

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 analysis of single fiber samples within the Trace Unit.

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
- Small crucible with lid
- Fiber reference library

4.3 Reagents

- Nail polish, evaporated to approximately 50 % concentration
- Xylene Substitute
- Xylene
- Glycerol (low fluorescing)

- Permout
- Epoxy
- Norland Optical Adhesive
- Acetonitrile
- Chloroform

5.0 Procedure

5.1 Analytical Approach

5.1.1 General guidelines:

5.1.1.1 The two types of examinations often requested of a fiber-trained Forensic Scientist are the identification of the generic fiber class and the comparison of questioned fibers to known fiber standards.

5.1.1.2 The Forensic Scientist shall approach a fiber comparison by attempting to show that the samples are different. The failure to detect any significant differences, after exhausting the methodology available to the Forensic Scientist, results in the conclusion that the known and questioned fibers could have had a common origin.

5.1.1.3 The questioned and known fibers are analyzed using the same techniques and are compared at every step throughout the process. If a difference is found, the analysis may be concluded at any step.

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

5.1.1.3.2 Some of the tests available to fiber-trained Forensic Scientists are destructive. When sample size is limited, destructive testing, if necessary, shall only be performed after all non-destructive testing is complete.

5.1.1.4 All results shall be based on the Forensic Scientist's knowledge and experience and the case being examined. Results shall be in agreement with the technical reviewer.

5.1.2 Identification of Generic Fiber Class

5.1.2.1 Review the request for analysis.

5.1.2.2 Open evidence container and describe the evidence present.

5.1.2.3 Perform a preliminary evaluation and record the overall macroscopic characteristics, to include general fiber type (animal, man-made, vegetable).

5.1.2.4 Mount the fibers as described in **5.2**.

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- 5.1.2.5** Perform a microscopic examination of the fibers as detailed in **5.3** (natural fibers) and/or **5.4** (man-made fibers).
 - 5.1.2.6** Identify the composition of man-made fibers using one or more of the following Trace Unit techniques:
 - 5.1.2.6.1** [Technical Procedure for Infrared Spectroscopy](#)
 - 5.1.2.6.2** [Technical Procedure for Pyrolysis - Gas Chromatography - Mass Spectrometry](#)
 - 5.1.2.6.3** Solubility Testing. See **5.5**.
 - 5.1.2.7** Once all visual, microscopical, chemical and instrumental examinations have been completed, the Forensic Scientist shall issue a report stating his or her findings.
 - 5.1.3 Comparison of Questioned and Known Fibers**
 - 5.1.3.1** Review the request for analysis.
 - 5.1.3.2** Open evidence container and describe the evidence present.
 - 5.1.4** Collect standards of the known. If examining the questioned item for fiber transfer, process the item to remove any adhering fiber evidence following the Trace Unit [Technical Procedure for the Collection and Preservation of Evidence](#).
 - 5.1.4.1** Perform a preliminary evaluation of the known standard and record the overall macroscopic characteristics, to include general fiber type (animal, man-made, vegetable).
 - 5.1.4.2** Mount the fibers from the known standard as described in **5.2**.
 - 5.1.4.3** Using a stereomicroscope, search the unknown for any fibers similar to the known standard. Remove the fibers selected for microscopic analysis and mount them as described in **5.2**.
 - 5.1.4.4** Perform a microscopic examination and/or comparison of both the questioned and the known fibers as detailed in **5.3** (natural fibers) and/or **5.4** (man-made fibers). If any fibers are found to be visually 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.
 - 5.1.4.5** Cross-sections may be created of the known and unknown samples for comparison and analysis as described in **5.2**.
 - 5.1.4.6** Identify the composition of the man-made fibers using one or more of the following Trace Unit techniques:

5.1.4.6.1 [Technical Procedure for Infrared Spectroscopy](#)

5.1.4.6.2 [Technical Procedure for Pyrolysis - Gas Chromatography - Mass Spectrometry](#)

5.1.4.6.3 Solubility Testing. See **5.5**.

5.1.4.7 Perform a color comparison using one or more of the following Trace Unit techniques:

5.1.4.7.1 [Technical Procedure for Microspectrophotometry](#)

5.1.4.7.2 Thin Layer Chromatography. See **5.6**.

5.1.4.8 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 qualified Forensic Scientist shall verify the fiber association. This Forensic Scientist shall initial the microscope slides involved and complete a verification review in Forensic Advantage.

5.2 Sample Preparation

5.2.1 Mounting of Fibers

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

5.2.1.2 Occasionally, a permanent mount may be required. In this case, fibers shall be mounted on a glass slide using a mounting medium, such as Permout or Norland Optical Adhesive.

5.2.1.2.1 A mounting medium, such as Cytoseal or Norland Optical Adhesive, may be used if, based on the Forensic Scientist's training and experience, it flows properly and has not yellowed.

5.2.2 Preparation of Cross Sections

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

- Joliff card method
- Pipette tip method
- Polyethylene film method

5.3 Natural Fiber Analysis

5.3.1 Vegetable Fibers

- 5.3.1.1** Vegetable fibers are generally found in bundles. For microscopic analysis, the fibers must be broken down to the ultimates. Some may be pulled apart manually, while others may require chemical maceration.
- 5.3.1.2** Examine the internal and external characteristics (both longitudinally and in cross section) 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.
- 5.3.1.3** Using the fluorescence capabilities of the comparison microscope, observe the color and intensity of a fiber's fluorescence.
- 5.3.1.3.1** The two different fluorescence cubes used are A (wide UV, 340-380 nm) and H3 (wide band blue, 420-490 nm).
- 5.3.1.3.2** Non-fluorescing mounting media shall be used.
- 5.3.1.4** Perform either the Dry Twist Test or the Herzog test on the fiber ultimates. These tests are not required for cotton.
- 5.3.1.5** 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.

5.3.2 Animal Fibers

- 5.3.2.1** 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, analysis shall follow the Trace Unit [Technical Procedure for Hair Analysis](#).
- 5.3.2.2** 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

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

5.4 Man-Made Fiber Analysis

- 5.4.1** Using a light microscope, examine the general fiber properties to include size, optical cross-section, surface markings and striations, inclusions (including delustrant) and dyes or colorants.
- 5.4.2** Using a PLM, examine the optical properties of the fibers. These include, but are not limited to, sign of elongation, extinction points, birefringence, and pleochroism.
- 5.4.3** If the fibers are consistent in the longitudinal orientation, they shall be cross-sectioned. See **5.2.2**.
- 5.4.4** Using the fluorescence capabilities of the comparison microscope, observe the color and intensity of a fiber's fluorescence.
- 5.4.4.1** The two different fluorescence cubes used are A (wide UV, 340-380 nm) and H3 (wide band blue, 420-490 nm).
- 5.4.5** 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 Solubility Testing

- 5.5.1** If sufficient sample is present, solubility testing may be performed.
- 5.5.1.1** Acetonitrile at room temperature (approximately 75 °F)
- Acetate – Soluble
 - Triacetate – Insoluble
- 5.5.1.2** Butyrolactone at room temperature (approximately 75 °F)
- Acrylic – Insoluble
 - Modacrylic – Soluble
- 5.5.2** 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.

5.6 Thin Layer Chromatography (TLC)

- 5.6.1** If sufficient sample is present, TLC may be used for dye separation.
- 5.6.2** A minimum of one solvent system is required. Suggested extraction and eluent systems can be found in the attachment “Suggested Extraction and Eluent Systems for Thin Layer Chromatography.”
- 5.6.3** The known and questioned samples shall be run on the same plate at the same time.

5.6.4 The resulting chromatographic plate shall be viewed under both ambient light and UV light. Note and compare the number, location, color and R_f value of all resulting spots.

5.6.5 To document the results, photocopy, scan or photograph the TLC plate.

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 Positive

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 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 an item.

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

5.7.3 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 Negative

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.

5.7.5.2 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 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 at any point during the course of examination the items are found to be inconsistent with one another, analysis may be halted and a lab report shall be issued stating a negative finding.
- 5.11.3 If no fiber standards are submitted, the evidence may be returned to the agency unanalyzed.
- 5.11.4 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 - Crucibles removed from the muffle furnace 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 D276, 2000a, “Standard Test Methods for Identification of Fibers in Textiles.” ASTM International, West Conshohocken, PA, 2000.

ASTM Standard E175, 1982 (2005e1), “Standard Terminology of Microscopy.” ASTM International, West Conshohocken, PA, 2005.

ASTM Standard E227, 2002, “Standard Guide for Forensic Examination of Non-Reactive Dyes in Textile Fibers by Thin-Layer Chromatography.” ASTM International, West Conshohocken, PA, 2002.

ASTM Standard E2224, 2002, “Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy.” ASTM International, West Conshohocken, PA, 2002.

ASTM Standard E2228, 2002, “Standard Guide for Microscopic Examination of Textile Fibers.” ASTM International, West Conshohocken, PA, 2002.

SWGMA. Forensic Fiber Examination Guidelines. For. Sci. Comm. 1999: 1(1).

8.2 Books

Billmeyer, F.W. and M. Saltsman. *Principles of Color Technology*. 2nd Ed. New York: John Wiley and Sons, 1981.

Caitling, D. and J. Grayson. *Identification of Vegetable Fibres*. New York: Chapman and Hall, 1982.

Hall, D.M. *Practical Fiber Identification*. 2nd Ed. Auburn, AL: Auburn University, 1982.

Hatch, K.L. *Textile Science*. New York: West Publishing Company, 1993.

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Joseph, M.L. *Introductory Textile Science*. 4th Ed. New York: Holt, Rinehart and Winston, 1981.

Robertson, J. and M. Grieve, eds. *Forensic Examination of Fibres*. 2nd Ed. London: Taylor and Francis, 1999.

Saferstein, R. *Forensic Science Handbook Volume II*. Englewood Cliffs, NJ: Prentice Hall, 1988.

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The Textile Institute. *Identification of Textile Materials*. 7th Ed. Portsmouth: Eyre & Spottiswoode Limited, 1975.

8.3 Journal Articles

Deedrick, Douglas W. and Sandra L. Koch. “Microscopy of Hair Part II: A Practical Guide and Manual for Animal Hairs”, *Forensic Science Communications* 6.3 (July 2004).

Grieve, M.C. and L.R. Cabiness. “The Recognition and Identification of Modified Acrylic Fibers.” *Forensic Science International* 29 (1985): 129-146.

Palenik, S. and Fitzsimons. “Forensic Microscopy, Fiber Cross-Sections: Part II.” *Microscope* 38 (1990): 313-320.

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

- A General Table of Solubility for the Qualitative Analysis of Textile Fibers
- Suggested Extraction and Eluent Systems for Thin Layer Chromatography

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original ISO Document
10/18/2013	2	Added issuing authority to header
08/29/2014	3	Updated header to Physical Evidence Section – Trace Unit, issuing authority to Physical Evidence Section Forensic Scientist Manager. Updated all references in procedure from Trace Evidence Section to Trace Unit Changed unworked to unanalyzed throughout document Update header information 4.1 – removed HPLC, Microtome; added Alternate light source 4.2 – removed capsule mold; added fiber reference library 4.3 – added xylene Added: 5.1.1.1, 5.1.2 (and all subheadings), 5.1.3, 5.1.3.8, 5.2.1 (indented subheadings), 5.2.2 (and all subheadings), 5.5.1 Combined 5.1.5 with 5.1.5.1; 5.1.7 with 5.1.8; 5.3.1.2 with 5.3.1.3; 5.6.5 with 5.6.2 Deleted: 5.1.5.3 5.1.3.3 – added questioned 5.1.3.5 – added from the known standard 5.1.3.9 – added man-made and one or more 5.1.3.10 – added one or more 5.1.3.11 – added and the results compared, microscopically, and Forensic Advantage Removed “based” from: 5.3.1, 5.3.1.1, 5.3.2, 5.3.2.1 5.3.1.5 – added examine resulting ash for crystals 5.3.2.1 – removed essentially 5.4.3 – added see 5.2.2 Expanded: 5.6.4 5.6.5 – removed calculate the R _f values

Suggested Extraction and Eluent Systems for Thin Layer Chromatography

Suggested Extraction Systems:

Dye classification indicates the best extraction solution for a particular fiber. Useful published information is summarized below:

Dye Class	Fiber Type	Extraction Solution
Acid ¹	Polypropylene	Pyridine / Water (4:3)
Acid	Nylon	Pyridine / Water (4:3)
Acid	Wool	Pyridine / Water (4:3)
Acid	Cotton	Glacial Acetic Acid
Basic	Polyester	Pyridine / Water (4:3)
Direct	Cotton	Pyridine / Water (4:3)
Disperse	Polyester	Pyridine / Water (4:3)
Disperse	Polypropylene	Pyridine / Water (4:3)
Disperse	Acetate / Triacetate	Pyridine / Water (4:3)
Metalized	Wool	2% Aqueous Oxalic Acid then pyridine / water (4:3)
Metalized	Polypropylene	Pyridine / Water (4:3)
Basic	Acrylic	Formic Acid / Water (1:1)
Reactive, Sulfur, Vat, Diazo, Ingrain and pigmented dyes do not extract		

¹ Dyes rarely extract from Polypropylene, but if they do, they are most likely to be Acidic.

Choice of Eluent System:

The following list summarizes eluent systems that have been recommended in the relevant forensic literature. This list is not meant to be totally inclusive or exclusive.

Eluent	Solvents ²	Proportions (v/v)
1	n-Butanol, acetone, water, ammonia	5:5:1:2
2	Pyridine, amyl alcohol, 10 % ammonia	4:3:3
3	n-Butanol, ethanol, ammonia, pyridine, water	8:3:4:4:3
4	Methanol, amyl alcohol, water	5:5:2
5	Toluene, pyridine	4:1
6	Chloroform, ethyl acetate, ethanol	7:2:1
7	n-Hexane, ethyl acetate, acetone	5:4:1
8	Toluene, methanol, acetone	20:2:1
9 ³	n-Butanol, acetic acid, water	2:1:5
10	n-Butanol, ethanol, ammonia, pyridine	4:1:3:2
11	Chloroform, butanone, acetic acid, formic acid	8:6:1:1
12 ³	n-Butanol, acetic acid, water	4:1:5

² The ethanol used is 99 %; the ammonia 0.880 SG unless otherwise stated.

³ These eluents form an upper and lower phase. Use the upper phase as the eluent.

Eluents Recommended for Certain Dye Classes:

Certain fiber type/dye class combinations have been found to give better separation in certain eluents. These are shown in the table below and are recommended as a first choice.

Fiber Type	Dye Class	Eluent Number
Wool	Acid or Metallized	1,2
Cotton and Viscose	Direct	1,4,3
Acrylic	Basic	11,12,1
Polyester	Disperse	6,7,8,5
Polyamide	Acid	9,10
Polypropylene	Polypropylene rarely contains an extractable dye. If the dye can be extracted an eluent appropriate to the dye class is used.	

Adapted from:

ASTM Standard E227, 2002, “Standard Guide for Forensic Examination of Non-Reactive Dyes in Textile Fibers by Thin-Layer Chromatography, ASTM International, West Conshohocken, PA, 2002.