# Procedures for the Collection of Biological Material

## 1 Scope

These procedures apply to DNA personnel that collect samples from various types of evidentiary items that will undergo DNA analysis by the DNA Casework Unit (DCU) and Biometric Analysis Unit (BAU). Because it is not practical to anticipate all items of evidence received, or their condition, these procedures are put forth as guidelines. 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 procedures (e.g., calcified tissue samples, hair). Any collection conducted in a manner not consistent with the following guidelines must be described in the case notes.

## 2 Equipment/Materials/Reagents

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

## 3 Procedures

Refer to the DNA procedures introduction (i.e., DNA QA 600) and follow applicable general precautions and cleaning instructions.

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| **3.1** | For each item examined, an indication of the packaging condition (e.g., properly sealed envelope) and a description of the item(s) and the sample(s) collected from the item, if appropriate, will be captured in the case notes.<br>-The description of the item should be enough to distinguish it from other items in the case.<br>-The description of the collection should be enough to allow another biologist or an examiner to determine approximately where the sample was collected from. | |

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.

Multiple samples may be combined into a single tube, as appropriate.

## 3.2 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.

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| **3.2.1** | 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. | |

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| **3.2.2** | Gently invert the blood tube until the contents are thoroughly mixed.<br>Record the fill-level of the tube by marking its meniscus on the outside of the tube. | |

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| **3.2.2.1** | *If the blood is coagulated:*<br>Slowly transfer the clotted blood into the reservoir of an autoclaved tissue homogenizer.<br>Insert the glass pestle into the reservoir and gently homogenize the clotted material and serum. | |

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| **3.2.3** | 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.<br>Record the volume of blood used to prepare the stain in the case notes. | |

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| **3.2.3.1** | *If the blood was homogenized:*<br>Return the unused homogenate to the original blood tube.<br>Ensure the glass homogenizer and pestle are rinsed with 10% bleach solution, washed, and rinsed with reagent grade water. | |

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| **3.2.4** | 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.

### 3.3 Cutting Dried Stains (e.g., blood stains, semen stains, sexual assault kit swabs, buccal swabs, other evidentiary swabs)

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| **3.3.1** | Cut ~5 mm x 5 mm from the dried stain or ~ ⅓ to ½ of swab head and place it into the appropriate type of labeled tube. | |

For sexual assault kit swabs, typically ~ ¾ of each swab from an orifice is cut and the cuttings from up to 2 swabs are placed in a single tube for extraction.

### 3.4 Swabbing Dried Stains (e.g., blood stains, semen stains)

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| **3.4.1** | Using a sterile swab moistened with reagent grade water, swab a sufficient portion of the stain. | |

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| **3.4.2** | Remove the entire swab head (or a portion of the swab head) and place it into the appropriate type of labeled tube. | |

### 3.5 Recovery from Liquid Blood

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| **3.5.1** | Gently invert the blood tube until the contents are thoroughly mixed. | |

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| **3.5.2** | Transfer an appropriate amount of blood (generally ~ 5 µL) into the appropriate type of labeled tube. | |

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| **3.4.3** | Close and tape seal the tube, and initial the tape seal. | |

### 3.6 Recovery from Moisture-Activated Envelope Flaps or Stamps

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| **3.6.1** | Carefully open any sealed envelope flap and/or remove any affixed stamp as appropriate using steam generated from water boiled in a household teapot. | |

*Purified water available at laboratory sinks may be used for steaming.*

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| **3.6.2** | Using a sterile swab moistened with reagent grade water, swab the adhesive area of flap and the corresponding region on the envelope. | |

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| **3.6.3** | Remove the entire swab head and place into the appropriate type of labeled tube. | |

### 3.7 Recovery from Cigarette Butts

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| **3.7.1** | Remove ~5 mm from the filter end of the cigarette butt and place it into the appropriate type of labeled tube. | |

### 3.8 Recovery from Bottles, Cans, Cups

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| **3.8.1** | 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.*

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| **3.8.2** | Remove the entire swab head and place into the appropriate type of labeled tube. | |

### 3.9 Recovery from Tape

#### 3.9.1 By Swabbing

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| **3.9.1.1** | Using a sterile swab moistened with reagent grade water, swab the tape where appropriate. | |

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| **3.9.1.2** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

#### 3.9.2 By Cutting

*Tape may be adhered to a plastic sheet for the preservation of latent prints. Collection can occur after latent processing.*

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| **3.9.2.1** | Take an ~1 inch cutting from the appropriate area(s) (e.g., from the unexposed end) of the tape. | |

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| **3.9.2.2** | Place the cutting with adhesive side facing out into the appropriate type of labeled tube. | |

### 3.10 Recovery from Clothing Items

#### 3.10.1 Wearer Swabbing

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| **3.10.1.1** | 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.*

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| **3.10.1.2** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

### 3.10.2 Male Underwear in Sexual Assault Scenarios

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| **3.10.2.1** | View the underwear with the alternate light source. Circle areas of fluorescence with a dotted line. | |

*A photograph should be taken of the item.*

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| **3.10.2.2** | Using a sterile swab moistened with reagent grade water, swab the inside front area(s) of the underwear circled with a dotted line. | |

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| **3.10.2.3** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

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| **3.10.2.4** | Using a sterile swab moistened with reagent grade water, swab the outside front area(s) of the underwear circled with a dotted line. | |

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| **3.10.2.5** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

*Should such areas 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.*

### 3.11 Recovery of Handler DNA from Firearms, Knives, and Weapons

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| **3.11.1** | 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). | |

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| **3.11.2** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

## 3.12 Recovery from Fingernail Clippings

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| **3.12.1** | 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. | |

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| **3.12.2** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

## 3.13 Recovery from Microscope Smear Slides

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| **3.13.1** | Using a sterile swab moistened with reagent grade water, remove ~½ to ¾ of the smear from the microscope slide. | |

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| **3.13.2** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

## 3.14 Recovery from Microscope Smear Slides with Cover-Slips

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| **3.14.1** | Attempt to remove the cover-slip from the microscope slide, using a scalpel if necessary. | |

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| **3.14.2** | 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.*

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| **3.14.3** | Using a sterile swab moistened with reagent grade water, remove ~½ to ¾ of the smear from the slide and the cover-slip. | |

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| **3.14.4** | Remove the entire swab head and place it into the appropriate type of labeled tube. | |

## 3.15 Recovery from Liquid Rinses or Washes

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| **3.15.1** | 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.*

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| **3.15.2** | Transfer the supernatant from over the pelleted material into an appropriate tube. | |

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| **3.15.3** | Collect pelleted material onto a sterile swab. Remove the entire swab head and place it into the appropriate type of labeled tube. | |

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| **3.15.4** | Transfer the supernatant back into the original tube. | |

### 3.16 Recovery from Biological Tissue

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| **3.16.1** | Dissect ~1 $cm^3$, if available, from the tissue mass and mince. | |

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| **3.16.2** | Place minced tissue into the appropriate type of labeled tube(s) so that tube(s) is ~ ⅓ to ½ full. | |

### 3.17 Recovery from Paraffin-Embedded Biological Tissue

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| **3.17.1** | Dissect ~1 $cm^3$, if available, from the tissue mass and mince. | |

*Remove any excess paraffin, if possible.*

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| **3.17.2** | Place minced tissue into the appropriate type of labeled tube(s) so that tube(s) is ~ ⅓ to ½ full. | |

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| **3.17.3** | 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. | |

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| **3.17.4** | Spin to pellet (generally 9,000 to 13,000 rpm for 5 minutes). | |

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| **3.17.5** | In a fume hood, decant the supernatant into an appropriately labeled container suitable for xylene waste. | |

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| **3.17.6** | Repeat the xylene wash procedure. | |

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| **3.17.7** | Add 95% ethanol equivalent to half the volume of xylene and vortex. | |

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| **3.17.8** | Spin to pellet (generally 9,000 to 13,000 rpm for 5 minutes). | |

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| **3.17.9** | Decant the supernatant into an appropriately labeled container suitable for ethanol waste. | |

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| **3.17.10** | Repeat the ethanol wash procedure. | |

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| **3.17.11** | 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.*

### 3.18 Recovery from Aborted Tissue

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| **3.18.1** | 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.*

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| **3.18.2** | 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).*

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| **3.18.3** | Dissect ~1 $cm^3$, if available, from the selected tissue and mince. | |

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| **3.18.4** | Place minced tissue into the appropriate type of labeled tube(s) so that tube(s) is ~⅓ to ½ full. | |

### 3.19 Recovery from Hair Samples

*Refer to the DNA procedure for the extraction of DNA from hair and keratinized tissue (i.e., DNA 402) for additional collection and wash guidance for hairs to be extracted for mitochondrial DNA processing.*

#### 3.19.1 Recovery from Hair Samples to be Used as a Reference Sample

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| **3.19.1.1** | Rinse the hair thoroughly with 95% ethanol. Discard the wash into an appropriately labeled container suitable for ethanol waste. | |

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| **3.19.1.2** | Follow the ethanol rinse with a thorough rinse with reagent grade water. | |

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| **3.19.1.3** | Remove ~1 cm from the root end and place it into the appropriate type of labeled tube. | |

### 3.19.2 Recovery from Hair Samples of Unknown Origin

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| **3.19.2.1** | Remove ~1 cm from the root end and place it into the appropriate type of labeled tube. | |

### 3.19.3 Recovery from Mounted Hair Samples

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| **3.19.3.1** | Attempt to remove the cover-slip from the microscope slide, using a scalpel if necessary. | |

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| **3.19.3.2** | 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. | |

*Xylene must be handled in a fume hood.*

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| **3.19.3.3** | Rinse the hair with ~5 mL 95% ethanol. Discard the wash into an appropriately labeled waste container suitable for ethanol waste. | |

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| **3.19.3.4** | Follow the ethanol rinse with a thorough rinse with reagent grade water. | |

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| **3.19.3.5** | Remove ~1 cm from the root end and place it into the appropriate type of labeled tube. | |

## 4 Sampling or Sample Selection

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.

## 5 Standards and Controls

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

## 6 Calculations

Not applicable.

## 7 Measurement Uncertainty

Not applicable.

## 8 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.

## 9 Safety

**9.1** 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. Follow the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual*.

**9.2** Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

**9.3** 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.

## 10 References

*FBI Laboratory Quality Assurance Manual*

*FBI Laboratory Operations Manual*

*FBI Laboratory Safety Manual*

*DNA Procedures Manual*

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.

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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.

| Rev. # | Issue Date | History |
|---|---|---|
| 12 | 03/08/16 | This procedure may also be used by BAU DNA personnel.<br>Minor wording edits throughout.<br>Added ALS to 2.<br>Added reference to evidence management and examination practices and procedures.<br>Revised 3.1 to give guidance for items/sample descriptions.<br>3.8.2 Added specific guidance for cutting tape.<br>3.10 Specified for handler DNA.<br>3.16 Reworded removing excess paraffin guidance.<br>4 Reworded sample selection, applies to DNA personnel. |
| 13 | 02/28/18 | 1 Reworded scope<br>2 and 3.2 Added blood spotting guidance from SOP 108-5.<br>Renumbered remaining sections.<br>3.1 Changed info captured in case notes from should to will.<br>3.3.1 Added guidance for typical SAK swab collection.<br>3.6.2 and 3.8.1 Removed repeat with second swabbing. |

**Approval**

Redacted - Signatures on File

DNA Technical Leader Date: 02/27/2018

DCU Chief Date: 02/27/2018

BAU Chief Date: 02/27/2018

**QA Approval**

Quality Manager Date: 02/27/2018