Collection of Biological Material

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1 Introduction

While it is not practical to anticipate all items of evidence received, or their condition, these procedures are put forth as guidelines for collection of samples from various types of evidentiary items. If an evidentiary item is encountered that is not specifically cited, samples should be collected using the procedures for the item most similar in composition and/or nature. Additional guidance may be included in substrate-specific DNA extraction procedures (e.g., calcified tissue samples, hair).

2 Scope

These procedures apply to DNA personnel that collect samples from various types of evidentiary items that will undergo DNA analysis.

3 Equipment

- General laboratory supplies (e.g., pipettes, scalpel, ruler)
- Sample Tubes
- Sterile Applicators (swabs)
- Water, Molecular Grade or equivalent
- Ethyl Alcohol (i.e., ethanol), 95%
- Xylene, Xyless or Xylene substitute
- Alternate light source (Polilight Flare Plus or equivalent)
- Tissue homogenizer (Dounce tissue grinder or equivalent)
- Fabric swatch (cotton sheeting or equivalent)
- Stereomicroscope

4 Standards and Controls

Controls are initiated at extraction. Refer to the appropriate DNA extraction procedure for the introduction of the appropriate controls.

5 Sampling

DNA personnel rely on knowledge, training and experience to select the appropriate samples and/or stains to test. In addition, an examiner may rely on the results of the serological testing and/or observations noted by the biologist regarding the selection of appropriate stains (e.g., dark red stain versus faint red stain, swab with yellow staining versus swab with no staining). Where information does not allow two stains/samples to be distinguished from one another (e.g., two swabs with no staining observed on either), a stain/sample may be selected at random.

These procedures provide guidance for sample size; however, DNA personnel should use the amount of evidence considered necessary to provide DNA typing results. Requirements for evidence consumption are described in the DNA Quality Assurance Manual (BIO-101).

6 PROCEDURE

- Refer to the DNA procedures introduction (BIO-100) and follow applicable general precautions and cleaning instructions.
- Refer to the Laboratory Operations Manual (LAB-200) and DNA Evidence Management Procedures (BIO-201) for evidence handling requirements.
- Ensure the appropriate fields in the Sample Tracking and Control Software (STACS) are completed, as necessary.

6.1 Description in Notes

The case notes for each item examined will include:

A. an indication of the packaging condition (e.g., properly sealed envelope)
    - If packaging and/or an item was previously described (e.g., during serology examinations), the description should be reviewed and any discrepancies or updates noted.

B. a description of the item(s)
    - The description of the item should be enough to distinguish it from other items in the case.

C. a description of the sample(s) collected from the item, if appropriate.
    - The description of the collection should be enough to allow another biologist or an examiner to determine approximately where the sample was collected from.
    - Multiple samples may be combined into a single tube, as appropriate.

6.2 Preparation of Dried Blood Sample from Liquid Blood

A dried blood sample may be prepared from a liquid blood sample. If a liquid blood sample is clotted, the blood should be homogenized and a dried blood sample prepared from the homogenized blood prior to a collection. Recovery from liquid blood should not be attempted for a clotted sample.

1.	• Label a fabric swatch with the Laboratory number, item number, name of the individual from whom the blood is identified as having been collected, the date of swatch preparation, and the initials of the individual preparing the bloodstain. • Record the type of blood tube used to prepare the exemplar (e.g., purple top tube) in the case notes.

2.	• Gently invert the blood tube until the contents are thoroughly mixed. • Record the fill-level of the tube by marking its meniscus on the outside of the tube.

3.	*If the blood is coagulated:* • Slowly transfer the clotted blood into the reservoir of an autoclaved tissue homogenizer. • Insert the glass pestle into the reservoir and gently homogenize the clotted material and serum.

4.	• In a hood, slowly pipette approximately 500 µL of liquid blood onto the fabric swatch. If the liquid blood tube contains limited volume, a smaller volume of blood should be applied to the swatch. • Record the volume of blood used to prepare the stain in the case notes.

5.	*If the blood was homogenized:* • Return the unused homogenate to the original blood tube. • Ensure the autoclaved glass homogenizer and pestle are rinsed with 10% bleach solution, washed, and rinsed with reagent grade water.

6.	• Allow the bloodstain to thoroughly air dry within the hood (approximately 1 hour). • Place the dried stain in a labeled coin envelope and store refrigerated.

Multiple samples may be dried in the same hood provided they are sufficiently far apart to prevent their touching each other during drying.

6.3 Collection Guidance

A. Alternative collection techniques or collections from an item without guidance below will be clearly described in the notes.
B. If a smaller or larger cutting is necessary, the size of the cutting will be captured in the notes.
C. Refer to the DNA Quality Assurance Manual (i.e., BIO-101) for additional information on examination of evidence including consumption.
D. The appropriate type of tube is generally dependent upon the intended extraction technique and compatibility with applicable equipment.
E. Tubes will be labeled with a unique identifier (i.e., portion of lab number and sample identifier and/or barcode)
F. Approximately 50 µL of reagent grade water is typically sufficient to moisten a sterile swab.

6.3.1 <u>Dried Stains (e.g., blood stains, semen stains) on items or Submitted swabs (e.g., sexual assault kit swabs, buccal swabs, other evidentiary swabs)</u>

A. Cutting a stain

1.	Cut ~5 mm x 5 mm from the dried stain and place it into a labeled tube.

B. Swabbing a stain

1.	Using a sterile swab moistened with reagent grade water, swab a sufficient portion of the stain.
2.	Remove the entire swab head (or a portion of the swab head) and place it into a labeled tube.

C. Sexual Assault Kit swabs

1.	Cut ~ ¾ of each swab from an orifice and place the cuttings from up to 2 swabs into a labeled tube.

D. Other submitted swabs

1.	Cut ~ ⅓ to ½ of swab head and place it into a labeled tube.

At the discretion of the examiner, it may be necessary to cut an entire swab head for items suspected to have low amounts of DNA. Refer to the guidance for consumption (See BIO-101), when applicable.

6.3.2 <u>Liquid Blood</u>

1.	Gently invert the blood tube until the contents are thoroughly mixed.
2.	Transfer an appropriate amount of blood (generally ~ 5 µL) into a labeled tube.
3.	Close and tape seal the blood tube and initial the tape seal.

6.3.3 <u>Moisture-Activated Envelope Flaps or Stamps</u>

1.	Carefully open any sealed envelope flap and/or remove any affixed stamp as appropriate using steam generated from water boiled in a household teapot.

The purified water available at laboratory sinks (e.g., spigot sometimes labeled "DE") *is generally used for steaming, but reagent grade water may be used if necessary.*

2.	Using a sterile swab moistened with reagent grade water, swab the adhesive area of flap or stamp and the corresponding region on the envelope.
3.	Remove the entire swab head and place into a labeled tube.

6.3.4 *Cigarette Butts*

1.	Remove ~5 mm from the filter end of the cigarette butt and place it into a labeled tube.

6.3.5 *Bottles, Cans, and Cups*

1.	Using a sterile swab moistened with reagent grade water, swab the mouth of the item.

If a dry residue may contain inhibitors (e.g., coffee, cola), this residue should be collected on a separate swab and processed as a separate sample.

If fluid remains in the container, the inside surfaces of the container should not be sampled. The liquid volume in such a container may be collected and processed as a liquid rinse or wash.

2.	Remove the entire swab head and place into a labeled tube.

6.3.6 *Tape*

Tape may be adhered to a clean/UV'd plastic sheet for the preservation of latent prints. Collection can occur after latent processing.

A. By Swabbing

1.	Using a sterile swab moistened with reagent grade water, swab the tape where appropriate (e.g., adhesive side suspected of binding a victim, edges suspected of being handled by subject).
2.	Remove the entire swab head and place it into a labeled tube.

B. By Cutting

1.	Take an ~1 inch cutting from the appropriate area(s) of the tape (e.g., from the unexposed end or area indicated, if previously removed from another item of evidence).
2.	Place the cutting with adhesive side facing out into a labeled tube.

6.3.7 *Wearer Swabbing of Clothing Items*

1.	Using a sterile swab moistened with reagent grade water, swab the contact areas of the item (e.g., the sweatband of baseball style cap; the inside collar, cuffs, and/or underarm areas of a shirt; the region around the eye, nose, and/or mouth holes of a mask).

Should such areas not be identifiable, a general swabbing may be taken. Begin in areas likely to have less DNA and end in areas likely to have more DNA.

Alternatively, fabric cuttings from one or more of these areas may be taken and processed.

2.	Remove the entire swab head and place it into a labeled tube.

6.3.8 <u>Male Underwear in Sexual Assault Scenarios</u>

1.	View the underwear with the alternate light source (e.g., 455 nm with orange filter/goggles). Circle area(s) of fluorescence with a dotted line.

A photograph should be taken of the item to record the area(s) of fluorescence in the case notes.

2.	Using a sterile swab moistened with reagent grade water, swab the inside front area(s) of the underwear circled with a dotted line.
3.	Remove the entire swab head and place it into a labeled tube.
4.	Using a sterile swab moistened with reagent grade water, swab the outside front area(s) of the underwear circled with a dotted line.
5.	Remove the entire swab head and place it into a labeled tube.

Should such items not display fluorescence, a general swabbing of the inside front of the underwear and a general swabbing of the outside front of the underwear may be taken.

6.3.9 <u>Handler DNA from Firearms, Knives, IED components, and other Weapons</u>

1.	Using a sterile swab moistened with reagent grade water, swab those portions of the weapon that would most likely have been handled by the individual (e.g., the handle of the knife; the textured portions of the grip, trigger, or hammer of the firearm; pin from grenade).
2.	Remove the entire swab head and place it into a labeled tube.

6.3.10 <u>Fingernail Clippings</u>

1.	Using a sterile swab moistened with reagent grade water, swab the underside of all clippings. Generally, separate swabs should be taken from the clippings of each hand if submitted.
2.	Remove the entire swab head and place it into a labeled tube.

6.3.11 Microscope Smear Slides

1.	Using a sterile swab moistened with reagent grade water, remove ~½ to ¾ of the smear from the microscope slide.
2.	Remove the entire swab head and place it into a labeled tube.

6.3.12 Microscope Smear Slides with Cover-Slips

1.	Attempt to remove the cover-slip from the microscope slide, using a scalpel if necessary.
2.	If necessary, cover the appropriate portion of the slide in xylene until the cover slip can be removed. Alternatively, the entire slide may be soaked in xylene. Discard the liquid into an appropriately labeled container suitable for xylene waste. Allow the slide to evaporate to dryness.

Xylene must be handled in a fume hood and the slide should be kept in the fume hood until dry. A xylene substitute (i.e., Xyless) may be used in place of xylene.

3.	Using a sterile swab moistened with reagent grade water, remove ~½ to ¾ of the smear from the slide and the cover-slip.
4.	Remove the entire swab head and place it into a labeled tube.

6.3.13 Liquid Rinses or Washes

1.	Centrifuge the tube containing the rinse or wash in the appropriate centrifuge for 10 minutes at maximum speed.

If the rinse is in a tube not compatible with the centrifuge, transfer the rinse to an appropriately labeled tube prior to centrifugation and the supernatant can be transferred back to the original tube after centrifuging.

2.	Transfer the supernatant into an appropriate tube.
3.	Collect pelleted material onto a sterile swab. Remove the entire swab head and place it into a labeled tube.
4.	Transfer the supernatant back into the original tube.

6.3.14 Biological Tissue

1.	Dissect ~1 cm^3, if available, from the tissue mass and mince.
2.	Place minced tissue into a labeled tube(s) so that tube(s) is ~ ⅓ to ½ full.

6.3.15 Paraffin-Embedded Biological Tissue

Xylene must be handled in a fume hood and the sample should be kept in the fume hood until dry. A xylene substitute (i.e., Xyless) may be used in place of xylene.

1.	Dissect ~1 cm^3, if available, from the tissue mass and mince.

Remove any excess paraffin, if possible.

2.	Place minced tissue into a labeled tube(s) so that tube(s) is ~ ⅓ to ½ full.
3.	In a fume hood, add enough xylene to the tube to fully immerse the sample, vortex, and incubate for ~30 minutes at room temperature with agitation.
4.	Spin to pellet (generally 9,000 to 13,000 rpm for 5 minutes).
5.	In a fume hood, decant the supernatant into an appropriately labeled container suitable for xylene waste.
6.	Repeat the xylene wash procedure.
7.	Add 95% ethanol equivalent to half the volume of xylene and vortex.
8.	Spin to pellet (generally 9,000 to 13,000 rpm for 5 minutes).
9.	Decant the supernatant into an appropriately labeled container suitable for ethanol waste.
10.	Repeat the ethanol wash procedure.
11.	Allow the de-paraffinized sample to air dry at room temperature (generally for 2-4 hours) in a hood.

If necessary, transfer the sample to a secondary container in a hood to facilitate drying. Transfer the sample back to the labeled tube following drying.

6.3.16 Aborted Tissue

1.	If frozen, allow the sample to thaw at room temperature for several hours or at 4°C overnight.

The liquid surrounding the specimen may contain a large proportion of maternal blood. Sample may be transferred to a secondary container to facilitate collection.

2.	If possible, select recognizable pieces from the tissue, transfer them to another container, and rinse with reagent grade water. Additional reagent grade water can be used to facilitate the collection process.

Aborted tissue may contain both embryonic/fetal and maternal tissues. Both the embryonic/fetal DNA and the maternal DNA may be isolated. Additional information can be found in Johnson, et. al. (2010).

3.	Dissect ~1 cm^3, if available, from the selected tissue and mince.
4.	Place minced tissue into a labeled tube(s) so that tube(s) is ~⅓ to ½ full.

6.3.17 Hair

Collection of hairs should be performed in a PCR workstation or laminar air flow hood. ***Hood air flow must be turned off while transferring/handling hair.*** *Any step involving manipulations of difficult hairs may occur outside of hood with aid of stereomicroscope.*

6.3.17.1 Hair Samples to be Used as a Reference Sample for Nuclear DNA Extraction

1.	Rinse the hair thoroughly with 95% ethanol. Discard the wash into an appropriately labeled container suitable for ethanol waste.
2.	Follow the ethanol rinse with a thorough rinse with reagent grade water.
3.	Remove ~1 cm from the root end and place it into a labeled tube.

6.3.17.2 Hair Samples of Unknown Origin with potential root material for Nuclear DNA Extraction

1.	Remove ~1 cm from the root end and place it into a labeled tube.

Alternatively, a hair may be received in a tube and that tube will be appropriately labeled (i.e., barcoded) for extraction.

6.3.17.3 Hair Samples for Mitochondrial DNA Extraction

Refer to the hair extraction procedure (i.e., BIO-513) for guidance for collection and cleaning of hairs for mitochondrial DNA.

6.3.17.4 Accessing a Mounted Hair Sample

Xylene must be handled in a fume hood. A UV-treated xylene substitute (i.e., Xyless) may be used in place of xylene.

1.	Attempt to remove the cover-slip from the microscope slide, using a scalpel if necessary.
2.	• If necessary, cover the appropriate portion of the slide in xylene until the cover slip can be removed. • Alternatively, the entire slide may be soaked in xylene. Discard the liquid into an appropriately labeled container suitable for xylene waste.
3.	• Rinse the hair with 95% ethanol. • Discard the wash into an appropriately labeled waste container suitable for ethanol waste.
4.	Follow the ethanol rinse with a thorough rinse with reagent grade water.
5.	Refer to the appropriate section above for collection of the hair sample.

7 Limitations

These procedures do not exhaust the possible list of evidentiary items that may be encountered. For those items not specifically cited, samples should be collected using the procedures most similar in composition and/or nature.

8 Safety

- All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material.
- Refer to the FBI Laboratory Safety Manual for information on personal protection, the proper disposal of the chemicals used in these procedures, as well as the biohazardous wastes generated.
- Procedural Specific Chemical Hazards:
    - Ethyl alcohol and xylene are hazardous materials. Use only in a fume hood. Wear appropriate protective clothing and eyewear when handling both. Be careful not to expose face or hands to splashes.

9 References

An SF and Fleming KA. Removal of inhibitor(s) of the polymerase chain reaction from formalin fixed, paraffin wax embedded tissues, *Journal of Clinical Pathology* (1991) 44: 924-927.

Grünewald K, Feichtinger H, Weyrer K, Dietze O, and Lyons J. DNA isolated from plastic embedded tissue is suitable for PCR, *Nucleic Acids Research* (1990) 18: 6151.

Impraim CC, Saiki RK, Erlich HA, and Teplitz RL. Analysis of DNA extracted from formalin-fixed, paraffin-embedded tissues by enzymatic amplification and hybridization with sequence-specific oligonucleotides. *Biochem Biophys Research Comm* (1987) 142: 710-716.

Johnson DJ, Matthies LK, Roberts KA, Yorker BC. Isolation and individualization of conceptus and maternal tissues from abortions and placentas for parentage testing in cases of rape and abandoned newborns. *Journal of Forensic Sciences* (2010) 55(6): 1430-1436.

Shimizu H and Burns JC. Extraction of nucleic acids: sample preparation from paraffin-embedded tissues. In: *PCR Strategies*. M Innis, D Gelfand and J Sninsky eds. Academic Press, NY, 1995, pp. 32-38.

Wright DK and Manos MM. Sample preparation from paraffin-embedded tissues. In: *PCR Protocols*: *A Guide to Methods and Applications.* MA Innis, DH Gelfand, JJ Sninsky and TJ White, eds. Academic Press, NY, 1990, pp. 153-158.

10 Revision History

Revision	Issued	Changes
00	06/15/2022	Reformatted DNA 201-13 into new template and assigned new Doc ID. Minor revisions to content.
01	04/01/2024	Update reagents to Molecular grade water and added Xyless Added Consumption reference. Added evidence handling references.