

## FBS01 – Guidelines for Forensic Biological Evidence Examination

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### 1. Scope

- 1.1. Analysts should follow the guidelines listed below when analyzing evidentiary items for the presence of biological fluids.

### 2. Background

- 2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.

### 3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

## 4. Materials Required

- 4.1. Not applicable

## 5. Standards and Controls

- 5.1. All sample collection procedures must be performed in dedicated laboratory space to maintain their separation from all sources of amplified DNA product.
- 5.2. All sample collection procedures must be performed using pipettes dedicated to pre-PCR amplification set-up activities.
- 5.3. To maintain a separation in time and space between questioned and known samples, all questioned items of evidence must be fully examined (inventoried, processed, cut, and re-packaged) before the known samples are examined (inventoried, processed, cut, and re-packaged).
- 5.4. To maintain separation in time and space between individual samples, each sample collected must be placed in a sample tube, and that tube closed, before any other sample from that item can be collected. If no other sample is to be taken from an item, then that item must be repackaged before the next item may be opened and processed for sample collection.
- 5.5. Only one case will be examined at a time and only one sample will be opened and processed at any one time.
- 5.6. Casework notes must be recorded contemporaneously with each procedure.

## 6. Calibration

- 6.1. Not applicable

## 7. Procedures

- 7.1. Prior to the analysis of evidentiary material, an evaluation of the important elements of each case shall be obtained through communication (e.g., police reports, medical reports, discussions with investigators, etc.) with the submitting agency and/or attorneys. This evaluation should include an assessment of the

evidence and its relevance. A Schedule of Analysis will be prepared based on this information directing which items are to be tested for the case.

- 7.2. Each evidence item will be examined separately on a clean work surface. (NOTE: examining each item individually will avoid handling errors and reduce the potential for cross-contamination.) The examination of each evidence item will include an assessment for all potential physical evidence (e.g., physiological fluid stains, hairs, fibers, gunshot residue, latent prints, etc.).
- 7.3. If trace evidence examination has been requested or is warranted, the evidence should be examined by the Materials Science Unit prior to the Forensic Biology Unit, Firearms & Tool Marks Unit, or Latent Fingerprint Unit examinations.
- 7.4. If trace evidence is encountered during an examination, the evidence should be collected prior to any further examinations, labeled to indicate what it is and where it was located, and then properly packaged to prevent loss.
- 7.5. The FBU will only make gross observations regarding evidence suspected of being hairs or fibers. Analysts will refer to this type of evidence as “possible hairs” or “possible fibers” in case notes and reports. Characteristics such as color, length, convolution and presence/absence of adhering material may be noted. Detailed characterization (e.g., stage of root growth, species of origin, condition, fiber type, etc.) and comparisons will only be made by qualified trace evidence analysts.
- 7.6. Evidence items should be processed for latent prints prior to submission to the FBU for examination. If an item is to be examined by the FBU prior to latent print processing, the analyst should take all necessary precautions to ensure any latent prints are not compromised during the examination (e.g., wear cotton gloves under disposable gloves).
- 7.7. Detailed information including item description, size, color, condition, visible stains, etc., should be recorded on an appropriate worksheet. In addition, record location of apparent stab or bullet holes and/or other obvious damage. All stains on the item (evidentiary or otherwise) must also be documented to note the location, size, color, condition, visibility, etc. Photographs and/or diagrams may also be included to document the condition of the evidence prior to, or during examination(s).

7.8. Evaluate each stain in the following systematic manner and record observations on the appropriate worksheets. Depending on the information obtained from the submitter, not all questions will be addressed.

7.8.1. Is the stain blood?

7.8.1.1. This question is answered by visual, chemical, and immunological testing. Normally, bloodstains are fairly easy to locate due to their distinctive red/brown color. However, if they occur on a dark colored background, are faint or small in size, they may prove to be difficult to locate without the use of careful searching techniques and specialized lighting. The shape and size of the stain can be important evidence and should be documented (e.g., notes, diagrams, photography) prior to actual sampling, which might alter the interpretive value of the stain pattern. The information recorded should include location, size, color, and may contain additional information such as: shape, concentration (e.g. diluted), and/or stain type (e.g., smear, transfer, droplets, etc.).

7.8.1.2. A chemical test, such as phenolphthalein (FBS02), determines the presumptive presence of blood. A positive presumptive test alone should not be interpreted as a positive identification of blood. However, a negative presumptive test provides proof of the absence of detectable quantities of blood. The presence of blood may be confirmed by Hematrace testing. When performing these tests and the additional tests listed below, appropriate control samples shall be used to evaluate the chemical reagents.

7.8.2. Does the stain contain semen or seminal fluid?

7.8.2.1. This question is answered by visual, microscopic, chemical and/or immunological testing. Potential semen stains can be visually located with the use of careful searching techniques and specialized lighting (FBS04). Chemical tests can be used to determine if a stain presumptively contains semen by detecting the presence of the enzyme acid phosphatase (FBS05). The presence of semen may be confirmed through immunological testing (FBS06) or the identification of spermatozoa (FBS07). When performing these tests, appropriate control samples shall be used to evaluate the chemical reagents.

7.8.3. Is the stain a mixture of body fluids?

7.8.3.1. This question can be answered by performing a panel of chemical, microscopic and immunological tests.

7.8.4. Is the stain human?

7.8.4.1. This question is answered through the use of a human specific PCR test (e.g., Quantifiler<sup>®</sup> Duo kit, AmpF $\ell$ STR<sup>®</sup> PCR Amplification Kits, etc.), it may be inferred that the biological material is of human origin.

7.8.5. What samples should be collected for further testing?

7.8.5.1. Based on the provided case information, the analyst should evaluate the evidence and determine which samples have probative value. A portion of any probative sample should be collected for DNA analysis, placed into a sterile microcentrifuge tube, appropriately labeled and stored frozen. The size of the sample collected will depend on various factors including the stain concentration, size, and/or the results of presumptive and confirmatory testing. Every effort will be made to retain a portion of the evidence, either in its original form or as an extract. If a stain must be consumed in order to obtain the most complete DNA profile possible, written permission must be given by the appropriate submitter or attorney.

7.8.5.1.1. Refer to LOM01 – Practices for the Examination of Evidence, Section 5.3.2 for evidence subdividing procedure for creation of unique identifying numbers for stain/swab cuttings to be taken forward for DNA analysis.

7.8.5.2. Available reference samples should also be collected for typing and comparison. These samples are typically received as liquid blood, bloodstains, saliva stains or buccal swabs. If received as liquid blood, a portion should be spotted onto a bloodstain card and dried for preservation purposes.

7.8.6. Who could have contributed the stain(s)?

7.8.6.1. This question is answered through the use of DNA typing systems (STR polymorphisms). The cutting or swabbing is taken through a series of temperature and chemical processes in order to extract the DNA from the cells, assess the quantity of the DNA obtained,

generate multiple copies of the DNA and finally establish the DNA profile(s) of the contributor(s) of the original biological material.

- 7.8.7. What is the distribution of the genetic profile among the general population?
- 7.8.7.1. Statistical interpretation of the DNA profiles requires population studies of different racial or ethnic groups in the DNA testing systems. These studies establish genotypic frequencies of polymorphic loci in various population groups and are found in the literature and/or are compiled by individual laboratories.
  - 7.8.7.2. The significance of a DNA profile is expressed in terms of match probability. This is accomplished by determining the frequency of occurrence for the particular combination of genotypes in the DNA profile. The frequency of occurrence is simply the product of the single locus genotype frequencies, provided each locus is inherited independently.
  - 7.8.7.3. The significance of a DNA mixture is expressed in terms of combined probability of inclusion (CPI). This is accomplished by determining the frequency of the combination of alleles occurring at each locus of a DNA mixture.
  - 7.8.7.4. The reliability of a particular test or analysis can be demonstrated through the use of appropriate methods, controls, standards, blanks and proficiency tests. Unexpected results and/or results that cannot be interpreted or compared should, as appropriate, receive further evaluation (refer to *FBS15 – Identifiler Plus Interpretation Guidelines* for specifics), such as:
    - 7.8.7.4.1. Re-evaluation of sampling method
    - 7.8.7.4.2. Evaluation of sample age and possible substrate interferences
    - 7.8.7.4.3. Re-analysis using the same conditions
      - 7.8.7.4.3.1. The original unique identifying number for samples reinjected from the same plate will be maintained
      - 7.8.7.4.3.2. Samples rerun on a new plate will have the designation “r1” added to the end of their unique identifying number

7.8.7.4.3.2.1. Multiple reruns of the same sample in different wells on the new plate will receive sequential "r[number]" designations

7.8.7.4.3.3. Subsequent reruns on new plates will receive sequential "r[number]" designations

7.8.7.4.4. Re-analysis using different conditions or alternative methods

7.8.7.4.4.1. Additional cuttings from a stain or swab will have the following designation added to the end of their unique identifying number:

7.8.7.4.4.1.1. First recutting: "c1"

7.8.7.4.4.1.2. Subsequent recuttings will receive sequential "c[number]" designations

7.8.7.4.4.2. Reamplifications (reamps/reamp) will have the following designation added to the end of their unique identifying number:

7.8.7.4.4.2.1. First reamp: "a1"

7.8.7.4.4.2.2. Subsequent reamps will receive sequential "a[number]" designations

7.8.7.4.5. Recognition of ambiguous results; report as "inconclusive"

7.8.7.4.6. Refer to supervisor for direction

7.8.7.4.7. Independent re-analysis by another qualified analyst

7.8.7.4.8. Evaluation of the amount of remaining evidence and value of re-analysis

7.9. Casework notes are intended to: (a) refresh the analyst's memory; (b) document the approach, observations, methodology, results and conclusions; and (c) allow interpretations to be made.

- 7.10. Casework notes should be made contemporaneously to the testing and examinations, and must reflect analytical results and observations. Notes are permanent records that reflect evidence conditions, techniques and methodology used, data and conclusions. Casework notes are the basis for the written report. Reports are prepared in accordance with laboratory policy.
- 7.11. Any deviation from laboratory protocol must first be approved by the DNA Technical Leader or designee and must be indicated in the case notes. Otherwise, it may be assumed that the analysis proceeded according to the laboratory policies and procedures.
- 7.12. The final step in the process is to write a report which reflects all of the testing and interpretations from the case. The entire case file and report are then technically and administratively reviewed to verify that all conclusions are appropriate and supported by documentation.
- 7.13. If there is a conflict, uncertainty or dispute between the conclusions of the analyst and reviewer, the case will be discussed and reviewed with the DNA Technical Leader. If necessary, the final conclusion of the Technical Leader will stand as the official conclusion for the report, and must be adhered to by the original analyst.

## **8. Sampling**

- 8.1. Not applicable

## **9. Calculations**

- 9.1. Not applicable

## **10. Uncertainty of Measurement**

- 10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6)*.

## 11. Limitations

- 11.1. Appropriate measures should be taken throughout the testing process to avoid contamination. The precautions listed below should be followed during each procedure to guarantee quality of work and accuracy of results.
  - 11.1.1. All work surfaces are thoroughly cleaned with 10% bleach and may be followed by 70% ethanol prior to and after the examination of each separate item in a case. This practice can also be performed more often if examining a heavily soiled item of evidence.
  - 11.1.2. Disposable bench paper is used to prevent the accumulation of biological material on permanent work surfaces. At a minimum, the paper is changed between items of evidence or more frequently if examining a heavily soiled item of evidence. Disposable bench paper is discarded in appropriate containers. Disposable bench paper will be placed in biohazard trash only when visibly soiled with other potentially infectious materials (OPIM).
  - 11.1.3. Wear appropriate personal protective equipment (e.g., lab coat, gloves, masks, eye protection, and/or hair net). Change gloves between items of evidence or more frequently when visibly soiled. Gloves are discarded in biohazard containers if visibly soiled. Hands should be thoroughly washed when leaving laboratory space.
  - 11.1.4. Lab coats are worn at all times. A dedicated laboratory coat must be worn for all pre-amplification sample handling activities. A separate, dedicated laboratory coat must be worn when handling samples that may potentially contain amplified DNA.
  - 11.1.5. Instruments used during examinations are thoroughly cleaned with 10% bleach and may be followed by 70% ethanol before and after coming in contact with an item of evidence. Metal instruments may also be autoclaved to sterilize. After use, disposable utensils are discarded in the appropriate trash.
  - 11.1.6. Any evidence of considerable size (bed sheets, comforters, etc.) should be examined in a size appropriate space. Disposable paper may be placed on the floor under such items if they are suspended for examination.
  - 11.1.7. Biological hoods are thoroughly cleaned with 10% bleach and may be followed by 70% ethanol prior to and after each use. Biological hoods are irradiated with the interior ultraviolet (UV) light after each use.
  - 11.1.8. Pipettes are thoroughly decontaminated with 10% bleach and may be followed by a 70% ethanol before and after use or as needed.

- 11.1.9. Where specified, reagents are autoclaved prior to initial use.
- 11.1.10. In-use reagents are stored in small aliquots to minimize the number of times the stock reagent is opened.
- 11.1.11. All evidence items under active examination are analyzed as far away as possible from other items of evidence under examination by other individual(s) working within a common laboratory space,
- 11.1.12. To prevent indirect transfer of biological material to telephones, computer keyboards, etc., disposable gloves must be removed prior to handling such laboratory equipment.
- 11.1.13. Sterile disposable pipette tips must be used when handling liquid reagents or samples. Always use a new pipette tip when removing extract from a sample tube or when introducing a reagent into a tube containing extract. Discard the tip after use.
- 11.1.14. Before opening a sample tube(s) removed from the refrigerator or freezer storage, quick-spin the tube(s) in a microcentrifuge to return all liquid to the bottom of the tube.
- 11.1.15. During a common procedure step, sample tubes must remain closed unless being processed. Only one sample or reagent tube can be open at a given time during the processing of the individual samples of a case or batch.
- 11.1.16. Prior to leaving the laboratory area, always remove laboratory coat, dispose of gloves and wash hands.

## 12. Documentation

- 12.1. FBU Examination Worksheets
- 12.2. FBU Report of Examination

## 13. References

- 13.1. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)
- 13.2. *DFS Departmental Operations Manual* (Current Version)
- 13.3. *FSL Laboratory Operations Manual* (Current Version)