FBS01 – General Procedures for Forensic Biological Evidence Examination

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1. Scope

1.1. Analysts will follow the procedures listed below when analyzing evidentiary items for the presence of biological fluids and/or DNA.

2. Background

2.1. This procedure is used to established general practices for documenting the examination of biological evidence.

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. A separation in time and space must be maintained between questioned and known samples during inventory, examinations, cutting, and re-packaging.

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. Appropriate measures will be taken throughout the testing process to avoid contamination. The precautions listed below will be followed during each procedure to ensure the quality of work and accuracy of results:

7.1.1. All work surfaces are thoroughly cleaned with 10% bleach 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.

7.1.2. 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).

7.1.3. 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 will be thoroughly washed when leaving laboratory space.

7.1.4. 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.

7.1.5. Metal tools (e.g., forceps, scissors, etc.) used during examinations are thoroughly cleaned with 10% bleach followed by 70% ethanol before and after coming in contact with an item of evidence. Metal tools may also be autoclaved to sterilize. After use, disposable utensils are discarded in the appropriate trash.

7.1.6. Any evidence of considerable size (bed sheets, comforters, etc.) will be examined in a size appropriate space. Disposable paper may be placed on the floor under such items if they are suspended for examination.

7.1.7. All evidence items under active examination are analyzed in distinct workspaces away from other items of evidence under examination by other individual(s) working within a common laboratory space.

7.1.8. To prevent indirect transfer of biological material to telephones and vestibule door handles, clean disposable gloves must be worn to handle such laboratory equipment.

7.1.9. 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.

7.1.10. Prior to leaving the laboratory vestibule area and entering non-laboratory areas, always remove laboratory coat, dispose of gloves and wash hands.

7.2. Prior to the analysis of evidentiary material, when practicable the relevant elements of each case will be evaluated through communication (e.g., police reports, medical reports, discussions with investigators, etc.) with the submitting agency and/or attorneys. This evaluation will include an assessment of the evidence and its relevance. A Schedule of Analysis may be prepared based on this information directing which items are to be tested for the case.

7.3. Each evidence item will be examined separately on a clean work surface. 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.).

7.4. If trace evidence (e.g. hairs, fibers) is encountered during an examination, the evidence will 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. Ideally evidence items will 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 will 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., will be recorded on an appropriate worksheet (i.e., Serology Examination Worksheet [Document Control Number: 1569], Physical Evidence Recovery Kit Worksheet [Document Control Number: 2154], Evidence Listing [Document Control Number: 1316], Reference Sample Inventory Worksheet [Document Control Number: 2250], Swab Inventory with Serology Worksheet [Document Control Number: 2249], Swab Inventory Worksheet [Document Control Number: 2248]) 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 (Note: An analyst may evaluate and test representative stains; however, the evidence exam notes must clearly state that representative stains were taken). Depending on the information obtained from

the submitter, not all questions will be addressed. For items not requiring biological fluid examination(s) skip to section 7.8.4.

7.8.1. Is the stain blood?

7.8.1.1. This question is answered by visual and chemical 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 will be documented (e.g., notes, diagrams, photography) prior to actual sampling, which might alter the interpretive value of the stain pattern. The information recorded will 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.).

7.8.2. 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).

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. Does the sample contain human DNA?

7.8.4.1. It may be inferred that the biological material is of human origin through the use of a human specific PCR test (e.g., Plexor® HY kit, AmpF*ℓ*STR® PCR Amplification Kits, etc.).

7.8.5. Which samples will be collected for further testing?

7.8.5.1. Based on the provided case information, the analyst will evaluate the evidence and determine which samples may have probative value. A sampling of any probative sample may be collected for DNA analysis and placed into an appropriately labeled, sterile microcentrifuge tube. If not immediately processing sample, store in the appropriate location (room temperature, refrigerator, freezer). 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.

7.8.5.1.1. Refer to *LOM01 – Practices for the Examination of Evidence* [Document Control Number: 1315], 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.

7.8.5.1.2. Available reference samples will also be collected for typing and comparison. These samples are typically received as liquid blood, bloodstains, saliva stains or buccal swabs.

7.8.5.1.2.1. If received as liquid blood, a portion will be spotted onto a Whatman FTA® card and dried for preservation purposes and documented on the Liquid Blood Examination Sheet (Document Control Number: 1568).

7.8.6. When samples are to be outsourced or further downstream analyses are to be conducted by different FBU analyst(s), the appropriate casework documentation (e.g., worksheets, data results print-outs, electropherographs, examination report(s), etc.) will be submitted to the outsource lab or assigned FBU analyst(s) accompanying the samples.

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

7.8.7.1. 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 specific areas of the DNA and finally establish the DNA profile(s) of the contributor(s) of the original biological material. The evidence DNA profile(s) are then evaluated to determine if they are suitable for interpretation. If they are suitable for interpretation, the profile(s) may be uploaded to CODIS and/or compared to known/reference DNA profiles to determine if an individual of interest is included or excluded as a possible source of the genetic material.

7.8.8. What is the significance of an inclusion?

7.8.8.1. In 2000 the DNA Advisory Board stated, “When a comparison of DNA profiles derived from evidence and reference samples fails to exclude an individual(s) as a contributor(s) of the evidence sample, statistical assessment and/or probabilistic reasoning are used to evaluate the significance of the association”. SWGDAM Interpretation Guidelines 2010 states, “The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis.”

7.8.8.2. Statistical interpretation attempts to provide meaning/weight to the findings. The significance of an inclusion is expressed in terms of a likelihood ratio (LR). A likelihood ratio is a ratio of the probability of obtaining the evidence (DNA profile) given competing propositions/hypotheses.

$$LR = \frac{\text{Proposition of prosecution (Hp)}}{\text{Proposition of defense (Hd)}}$$

7.8.8.3. The likelihood ratio is calculated using allele frequencies referenced from the AmpFlSTR® Identifiler® Plus Kit population data set and the sub-population model developed by Balding and Nichols in 1994.

7.9. The reliability of a particular test or analysis is demonstrated through the use of appropriate methods, controls, standards, blanks and proficiency tests.

7.10. Further evaluation of samples may be warranted when unexpected results and/or results that cannot be interpreted or compared occur (refer to *FBS21– Identifiler Plus Interpretation* [Document Control Number: 2519] for specifics), such as:

7.10.1. Re-evaluation of the sample selection (e.g. cutting, swabbing or scraping of stain/item)

7.10.2. Evaluation of sample age and possible substrate interferences

7.10.3. Re-analysis using the same conditions. The following labeling conventions will be used.

7.10.3.1. The original unique identifying number for samples reinjected from the same plate will be maintained

7.10.3.2. Samples rerun on a new plate will have the designation “r1” added to the end of their unique identifying number

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

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

7.10.4. Re-analysis using different conditions or alternative methods. The following labeling conventions will be used.

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

7.10.4.1.1. First recutting: "c1"

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

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

7.10.4.1.3.1. First reamp: "a1"

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

7.10.5. Note: All labeling conventions listed above refer to manual processing and documentation of samples. If a laboratory information management system (LIMS) is used for processing and documentation, the associated LIMS labeling conventions will be used.

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

7.10.7. Refer to supervisor for direction

7.10.8. Independent re-analysis by another qualified analyst

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

7.11. Casework notes are the basis for the written report. Reports are prepared in accordance with laboratory policy.

7.11.1. Casework notes will 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.

7.11.2. 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.

7.12. Any deviation from laboratory protocol must first be approved by the DNA Technical Leader or designee and must be documented in the case notes. Otherwise, it may be assumed that the analysis proceeded according to the laboratory policies and procedures.

7.12.1. Refer to FSL Quality Assurance Manual Section 4.9.3 (Document Control Number: 1300) for procedure regarding deviations.

7.13. The final step in the process is to write a report which reflects all of the testing results 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.

7.14. 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 DNA Technical Leader will stand as the official conclusion for the report, and must be adhered to by the original analyst.

7.14.1. The discussion and resolution of the conflict, uncertainty or dispute will be documented in the case file's Activity Communication Log (Document Control Number:1282).

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. Not applicable

11. Limitations

11.1. Not applicable

12. Documentation

12.1. FBU Examination Worksheets

12.2. FBU Report of Examination

13. References

13.1. Balding, D.J. and R.A Nichols, DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. Forensic Science International, (1994). 64: 125-140.

13.2. SWGDAM Interpretation Guidelines for Autosomal STR Typing for Forensic DNA Testing Laboratories, (2010). http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf.

13.3. DNA Advisory Board. (2000). Statistical and population genetic issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database(s). Forensic Sci. Comm, 2(3). https://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2000/index.htm/dnastat.htm.

13.4. Forensic Science Laboratory Quality Assurance Manual (Current Version)

13.5. Forensic Biology Unit Quality Assurance Manual (Current Version)

13.6. DFS Departmental Operations Manual (Current Version)

13.7. FSL Laboratory Operations Manual (Current Version)