Technical Procedure for the Examination of Fibers

1.0 Purpose – This technical procedure shall be followed for the examination of individual fibers.

2.0 Scope – This procedure applies to the microscopical analysis of single fiber samples within the Trace Evidence Section.

3.0 Definitions – N/A.

4.0 Equipment, Materials, and Reagents

4.1 Equipment

- Stereomicroscope
- Polarized light microscope (PLM)
- Comparison microscope
- FT-IR with microscope attachment
- Microspectrophotometer
- Pyrolysis-GC-MS
- Muffle furnace
- UV light
- Hotplate
- Alternate Light Source

4.2 Materials

- Forceps
- Probes
- Scalpel
- Razor blades
- Glass slides and cover slips
- Quartz slides and cover slips
- Joliff Cards
- Wire loop
- Filler fibers
- Polyethylene sheets
- Micropipette and tips
- Roller
- KBr discs and holder
- Pellet Press
- Small crucible with lid
- Fiber reference library

4.3 Reagents

- Nail polish, evaporated to approximately 50 % concentration
- Xylene Substitute

- Xylene
- Glycerol (low fluorescing)
- Permount
- Cytoseal
- Epoxy
- Norland Optical Adhesive
- Acetonitrile
- Butyrolactone
- Chloroform

5.0 Procedure

5.1 Analytical Approach

5.1.1 Review the request for analysis and determine the type(s) of examination required based on the request made by the agency and/or the evidence submitted. Examination(s) that may be performed are the identification of the generic fiber class and the comparison of questioned fibers to known fiber standards.

5.1.1.1 There may be times when an agency requests a variation on the above listed types of analyses. If the request falls within the scope of the technical procedures, the analysis may be permitted.

5.1.2 Evaluate the case information and evidence submitted.

5.1.2.1 If necessary, contact the submitting agency to obtain additional evidence, case information, and/or contact the submitting agency to request known fiber standards following the Laboratory Procedure for Obtaining Evidentiary Standards. If standards are not submitted, the examination may be limited or the evidence may be returned.

5.1.3 Most fiber cases involve some degree of evidence screening, either visually/microscopically or in combination with instrumental techniques.

5.1.3.1 Preliminary evaluation of the known's characteristics is warranted when screening is performed of the questioned fibers to identify those suitable for further comparison.

5.1.3.1.1 If the known standard is fabric or cordage see the Trace Evidence Section Technical Procedure for the Examination of Fabric and the Trace Evidence Section Technical Procedure for the Examination of Cordage for the preparation of fiber standards.

5.1.3.2 In cases in which the questioned fiber evidence is loose fibers or scraps of fabric (i.e., not collected as tapings or debris) or the requested examination is an identification of generic fiber class, beginning with the questioned item(s) is required unless case constraints dictate otherwise.

5.1.3.3 In all other fiber cases (e.g., tapings, clothing) the known standards may be opened prior to the questioned fiber evidence for an initial visual evaluation and macroscopic examination.

5.1.3.4 The Forensic Scientist shall approach a fiber comparison by attempting to show that the samples are different. The questioned and known fibers are analyzed using the same techniques and are compared at every step throughout the process. The failure to detect any significant differences, after exhausting the methodology available to the Forensic Scientist, results in the conclusion that the questioned and known fibers could have had a common origin. If a difference is found, the analysis may be concluded at any step.

5.1.3.5 The order of the examination is based on the quantity, quality, type of the evidence, and the Forensic Scientist's training and experience.

5.1.4 Open the questioned fiber evidence container and describe the evidence present. If necessary, process the item to remove any fiber evidence adhering to the item following the Trace Evidence Section Technical Procedure for the Collection and Preservation of Evidence.

5.1.4.1 If no questioned fiber evidence is present, the examination shall conclude.

5.1.4.2 If the evidence is a human hair, the fiber analysis examination is concluded. The evidence may be retained for further trace hair examination.

5.1.5 Begin the fiber examination by performing a preliminary evaluation of the questioned fibers and/or known fiber standards and record the overall macroscopic characteristics.

5.1.5.1. The following is a list of macroscopic characteristics that may be used for classification and comparison of any fibers that may be suitable for further analysis. The characteristics listed below are not all-inclusive and may or may not be present in every fiber.

- General fiber type (animal, man-made, vegetable)
- Color
- Luster
- Crimp
- Relative Longitudinal Diameter
- Other characteristics (e.g., debris adhered, apparent cross-section)

5.1.5.2. No further analysis may be performed on fibers found to be macroscopically not consistent with the applicable known standard.

5.1.6. Based upon the previous macroscopic screening, the fibers may be further screened microscopically.

5.1.6.1. All questioned fibers that are found to be macroscopically similar to the applicable known standard and may be suitable for further analysis are

mounted on microscope slides(s). See **Sample Preparation.**

5.1.6.2. Using the same mounting medium that was chosen for the questioned fibers, collect and mount a representative sample of fibers from the known standard(s). See **Sample Preparation.**

5.1.6.3. The following is a list of the general microscopic characteristics that may be used for classification and comparison of fibers. The microscopic characteristics listed below are not all-inclusive and may or may not be present in every fiber.

- Color
- Delustrant and/or Pigment
- Dyeing
- Fluorescence
- Other characteristics (e.g., longitudinal cross-section, longitudinal diameter)

5.1.6.4. No further analysis may be performed on fibers found to be microscopically not consistent with the applicable known standard or on fibers with limited microscopic properties (e.g., opaque fibers, heavily damaged fibers).

5.1.7 A full characterization of questioned fibers meeting all preliminary screening criteria shall continue following the applicable **Examination Procedures**. The full characterization of the known standards shall be recorded after the questioned fibers.

5.1.8 Some of the tests available are destructive (e.g., solubility testing, pyrolysis gas chromatography-mass spectrometry). When sample size is limited, destructive testing, if necessary, shall only be performed after all non-destructive testing is complete.

5.2 Examination Procedures

5.2.1 Identification of Generic Fiber Class

5.2.1.1 A limited microscopic examination of the fibers to verify natural or man-made may be performed as detailed in **Natural Fiber Analysis** and/or **Man-Made Fiber Analysis**.

5.2.1.2 Identify the composition of man-made fibers using one or more of the following Trace Evidence Section techniques, as applicable:

5.2.1.2.1 Technical Procedure for Infrared Spectroscopy

5.2.1.2.2 Technical Procedure for Pyrolysis - Gas Chromatography - Mass Spectrometry

5.2.1.2.3 Solubility Testing. See **Solubility Testing**.

5.2.1.3 Once all visual, microscopical, chemical, and instrumental examinations have been completed, the Forensic Scientist shall issue a report stating his or her findings.

5.2.2 Comparison of Questioned and Known Fibers:

5.2.2.1 Perform a microscopic examination and/or comparison of both the questioned and the known fibers as detailed in **Natural Fiber Analysis** and/or **Man-Made Fiber Analysis**. If any fibers are found to be microscopically consistent, then both the questioned and known fibers shall be subjected to further testing. If no fibers are found to be consistent, the analysis shall be concluded.

5.2.2.2 Cross-sections may be created of the known and unknown samples for comparison and analysis as described in **Sample Preparation**.

5.2.2.3 Identify the composition of the man-made fibers using one or more of the following Trace Evidence Section techniques, as applicable:

5.2.2.3.1 Technical Procedure for Infrared Spectroscopy

5.2.2.3.2 Technical Procedure for Pyrolysis - Gas Chromatography - Mass Spectrometry

5.2.2.3.3 Solubility Testing. See **Solubility Testing**.

5.2.2.4 For any dyed fibers, perform a color comparison using the comparison microscope and/or the Microspectrophotometer following the Trace Evidence Section Technical Procedure for Microspectrophotometry.

5.2.2.4.1 In some instances, the fibers may be too lightly or darkly dyed to perform a color comparison using the Microspectrophotometer. A notation shall be made in the notes indicating if a color comparison using the Microspectrophotometer was not possible.

5.2.2.5 Once all visual, microscopical, chemical, and instrumental examinations have been completed and the results compared, the Forensic Scientist shall issue a report stating his or her findings. If questioned and known samples are found to be microscopically consistent with each other, a second Forensic Scientist, who is qualified in fiber comparisons, shall confirm these microscopic comparisons. Additional verifications may be completed by other available Forensic Scientists who are qualified in fiber comparisons. A verification review shall be completed in Forensic Advantage (FA) and the microscope slides shall be initialed and dated by the verifying Forensic Scientist(s).

5.3 Sample Preparation

5.3.1 Mounting of Fibers

5.3.1.1 The majority of fiber examinations shall be conducted using a semi-permanent dry mount and observed using a temporary mounting medium such as xylene substitute.

5.3.1.2 Occasionally fibers may be mounted on a glass slide using a mounting medium, such as Permount or Cytoseal.

5.3.1.2.1 A mounting medium, such as Permount or Cytoseal, may be used beyond the expiration date if, based on the Forensic Scientist's training and experience, it flows properly and has not yellowed/discolored.

5.3.2 Preparation of Cross Sections

5.3.2.1 There are numerous ways to cross section fibers, some of which are listed below.

- Joliff card method
- Pipette tip method
- Polyethylene film method
- Norland Optical Adhesive method

5.4 Natural Fiber Analysis

5.4.1 Vegetable Fibers

5.4.1.1 Vegetable fibers are generally found in bundles (technical fibers). For microscopic analysis, technical fibers must be broken down to the ultimates. Some may be pulled apart manually, while others may require chemical maceration.

5.4.1.2 Examine the internal and external characteristics using the PLM. These characteristics include, but are not limited to, the following:

- Surface texture: smoothness, pitting, scales, dislocations, cross-hatching, etc.
- Plant interstructure: lumen, holes, voids, crystals, spiral elements, etc.

5.4.1.3 Using the fluorescence capabilities of the comparison microscope, observe the color and intensity of a fiber's fluorescence.

5.4.1.3.1 The two different fluorescence cubes used are A (wide UV, 340-380 nm) and H3/I3 (wide band blue, 420-490 nm/450 – 490 nm).

5.4.1.3.2 Non-fluorescing mounting media shall be used.

5.4.1.4 If the fibers are consistent in the longitudinal orientation, they shall be cross-sectioned. This test is not required for cotton. See **Sample Preparation**.

5.4.1.4.1 In some instances, the sample size is limited and it may not be possible to obtain a usable cross-section. A notation shall be made in the notes indicating if a cross-section was not obtained.

5.4.1.5 Perform either the Dry Twist Test or the Herzog test on the fiber ultimates. These tests are not required for cotton.

5.4.1.6 If the sample size permits, ash the fibers in the muffle furnace. Heat at approximately 600 °C for 3-4 hours or until completely ashed. Examine the resulting ash for the presence of crystals.

5.4.2 Animal Fibers

5.4.2.1 Animal fibers are animal hair. However, in the course of a fiber examination, only the most common animal hair types used in textile production shall be examined. If the hair in question is not one of the common textile fibers, it may be retained for further analysis by a hair examiner.

5.4.2.2 Hair-based features shall be examined under the light microscope. This includes, but is not limited to, the following:

- Surface features (scales)
- Cortex features (pigment, ovoid bodies, etc.)
- Medullary features (size and pattern)
- Root structure

5.4.3 Based on the microscopic characteristics and physical tests, an identification of the fiber type may be made.

5.5 Man-Made Fiber Analysis

5.5.1 Using a PLM, fully characterize and record the optical properties of the questioned fibers. These include, but are not limited to, sign of elongation, extinction, interference colors, and pleochroism. The questioned fibers are then microscopically compared to the known fiber standard(s).

5.5.2 If the fibers are consistent in the longitudinal orientation, they shall be cross-sectioned. See **Sample Preparation**.

5.5.2.1 In some instances, the sample size is limited and it may not be possible to obtain a usable cross-section. A notation shall be made in the notes indicating if a cross-section was not obtained.

5.5.3 Using the fluorescence capabilities of the comparison microscope, observe the color and intensity of a fiber's fluorescence.

5.5.3.1 The two different fluorescence cubes used are A (wide UV, 340-380 nm) and H3/I3 (wide band blue, 420-490 nm/450 – 490 nm).

5.5.3.2 Non-fluorescing mounting media shall be used.

5.5.4 Based on the microscopic characteristics, a tentative identification of the fiber type may be made. However, instrumental analysis shall be used to confirm all man-made fiber identifications.

5.5.4.1 In some instances, the sample size is limited and it may not be possible to perform instrumental analysis. In these instances, confirmation of man-made fiber identifications may not be possible.

5.6 Solubility Testing

5.6.1 If sufficient sample is present, solubility testing shall be performed on the following:

5.6.1.1 Acetonitrile at room temperature (approximately 75 °F)

- Acetate – Soluble
- Triacetate – Insoluble

5.6.1.2 Butyrolactone at room temperature (approximately 75 °F)

- Acrylic – Insoluble
- Modacrylic – Soluble

5.6.2 Additional solubility testing may be conducted as necessary. See Attachment "A General Table of Solubility for the Qualitative Analysis of Textile Fibers" for suggested reagents.

5.7 Guidelines for Fiber Analysis Result Statements

5.7.1 The reports shall read as follows. The wording of the results shall accurately describe the evidence at hand.

5.7.2 Association (Consistent)

5.7.2.1 This statement shall be used when the questioned and known samples are consistent in color and composition.

5.7.2.1.1 Example: Example: Item A was found to be consistent in color and composition with Item B. Therefore, Item A could have originated from [the same source as] Item B.

5.7.2.2 No comparison performed, only identification of generic fiber class.

5.7.2.2.1 Example: Item A was identified as _____.

5.7.2.3 Qualifying statements shall be added to the report as needed, especially regarding the commonality of certain types of fibers. This includes, but is not limited to, the following:

- White, undyed cotton fibers
- Blue and white variegated cotton fibers (denim)
- White, undyed wool fibers

5.7.2.3.1 Example: It should be noted that blue and white (variegated) cotton fibers are common in the environment.

5.7.3 Inconclusive

5.7.3.1 These statements shall be used when, based on the acquired data, no conclusion could be reached.

5.7.3.1.1 Example: Item A was found to be consistent in __ to Item B; however, slight differences were noted in ___. Therefore, no conclusion could be reached as to whether or not Item A could have originated from [the same source as] Item B.

5.7.3.1.2 Example: Due to the size/condition of Item A, no conclusion could be reached as to whether or not Item A could have originated from [the same source as] Item B.

5.7.4 No Association (Not Consistent)

5.7.4.1 This statement shall be used when one or more of the characteristics associated with the questioned and known fibers are different.

5.7.4.1.1 Example: Item A is not consistent with Item B. Therefore, Item A could not have originated from [the same source as] Item B.

5.7.4.2 No fiber associations between items.

5.7.4.2.1 Example: No fiber associations were noted between Item A and Item B.

5.7.5 No Analysis

5.7.5.1 No analysis is performed.

5.7.5.1.1 Example: The above listed evidence is being returned unanalyzed. If you have any questions, please contact the Forensic Scientist who issued this report.

5.7.5.1.2 Example: Based on the results of the fiber examination between Items A and B, no further fiber analysis was performed. If you have any questions, please contact the Forensic Scientist who issued this report.

5.7.5.2 No analysis is performed due to the results of the DNA analysis.

5.7.5.2.1 Example: Based on the results of DNA analysis, the above listed evidence is being returned unanalyzed. If you have any questions, please contact the Forensic Scientist who issued this report.

5.7.5.3 No analysis is performed due to the size/condition of the sample.

5.7.5.3.1 Example: Due to the size or condition of the fiber sample, no analysis could be conducted.

5.8 Standards and Controls – N/A

5.9 Calibrations – This procedure does not require any calibrations or performance checks. However, it does utilize instruments that require performance checks. See the individual technical procedures for the operations of those instruments.

5.10 Maintenance – No maintenance is required in this procedure. However, the procedure does utilize instruments that require maintenance. See the individual technical procedures for the operations of those instruments.

5.11 Sampling and Sample Selection

5.11.1 No sampling is performed. When sample selection occurs, it shall be based on the Forensic Scientist's training and experience.

5.11.2 If DNA analysis is being performed on the evidence in the case, based on the results of the DNA analysis, the fiber evidence may be returned unanalyzed.

5.12 Calculations – N/A

5.13 Uncertainty of Measurement – N/A

6.0 Limitations - Fibers are a manufactured material. It shall not be possible to identify a fiber as having come from a particular source to the exclusion of all others.

7.0 Safety

7.1 Items may have blood or other body fluids present. Use protective equipment when dealing with items that may contain biohazard material.

7.2 Care shall be exercised when using solvents such as xylene and xylene substitute. Consult Safety Data Sheets for information on safe use for reagents listed in this procedure.

7.3 Care shall be exercised when using Cytoseal 280. Consult Safety Data Sheet for information on safe use in this procedure. Refer to Appendix 1 for Chemical Hygiene and Safety Precautions.

7.4 Glass pipettes, razor blades, and probes are sharp and can be dangerous.

7.5 Crucibles, muffle furnace, and hot plates are very hot and may cause burns. Care shall be exercised when using these items.

8.0 References

8.1 ASTM / SWG Guidelines

ASTM Standard E1732, "Standard Terminology Relating to Forensic Science." ASTM International, West Conshohocken, PA, 2000.

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8.2 Books

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Valaskovic, G.A. "Polarized light in multiple birefringent domains: a study of the Herzog effect." *The Microscope* 39 (1991): 269-286.

8.4 Training Materials

Introduction to Hairs and Fibers, Training Materials, FBI.

Microscopy of Wood and Vegetable Fibers (Training Materials), McCrone Research Institute, John D. Shane, Instructor, March 2000.

9.0 Records – N/A

10.0 Attachments

- Appendix 1: Chemical Hygiene and Safety Precautions for Particularly and Extremely Hazardous Substances
- Appendix 2: A General Table of Solubility for the Qualitative Analysis of Textile Fibers

Revision History		
Effective Date	Version Number	Reason
06/21/2023	4	• Updated header to Trace Evidence Section, issuing authority to Trace Evidence Section Forensic Scientist Manager. • Updated all references in procedure from Trace Unit to Trace Evidence Section • 3.0 – Added ASTM E1732 reference • 4.2 – Added Pellet Press • 4.3 – Added Cytoseal and Butyrolactone • 5.1 through 5.6 reorganized and updated to clarify ISO 17025 requirements to examine questioned before known. • Old section 5.6 [Thin Layer Chromatography (TLC)] removed along with Suggested Extraction and Eluent Systems for Thin Layer Chromatography attachment • 5.7.2 – Renamed Association (Consistent) • Added 5.7.2.3.1 • 5.7.4 – Renamed No Association (Not Consistent) • Removed 5.11.2 and 5.11.3 • 7.0 – Updated and added 7.1 through 7.4 • Added new 7.3 • 8.1 – Added ASTM E1732 reference • Added new Appendix 1

Appendix 1: Chemical Hygiene and Safety Precautions for Particularly and Extremely Hazardous Substances

Cytoseal 280 **DANGER: PARTICULARLY HAZARDOUS SUBSTANCE**	
	**HEALTH 3** **FLAMMABILITY 3** **REACTIVITY 0**
Detection of Release	Colorless liquid; Aromatic Odor
Signs/Symptoms of Exposure	Skin, eye irritation; drowsiness or dizziness; Breathing difficulties. Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting.
PEL	ACGIH TLV 20 ppm; OSHA PEL 100 ppm (TWA); NIOSH IDLH 500 ppm
Associated Hazards	**May be fatal if swallowed and enters airways**; Highly flammable liquid and vapor. Causes skin irritation. **Suspected of damaging fertility or the unborn child. May cause damage to organs (Central nervous system) through prolonged or repeated exposure.**
Controls	Use only under a chemical fume hood. Use explosion-proof electrical/ventilating/lighting/equipment. Ensure that eyewash stations and safety showers are close to the workstation location. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with impervious gloves.
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist; Use explosion-proof equipment; Keep away from heat and sources of ignition; take measures to prevent the build-up of electrostatic charge. Dispose in Hazardous Chemical Waste.
Emergency Procedures	**Eye Contact:** Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention. Immediately flush with plenty of water. After initial flushing, remove any contact lenses and continue flushing for at least 15 minutes. Keep eye wide open while rinsing. If symptoms persist, call a physician. **Inhalation Exposure:** Move to fresh air. If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with a respiratory medical device. Immediate medical attention is required. Move to fresh air in case of accidental inhalation of vapors. If symptoms persist, call a physician. **Ingestion:** Do not induce vomiting. Call a physician or Poison Control Center immediately. Clean mouth with water and drink afterwards plenty of water. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person. Consult a physician. **Skin Contact:** Wash off immediately with plenty of water for at least 15 minutes. Obtain medical attention. Call a physician immediately. SPEEDY ACTION IS

	CRITICAL, GET MEDICAL AID IMMEDIATELY.. If symptoms persist, call a physician. If skin irritation persists, call a physician. Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Small contained spill: wearing appropriate PPE, collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container. Dispose in Hazardous Chemical Waste. Large spills: Evacuate area and call 911 (Haz Mat).

Appendix 2: A General Table of Solubility for the Qualitative Analysis of Textile Fibers