclusion could not be made (**Exclusion with Limitations**).*

Fabric impressions wording example:
*Exhibit 1 has a similar pattern to the questioned impression from Exhibit 2, but there were minor differences [list differences] in the pattern and therefore, Exhibit 1 may not be the source (**Exclusion with Limitations**).*

Fabric impressions wording example:
*The pattern seen in the questioned impression shows some similarity to the known item, however, test impressions made with the known could not replicate the exact pattern. Therefore, it is unlikely that the known pants made the questioned impression (**Exclusion with Limitations**).*

7.7. Exclusion

Fiber wording example:
*Fibers from Exhibit 1 were compared to the known fibers in Exhibit 2. These fibers were different in microscopic characteristics [insert characteristics that differ]. Therefore, the Exhibit 1 fibers did not come from Exhibit 2 (**Exclusion**).*

Fiber wording example:
*The fibers from Exhibits 1 and 2 were compared and although visibly similar in color (list color), their fiber types were different. Exhibit 1 was determined to be polyester, and Exhibit 2 was determined to be polypropylene. Therefore, the Exhibit 1 fibers did not come from Exhibit 2 (**Exclusion**).*

Fabric damage wording example:
*The damage in the Exhibit 1 shirt was examined under a stereomicroscope and determined to be cut. Sample cuts made by the Exhibit 2 scissors produce damage different from that found in Exhibit 2. Therefore, the damage in Exhibit 1 was not made by Exhibit 2 (**Exclusion**).*

Fabric impression wording example:
*The pattern seen in the Exhibit 1 questioned impression was different from the known fabric from Exhibit 2 and therefore, can be excluded as being a possible source for the Exhibit 1 impression (**Exclusion**).*

Glass wording example:
*The glass fragment in Exhibit 1 differs in physical and optical properties from the Exhibit 2 windshield; therefore, the known glass source represented as Exhibit 2 is not the source of Exhibit 1 (**Exclusion).***

Paint wording example:
*The paint chip in Exhibit 1 displayed a different color and layer structure than the known paint in Exhibit 2. Therefore, the Exhibit 1 paint did not come from the known sample in Exhibit 2 (**Exclusion**).*

Paint wording example:
*The paint chips in Exhibit 1, though visibly similar in color and layer structure, are different in chemical composition from the known paint in Exhibit 2. Therefore, the paint in Exhibit 1 did not come from the same source as the Exhibit 2 known paint (**Exclusion**). Different panels on the same vehicle may have different paint systems. Further comparisons can be performed if additional known samples are submitted.*

Tape wording example:
*The tape in Exhibit 1 displayed a different color and chemical composition than the known tape roll in Exhibit 2. Therefore, the Exhibit 1 tape did not come from the known tape in Exhibit 2 (**Exclusion**).*

Other wording example:
*The piece of plastic from Exhibit 1 was a different color than the Exhibit 2 bumper. Therefore, Exhibit 2 can be eliminated as the source of Exhibit 1 (**Exclusion**).*

8. References

ASTM International. *ASTM E620 Standard Practice for Reporting Opinions of Technical Experts*. ASTM International, West Conshohocken, Pennsylvania. Available: http://www.astm.org/Standards/E620.htm.

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Organization of Scientific Area Committees for Forensic Science (OSAC) Materials Subcommittee Draft Standard Practice for Interpretation and Report Writing in Forensic Comparison of Trace Materials, May 2020 [Online]. Available at:

https://www.nist.gov/system/files/documents/2020/04/02/ChSAC-Mat_Interpretation_Document_March2020_0.pdf

Department of Justice policy on Uniform Language for Testimony and Reports available at www.justice.gov

Abbreviations	**Description**
approx	Approximately
ATR	Attenuated total reflectance
B	Birefringence
BPB	brown paper bag
BPT	brown paper tape
BT	brown tape
C	contrast
cont	contained, contains, container
CTS	collaborative testing services
EDS	energy dispersive x-ray spectroscopy
Env, ENV	envelope
Ex, Exh	Exhibit
ER	Exam Request, Evidence Room
ET	evidence tape
Frag(s)	fragment(s)
FTIR	Fourier Transform Infrared Spectroscopy
GET	green evidence tape
GT	green tape
HS	heat sealed
I&D	initialed and dated
in	inches
IR	infrared
IU	initials unidentified
K	known
lg	large
ME	manila envelope
med	medium
min	minutes
mDNA, mtDNA	Mitochondrial DNA
MS	mass spectrometer
MSP, Microspec	Microspectrophotometer
µscope	Microscope
n/a	non-applicable
$n_{\parallel}$	refractive index - parallel
$n_{\perp}$	refractive index - perpendicular
nDNA,nucDNA	Nuclear DNA
neg, (-)	negative
NIR	near infrared
nm	nanometer
NSR	no significant reaction
PB	plastic bag, post blast

Abbreviations	**Description**
PC	paint chip
PLM	polarized light microscopy
pos, (+)	positive
poss	possible
prep	prepare
PGC, PyGC	pyrolysis gas chromatography
Q	questioned
QC	quality check, quality control
R	retardation, radial section
RET	red evidence tape
rep(s)	repetition
RB	red-brown
RI	refractive index
RL	reflected light
S	sealed
SBT	sealed blue tape
SD	standard deviation
SEM	scanning electron microscope
SGT	sealed green tape
sm	small
SOE	sign of elongation
SRT	sealed red tape
std	standard
temp	temperature
T	tangential section
TL	transmitted light
unk	unknown
UV	ultra-violet
w/	with
w/o	without
WET	white evidence tape
X	cross section
XRD	X-ray diffraction
XRF	X-ray fluorescence spectroscopy
YET	yellow evidence tape
ZLB	zip lock bag
(“abc”)	initialed with legible letters

Additional symbols on following page

Symbol	Description
λ	wavelength
~	about, approximately
=	equal to, consistent with
≃, ≃	similar to, approximately equal to, consistent with
⊥	perpendicular
‖	parallel
>>	Much greater than
>	Greater than
≥	Greater than, but nearly equal
≤	Less than, but nearly equal
<	Less than
<<	Much less than
∴	therefore

1. Scope

1.1. Many different types of crimes may involve polymeric evidence, such as polyvinyl chloride (PVC) pipe, plastic bags, or cable ties. In these cases, the examiner is commonly asked to compare questioned and known (Q and K) polymers based on their physical properties and chemical compositions. In conducting those comparisons, the examiner's goal is to assess the significance of any differences observed. The absence of exclusionary differences between the Q and K samples suggests that the polymers could have had a common source. Besides a Q and K comparison, the examiner may analyze a questioned polymer for descriptive purposes or to attempt to determine its end use for investigative leads.

1.2. Polymers are substances that have a molecular structure consisting of a large number of similar units bonded together. Examples of polymers include coatings, plastics, paint, tape, and fibers. Since *adhesives*, *coatings*, *fibers*, *paint*, and *tape* are considered specific subdisciplines of Trace Evidence, their analyses are covered in separate documents.

1.3. The properties of the questioned and/or known samples may include physical (e.g., color, layer structure, surface features, fluorescence), microscopical (e.g., layer structure) and chemical properties. Chemical composition may be determined and compared by a variety of techniques, including micro-solubility/micro-chemical tests, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy – energy dispersive spectroscopy (SEM-EDS), X-ray diffraction (XRD), X-ray fluorescence (XRF) and pyrolysis gas chromatography – mass spectrometry (PyGC-MS).

2. Instrumentation/Reagents

2.1. Scraping utensils

2.2. Tweezers, scalpel, and other appropriate tools

2.3. Clean paper

2.4. Evidence containers for repackaging trace evidence (e.g., plastic petri dishes, glassine envelopes)

2.5. Biohazard safety equipment (if necessary)

2.6. Vacuum and vacuum filters

2.7. Microscope slides

2.8. Temporary or permanent mounting media

2.9. Microtome and embedding media

2.10. Microscopes (*ATF-LS-TE01* / *ATF-LS-TE02*)

2.10.1. Polarized light microscope

2.10.2. Stereomicroscope

2.10.3. Comparison microscope

2.10.4. Fluorescence microscope

2.11. Camera or other Imaging Equipment

2.12. Instrumentation

2.12.1. FTIR (*ATF-LS-E6*)

2.12.2. MSP (*ATF-LS-TE03*)

2.12.3. Pyrolysis GC-MS or High Temperature GC-MS (*ATF-LS-TE04 / ATF-LS-FD2*)

2.12.4. Raman (*ATF-LS-TE07*)

2.12.5. XRD (*ATF-LS-E5*)

2.12.6. XRF (*ATF-LS-E4*)

2.12.7. SEM-EDS (*ATF-LS-E3*)

3. Safety Considerations

3.1. The examiner shall follow all biohazard procedures and use universal precautions.

3.2. Precautions need to be taken whenever working with chemicals which could pose potential health hazards.

3.3. Precautions need to be taken when using sharp objects.

4. Procedure or Analysis

4.1. Physical Fit for polymers

4.1.1. Refer to Examination of Physical Fit protocol (*ATF-LS-TE10*)

4.1.2. If a physical fit is determined between probative evidence items (e.g., Q and K items), no further chemical analysis is required.

4.2. Characterization of polymers

4.2.1. The analytical scheme for characterization will vary depending on the type of material (e.g., colorless polymer versus colored polymer), the circumstances of the case, and the examinations requested by the customer.

4.2.2. The analysis for the characterization of polymers includes:

4.2.2.1. Visual and/or microscopic examination to indicate a type of material (e.g., plastic cable tie,).

4.2.2.2. If any chemical information is reported, instrumental analysis is required (e.g., plastic composed of *acrylic* resin, *polyester* film).

4.2.2.3. If polymeric information is included in the exhibit description, it will be clear if instrumental analysis was not conducted to characterize the polymer. (e.g., *Exhibit 1 contained a white plastic end cap with molded markings including "PVC".*).

4.3. Comparison of polymers

4.3.1. The questioned item is evaluated to identify physical features (e.g., color, layer structure, markings) suitable for comparison prior to examination of the known. Any subsequent chemical and elemental analysis of the unknown item shall be conducted prior to the known item.

4.3.2. If at any time during the comparative scheme of analysis an exclusionary difference is observed between the Q and K samples, no further examinations need to be conducted and the samples can be reported as being dissimilar to one another (Exclusion).

4.3.3. If samples have been subjected to different conditions (e.g., age, weathering, burning) caution should be used when interpreting differences and additional testing may be needed to confirm an exclusion.

4.3.4. There are many techniques that are available for the comparison of polymers. Use a combination of techniques that have the greatest potential for discrimination. Table 1 lists the available techniques for polymer comparison with the shaded boxes representing techniques which are recommended. Depending on the sample (e.g., polymer type, color, size) certain techniques may not be available or may not offer any additional information or discrimination power. For instance, MSP would be utilized on a blue piece of plastic but not a gray piece of plastic.

Table 1. Techniques for the comparison of polymers.

Physical Features	Optical Properties	Color/Dye/Pigment Analysis	Instrumental Analysis
Stereomicroscopy	PLM	Comparison Microscopy	FTIR
Light Microscopy/ Comparison Microscopy	Light Microscopy/ Comparison Microscopy	MSP	SEM-EDS/XRF
SEM	Fluorescence Microscopy		PyGC-MS
			Raman

4.3.5. Instrumental Analysis

4.3.5.1. Follow instrument protocols and work instructions for required performance checks and appropriate parameters.

4.3.5.2. When comparing samples, the same analytical techniques and parameters should be used for both the Q and K samples.

4.3.5.3. Due to condition, size and/or type of sample, analysis using some of the instrumentation may not be appropriate or possible.

4.3.5.4. Generally, when sample size is limited, destructive testing is performed after all non-destructive testing is complete.

5. Quality Assurance and Controls

5.1. Reference collections of known polymers are available as well as reference data from the instruments. When using a known reference sample for analysis, include the unique reference number is included in the technical record.

5.2. Quality is assured through the proper training and testing of examiners, the laboratory's technical review process, and the use of appropriate equipment that is maintained and performance checked.

5.3. The techniques described above for polymer examinations are well known and scientifically accepted in the forensic community and in private industry. Relevant examples of related literature can be found in Section 6 (References).

6. References

6.1. ASTM International Standards

ASTM E3295 Standard Guide for Using Micro X-Ray Fluorescence (μ-XRF) in Forensic Polymer Examinations

6.2. Applicable OSAC Registry documents

ASTM E2809 Standard Guide for Using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy in Forensic Polymer Examinations

ASTM E3296 Standard Guide for Using Pyrolysis Gas Chromatography and Pyrolysis Gas Chromatography – Mass Spectrometry in Forensic Polymer Examinations

6.3. Other

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