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FB Casefile and Laboratory Report Review Training Log Form
FB Case Observation Training Log Form
FB Court Testimony Training Review Log Form
FB Evidence Handling Training Check List Form
FB Safety Training Check List Form
FB Serology Training Check List Form
FB Training Program Review Form
1 INTRODUCTION

Purpose and Scope

1. The purpose of this training manual is to provide a comprehensive training program for all techniques used in the analysis of casework evidence and to provide the trainee with a complete understanding of appropriate laboratory protocols and a historical appreciation of the scientific nature of all analyses conducted in the Forensic Biology Unit. The training program is designed to develop an individual with the required educational background into a qualified forensic analyst by providing the trainee with the knowledge and application of accepted procedures of forensic serology, as well as their legal significance and evidentiary value. This training program applies to Forensic Scientists, Senior Forensic Scientists, and when appropriate Laboratory Analysts.

2. The training program will provide exposure to tests, methods, techniques, and procedures presently used and accepted by the courts and forensic serologists. Additionally, it will provide for an exposure to the pertinent literature available in the field and to the laws governing the handling of evidential materials. The training will concentrate on the methods currently used in the Palm Beach County Sheriff’s Office Forensic Biology Unit thus allowing the trainee to become competent in these as applied to both known and case materials. The training will also provide exposure to court procedures and assistance in developing the skills necessary for effective expert witness testimony.

3. The sequence in which the tasks are presented in the outline should not necessarily be considered as a mandatory order of instruction. Exposure to legal aspects and testimony will be continuous throughout the training.

4. The training program is designed so that the trainee will conduct approximately 200 serological tests by the end of the training modules. The 200 serological tests are comprised of the following:
   a. Approximately 50 stains for presumptive blood testing
   b. Approximately 50 stains for confirmatory blood testing
   c. Approximately 45 stains for presumptive semen testing
   d. Approximately 45 stains for confirmatory semen testing

5. This training program will culminate with, a laboratory bench practical exam, a comprehensive written examination and satisfactory completion of a mock trial using a mock case from the laboratory bench practical prior to conducting case work analysis and/or proceeding with the DNA training. Each module of the competency examination must be successfully completed before the next module will be administered.

6. The laboratory bench practical examination is used to ascertain the trainee’s technical skills and abilities. The analyst will complete analysis of a series of five mock cases for an approximate total of 25 samples. Analysts must achieve 100 percent accuracy (competence test samples) for a passing grade. These samples will cover all aspects of serological methodology. If less than 100% is achieved on this competency, the Technical Leader will determine the appropriate number of cases/stains for re-testing. In the case of an experienced analyst, the number of stains analyzed will be at the discretion of the Technical Leader. The analyst will also be evaluated on evidence handling, documentation, and report writing. This examination may be combined with the Serological bench practical. An 85% or above must be attained to show competency in evidence handling, documentation, and report writing. Any area of deficiency that is identified may warrant additional testing at the discretion of the Technical Leader.
7. The written competency examination is used to ascertain the trainee’s technical knowledge. This examination may be oral and/or written. The results of this examination must be recorded and reviewed with the analyst by the Technical Leader or designee. A passing grade will be awarded for an 85% or greater. If less than an 85% is achieved, a second examination covering the areas of deficiency will be administered at the discretion of the Technical Leader.

8. The trainee will testify to the examinations performed on the mock case at a mock trial, thus likening this test to an actual courtroom situation. A mock case from the bench practical will be selected for the mock trial. Mock trials are to be held in a courtroom or courtroom-like setting including a representative judge, prosecutor, defense and jurors. The current Court Testimony Evaluation form will be completed by any attendee who would like to complete the form. The information on these forms must be formally reviewed with the trainee. All Court Testimony forms will be retained in the analysts training folder. Satisfactory completion of the comprehensive written examination, practical examination, and mock trial must occur prior to the trainee being released to perform independent case approach and identification of biological substances type analysis. The Technical Leader will determine if a passing grade was achieved or if a second mock trial is warranted.

9. Any questions or discrepancies in the training program should be brought to the attention of the trainer.

Training Program

1. The training coordinator will be the Technical Leader. The Technical Leader may delegate certain duties and blocks of instruction to other qualified analysts, but will be responsible for the overall training.

2. It is estimated that this training program can be completed in one to six months, which is to include successful completion of laboratory bench practical, written comprehensive serological exam, and mock trial. Some individuals may require less time than others, depending on such factors as experience and education. The qualifications of the trainee will be evaluated and modifications will be made to this training program as appropriate by the Technical Leader.

3. Unless otherwise stated, the individual's training will occur in the Forensic Biology Unit of the Palm Beach County Sheriff’s Office.

Mock Trial

1. Each case a forensic scientist analyzes has the potential of involving him/her as an expert witness in courtroom testimony. The trainee must never underrate this important aspect of case work. It is the trainer’s responsibility to ensure that the trainee is thoroughly prepared for legal questioning. This can be done by a combination of mock trials, prearranged as well as impromptu question and answer sessions, pertinent literature review, and observation of courtroom testimony given by experienced examiners.

2. A mock trial may take place after the trainee has completed a block of this training protocol and a practical examination of a case incorporating that block of the training. The case will be fabricated so that the trainer knows the correct answers. The fabricated case thus serves as a monitor of the trainee's competency in applying techniques and procedures to actual casework examinations.
3. All mock trials will cover both in-depth technical questioning appropriate for a courtroom setting, as well as the typical chain of custody and standard procedural questioning. The mock trial should serve as a constructive learning process and a good evaluation tool.

4. The scheduling of mock trials is to be done by the trainer. Mock trials may be conducted in a Palm Beach County courtroom or within the Forensic Sciences Division or Technical Services Division of the Crime Laboratory. The atmosphere of the trial will be formal. That is, it will be conducted in the same manner as a real courtroom situation. This includes conduct, protocol, and all other aspects.

5. Harassment of the expert witness by defense counsel or prosecutor will be kept to the minimum necessary to achieve the desired goal. Questioning by both the prosecutor and defense attorney(s) should be relevant and realistic.

6. It cannot be overemphasized that testimony training is just as important as the analytical training. The trainee must successfully meet acceptable performance standards in both areas before he/she is deemed to be qualified to conduct forensic examinations on evidential material.

7. The outcome of the trial evaluation will be:
   a. Satisfactory
   b. Not Satisfactory: If the Technical Leader determines that the trainee’s performance was not satisfactory, steps must be taken to effect the appropriate action.

8. Satisfactory performance on technical aspects and testimony must be achieved before the individual is qualified to perform the duties of an analyst.

### Transition from Trainee to Analyst

1. After the trainee has successfully completed all training (case approach, identification of biological substances, and DNA analysis when applicable), the Technical Leader or designee must ensure that the transition from trainee to qualified analyst takes place as smoothly as possible. Once an analyst is deemed qualified, supervised casework is not required.

2. For a period of time to be determined by the Technical Leader, all of the newly qualified analysts’ reports must be reviewed prior to release by the Technical Leader or designee. Once it is determined that there are no systemic issues, the newly qualified analyst will use the Internal Review log located on the PBSO portal for random staff reviews.

### Instructions for the trainer

1. The intent of the training program is to ensure that each and every trainee is provided with certain basic principles and fundamentals necessary for the complete education of an analyst in the Forensic Biology Unit. All of the listed topics must be incorporated into the training program. However, education and prior experience of the trainee will be used as a guide to determine the amount of time devoted to each topic. Some of the topics will suggest an order of events and this ranking should be followed. Any deviation from the contents of the training program must be approved by the Technical Leader.

2. The trainer must go over the training program in its entirety with the trainee prior to the initiation of training. The training program may be altered at the discretion of the Technical Leader.

3. The trainer will conduct a practical demonstration of all applicable Forensic Biology Unit serological procedures prior to the trainee conducting training assignments.

4. The completed checklists or logs will be signed by the Technical Leader and retained in analyst’s training folder.
5. The Technical Leader must approve and sign off on all training programs and training records prior to completion of training and initiation of case work.

6. When the trainee has satisfactorily completed all training requirements:
   a. The Technical leader will award certificates of completion for the training program stating that the trainee may conduct case work analysis.
   b. A memorandum will be issued by the Technical Leader to the Crime Laboratory Director stating what job duties or analyses that the analyst is authorized to perform.

7. The training should culminate so that the trainee has the following:
   a. Knowledge of the principles and practices of forensic serology as these relate to the analysis of case material.
   b. Knowledge of the theory and application of instrumentation and specialized techniques used to examine biological evidence.
   c. The ability to perform accurate forensic analyses independently and proficiently, to accurately document the findings of all analyses in accordance with Quality Assurance Manual and Forensic Biology Unit’s policies and procedures, and to accurately report those findings in a laboratory report.
   d. The ability to skillfully present and defend analytical findings in a court of law.

8. If the trainee cannot meet the criteria expected of him/her during the period allowed for training, steps will be taken to effect the appropriate action.

9. All serological, DNA training and new technologies, methodologies, or platforms utilized in the Forensic Biology Unit training records will be retained permanently in either the Forensic Biology Unit, the Crime Laboratory’s limited access storage location, or scanned to the limited access network.

Instructions for the Trainee

1. The trainee is expected to keep accurate records of all training data. It is important that this data be kept up-to-date and organized in a training notebook.

2. The notebook may be organized by subject or in chronological order. Once an organizational style is chosen, the trainee must maintain the organizational style throughout the course of training.

3. For each procedure performed, comments/notes should include the following, where appropriate: principle, sensitivity, specificity, interpretation of results, possible interferences/problems, and comments, including comparison to other methods.

4. Literature references are an important part of the training program. Required readings are listed in the Appendix of this manual and cover the material needed for an adequate understanding of the subject matter. Electronic copies of the appropriate literature are located in the Forensic Biology Unit’s electronic reference library. It is the trainee’s responsibility to read the pertinent literature prior to attending the training demonstration.

5. Literature references for the FB Unit Methods Manual are listed in the respective protocols and are located on the Forensic Biology Unit’s electronic reference library. The trainees should be familiar with the literature used to develop the Forensic Biology Unit protocols, however these references are not required reading.

6. All trainees will be directed to the location of the current Forensic Biology Unit Methods Manual and all appropriate Forensic Biology Unit Training Manuals on the PBSO document control center and in the laboratory. It is the trainees responsible to review the protocols prior to attending or conducting training modules. Hardcopies of any protocol or individual worksheet in the trainee’s training notebook are kept for reference purposes only.

7. Each trainee is responsible for reading the Quality Assurance Manual.
8. All training requirements are outlined in the Forensic Biology Unit’s training manual(s). The trainee must sign the FB Training Program Review form stating that they understand the requirements of the training program.

9. The trainee will be exposed to the evaluation of many forensic-type samples during training. This also includes the duties and responsibilities of the secondary reviewer including case file review.

10. The trainee will document the completion of each required training task on the designated checklist for that aspect of training.

11. The completed checklists will be retained by the trainee in the appropriate sections of his/her training notebook.

12. All appropriate safety, evidence handling, and biohazard protocols must be followed.

13. A list of study questions for each training topic is located at the end of each section in this manual. The trainee is encouraged to write out the answers to the questions after completing the required tasks and readings for the section.
2 SAFETY

Goals

1. Ensure that the trainee is familiar with work hazards.
2. Educate the trainee on how to protect themselves so that the incidence of illness and injuries due to hazardous chemicals and biological agents are reduced.

Hazards

1. An analyst in the Forensic Biology Unit must be acutely aware of the potential hazards inherent in his/her work. These hazards include, but are not limited to:
   a. infectious agents, such as those associated with:
      i. Hepatitis
      ii. AIDS
      iii. Sexually transmitted diseases
      iv. Parasitic infections
      v. Bacterial infections
   b. hazardous materials, such as acids, bases, carcinogens, mutagens, and teratogens.

Safety Procedures

1. All trainees are required to read and be familiar with the Crime Laboratory’s Safety Manual and Chemical Hygiene Plan.
2. All trainees must wear the appropriate personal protection equipment when conducting analytical procedures or secondary reviews in the laboratory. Appropriate personal protective equipment includes but is not limited to:
   a. Gloves
   b. Safety glasses
   c. Face masks
   d. Lab coats
   e. Bouffant caps
   f. Closed toe and closed heel shoes
3. All trainees must be aware of the location of the Material Safety Data Sheets (MSDS) and read and be familiar with the prescribed precautions for the handling of all chemicals used in a particular procedure before performing the procedure.
4. All trainees must follow the prescribed cleaning procedures for laboratory work areas and equipment.
5. All biological materials and containers/supplies that have come in contact with biological materials and/or hazardous chemicals will be placed in an appropriate biohazard container and will be disposed of according to approved guidelines.
6. All glassware for disposal will be placed in a broken glass containers or a biohazard sharps container and will be disposed of according to approved guidelines.
7. Hazardous chemicals will be retained in labeled containers in the Crime Laboratory’s chemical bunker until picked up by a disposal company.

Tasks
The safety training program is divided into three modules.

1. Introduction to the safety program:
   a. Purpose of training program
   b. Who is covered
   c. Employee responsibility

2. Work place Hazards:
   a. Why a written program is important
   b. Requirements of safety program
   c. MSDS labeling and chemicals in the work place

3. Personal Protective Equipment, Blood borne Pathogens, Exposures:
   a. Payment for Personal Protective Equipment
   b. Engineering controls
   c. Work practice controls
   d. Establishing a Personal Protective Equipment program
   e. Types of Personal Protective Equipment
   f. Blood borne pathogens
   g. PBSO exposure reporting information

4. The PBSO orientation program explains the blood borne pathogen program for all employees.

5. Exposure reporting must follow appropriate PBSO General Orders.

6. Read the FB safety protocol.

Note: Trainees who have previously received safety training are not required to complete the safety training module.

Training Evaluation

1. The trainee must attend the Forensic Biology Unit safety training lecture and PowerPoint presentation, when applicable.
2. Review of the trainee’s Safety checklist by the Technical Leader
3 RECEIVING AND HANDLING PHYSICAL EVIDENCE

Goals

1. To obtain a working knowledge of factors influencing the deterioration of evidence as these relate to proper vs. improper packaging, handling, and storage.
2. To develop a thorough understanding of evidence handling procedures, including preservation of chain of custody and the use of the Laboratory Information Management System (LIMS).
3. To acquire a basic knowledge of bloodstain patterns and surface deposition of stains, including how and when to group stains together for testing.
4. To acquire a thorough understanding of the design and use of Sexual Battery Evidence Collection Kits and Standard Collection Kits.
5. To acquire competency in the recovery and packaging of possible hairs and fibers.
6. To acquire competency in the recovery of biological material from porous and non-porous surfaces.
7. To develop a knowledge of court procedures involving identification and introduction of evidence.
8. To develop a thorough understanding of the necessity for:
   a. Detailed comprehensive notes
   b. Adequate labeling of evidentiary material
   c. Drawings/photographs

Tasks

The Evidence Handling training module is divided into three major parts:

1. Part I: introduction and orientation for PBSO Crime Laboratory Evidence Collection and Submission Procedures including:
   a. Review of the following FBUMM protocols:
      i. FB Case Acceptance
      ii. FB Evidence Control
      iii. FB Evidence Examination
      iv. FB Preservation of Evidence
   b. Review of the Evidence Unit Protocols for submission and retrieval of evidence samples (see Evidence Unit Methods Manual).
   c. Review of documentation associated with accepting evidence from the Evidence Unit.
   d. Review of evidence documentation associated with the Forensic Biology Unit protocols
   e. Tour of the laboratory facilities.
   f. Meeting with an Evidence Technician for tour/training of the Evidence Unit.
   g. Lecture on Quality Assurance and Quality Control.
   h. Lecture on evidence debris removal.
2. Part II: observation of appropriate protocols for the preparation and storage of:
   a. Preservation of SBECK/SAK materials
   b. On-going casework evidence screening procedures as conducted by a qualified analyst.
   c. Instruction from and observation of qualified analysts on how and when to group stains together for testing.
   d. Instruction from and observation of qualified analysts in the recovery of biological evidence from porous and non-porous surfaces.
FB Case Approach, Evidence Handling and Serology Training Manual

e. Documentation of case notes, controls, and test results regarding the appropriate procedures as outlined in the Forensic Biology Unit Methods Manual.

f. Instruction from and observation of qualified analysts in regard to sample size required for DNA testing.

g. Work with qualified analysts in assessing the suitability of forensic specimens for DNA analysis, including appropriate documentation.

3. Part III: Competency exam on evidence handling and serological analysis.

Training Evaluation

1. The trainee should observe a sufficient number of cases processed by a qualified analyst to develop and exhibit a sound technique for handling physical evidence with a wide variety of evidentiary materials. A log of case observation and any pertinent notes will be kept by the trainee.

2. The trainee should examine a sufficient number of cases to develop and exhibit sound technique for grouping stains, recovering and packaging hairs and fibers, recovering biological evidences from porous and non-porous surfaces, and examining Sexual Battery Evidence Collection Kits.

3. Evaluation of case notes and observation logs by the Technical Leader.


5. Review of the trainee’s evidence handling checklist by the Technical Leader.

Receiving and handling physical evidence study questions

1. What is LIMS?

2. What is LIMS used for?

3. How is evidence transferred from an Evidence Technician to an Analyst?

4. What are the appropriate evidence storage locations in the FBU?

5. How is evidence transferred from an analyst to an approved vendor?

6. How are items of evidence versus stains documented on the FB Evidence Worksheet form (known how to itemize evidence)?

7. What is chain of custody?

8. How is chain of custody maintained?

9. How is evidence stored in the laboratory?

10. How is evidence stored in your personal custody when you are not examining it?

11. Who has access to the various storage areas including your personal evidence locker?

12. What is a proper seal?
13. How is the disposition of evidence documented in the Forensic Biology Unit?

14. You receive a known blood sample in a purple top blood tube. How do you preserve this sample to ensure that no degradation occurs?

15. What key pieces of information should be included on every page of your notes/forms?

16. What is a SBECK?

17. An investigator calls and says he has a case that was analyzed by a FBU employee who has since left the laboratory. ABO and enzyme typing were previously done. Now he has a suspect for the case and wants ABO and enzyme typing conducted on the suspect’s sample so that it can be compared to the previous results. What do you tell him?

18. Your Technical Reviewer checked the lot number on the KM reagent and noticed that it had expired. You used this expired chemical in testing a high profile case and testing cannot be redone. Can you rely on the results you obtained? Why or why not?

19. You receive a call from an officer at the scene of an assault. He observes what he believes to be blood on the sidewalk, but doesn’t know how to collect it. What do you tell him?

20. You receive a call from an officer at the scene of a breaking and entering. Apparently the unknown perpetrator cut himself when he broke the window to gain entry. There is blood on glass on the floor and blood on glass still in the window. He needs to know how to collect these samples. What do you tell him about collecting, packaging, and submitting the blood to the lab?

21. Introducing Physical Evidence In Court (Predicate question):
   a. Do you recognize this item of evidence?
   b. How do you recognize it?
   c. What is it?
   d. How did it first come into your possession?
   e. Where did you obtain it?
   f. When did you obtain it?
   g. Is this item in substantially the same condition now as when you first saw it?
   h. What did you do with it?
4 DETECTION OF BLOOD

Goals

1. To develop a basic understanding of the use of presumptive and confirmatory tests.
2. To develop a thorough understanding of the procedures used by the Forensic Biology Unit.
3. To become familiar with the sensitivity and stability of Phenolphthalein (Kastle-Meyer) reagent.
4. To determine the specificity and limitations of the presumptive and confirmatory test for blood.
5. To be able to locate and evaluate stains on evidentiary material.
6. To acquire a thorough understanding of the use of and documentation of controls.

Tasks

1. Obtain the necessary reagents and materials for training:
   a. Ethanol pads
   b. Pipettes (10, 20, 200, 1000µl)
   c. Pipette tips
   d. Sterile cotton swabs
   e. Biohazard container and biohazard sharps container
   f. Disposable gloves
   g. Filter paper
   h. Microscope slides and cover slides
   i. Slide holders for storage
   j. Notebook
   k. Scalpels
   l. KM / HemDirect and AP and P30 positive controls
   m. Bench coats
   n. Sterile transfer pipettes
   o. Scissors
   p. Forceps
   q. 0.5 and 1.5 ml Microcentrifuge test tubes
   r. Test tubes with a recessed cap
   s. All protective gear (face masks, lab coats, bouffant caps, respirator, safety glasses etc.)
   t. Test tube racks
   u. Date stamp
   v. Case file stamp
   w. Review stamp
   x. Methanol
   y. Ethanol
   z. 50 ml conical test tube rack
   aa. 15/50ml conicals
   bb. 15 ml amber conicals
   cc. Bloodstain cards and foil envelopes
   dd. Blood safety tube caps
ee. Evidence tape
ff. Transparent tape/tape dispenser
gg. FBU Tyvek Sero/DNA bag envelopes
hh. Stapler
ii. Pens
jj. Sharpies (include silver or white paint marker for labeling dark items of evidence)
kk. Ruler
ll. Plastic squirt bottle for methanol ethanol, and water
mm. Sterile water
nn. Manila Coin envelopes
oo. Ink pad

2. Read applicable literature. See Appendix: Required Reading.
3. Read applicable protocols prior to the initiation of training assignments: Refer to FBUMM:
   a. FB Phenolphthalein Test
   b. FB Seratec HemDirect
   c. FB Reagents
   d. FB Crime-lite ML2
4. Attend blood detection training lecture(s) and demonstration(s).
5. Prepare reagents used for the Phenolphthalein (Kastle-Meyer) test.
6. Perform the following tests:
   a. Serial Dilution Series
      i. Prepare the following dilutions neat, 1;1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc. (until a negative KM test is achieved) using whole blood and sterile water. Spot each dilution onto a blood stain card, sterile swabs, or filter paper and allow to dry.
      ii. Analyze each dilution for the presence of blood
      iii. Test each stain with the KM reagent
      iv. Record the results
      v. Compare results to the literature and results obtained by other trainees
   b. Analysis of stains on mock evidence
      i. The trainee will place six (6) liquid blood dilutions on several different types of materials (listed below). The trainee must ensure that the dilutions cover the extremes of the KM test for positivity.
         1. Cotton
         2. Denim
         3. Nylon or Polyester
         4. Other
      ii. The trainee will test approximately 10 bloodstains on mock evidence for the presence of KM. The blood stains prepared should produce both strong and weak KM results.
   c. The analyst must conduct analysis and document the results on the following to test KM specificity.
      1. Horse-radish
      2. Celery
      3. Lettuce
      4. Rusty objects
      5. Mushroom
      6. Potato
7. Tomato
8. Cauliflower
9. Cabbage
10. Yeast
11. Other

7. Conduct presumptive blood testing using IR.
   a. Test IR controls with appropriate wave length and goggles as per protocol
   b. Screen mock evidence with IR
   c. Document observations
   d. Record results

8. Obtain Seratec HemDirect cards.

9. Perform the following tests:
   a. Serial Dilutions
      i. Use the dilutions prepared for the Phenolphthalein (Kastle-Meyer) training set.
      ii. Test each dilution.
      iii. Record results.
      iv. Compare the confirmatory test results to the presumptive test results.
      v. Compare the results to the literature, validation notebook, and results obtained by other trainees
   b. Analysis of stains on mock evidence
      i. Use the stains from the mock evidence prepared for the KM training set.
      ii. Record the results.
      iii. Compare the results to the KM results.

10. Competency exam on evidence handling and serological analysis.

Training Evaluation

1. Review of notes in training notebook by Technical Leader.
2. Review of trainee’s completed checklist/training log.
3. Observation by Technical Leader.
4. Mock trial and practical examinations.

Detection of Blood Using Catalytic Tests – Technical Notes

Most of the preliminary chemical tests for blood are based on the detection of hemoglobin by detecting its peroxidase-like activity. Ionic iron forms chelate (ring) structures with many organic compounds and very often such iron-chelates possess catalytic activity in oxidation reactions. An example of a biological catalyst is peroxidase which decomposes hydrogen peroxide or organic peroxides to form free hydroxyl radicals. The heme group of hemoglobin possesses peroxidase-like activity which may catalyze this breakdown of hydrogen peroxide. If no other organic oxidizable compound is present, these radicals decompose to form water and oxygen. If phenolphthalin is present, it will oxidize the colorless reagent to form a colored product.

The peroxidase-like activity of hemoglobin operates in both acidic and basic media, while some of the bacterial and plant enzymes (catalases and peroxidases) are more pH dependent. Fast positive reactions obtained with both tests on a red-brown or other appropriately colored substance can be
Seratec® HemDirect Hemoglobin Assay Confirmatory Test

The Seratec® HemDirect test is a chromatographic immunoassay. It contains two monoclonal murine anti-Hb (human hemoglobin) antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane as a line. The upstream control region contains immobilized polyclonal goat anti-rabbit antibodies that are also fixed on the membrane as a line. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad that contains the dried gold-labeled second monoclonal murine anti-hHb antibody that will bind to the hemoglobin present in the sample. Additionally, the pad contains gold-labeled rabbit antibodies. Through the capillary effect of the membrane, the reaction mixture moves across the membrane towards the test and the control region. The colored gold-labeled rabbit-antibodies will bind to the anti-rabbit-antibody at the control region resulting in the formation of the red control line in the upper part of the result well. This line indicates the correct performance of the test.

If sample contains hHb, the hHb-gold-labeled anti-hHb-antibody complex will bind to the immobilized monoclonal antibody of the test region that recognizes another epitope on the hemoglobin molecule (sandwich complex). The binding is indicated by the formation of an additional line in the test result region.

A high dose hook effect can be observed, if too much free hemoglobin that is not bound to the gold-labeled antibody, reaches the test result region. If the amount of hemoglobin is high, the antibody fixed at the test result region becomes saturated with free hemoglobin. This prevents the binding of the hemoglobin with the gold-labeled antibody, thus repressing the formation of the test result line. The test result appears negative in spite of the presence of hemoglobin in the sample.

Detection of Blood Study Questions

1. What is blood and what is it composed of?
2. What is the purpose of blood in the body?
3. How much blood does the average human have?
4. How long do red blood cell “live” in the body”?
5. What is the structure of Hemoglobin?
6. What is the Phenolphthalein (KM) test?
7. When is the KM test performed?
8. What is the mechanism behind the KM test?
9. What is the purpose of each chemical used for the KM testing?
10. What is the benefit of using a two-step presumptive test?

11. What does a positive KM result tell you?

12. What action would you take if your negative control was positive?

13. What is the purpose of a positive control?

14. Name 2 presumptive tests for blood and 2 confirmatory tests for blood not used by the Forensic Biology Unit.

15. Describe how to conduct the Seratec HemDirect test?

16. What are the principles behind how the Seratec HemDirect test works?

17. What does a negative test result tell you?

18. What is High Dose Hook Effect?

19. What would you do if you expected a sample gave a false negative as a result of High Dose hook effect?

20. Why does the Forensic Biology unit not use the Seratec HemDirect test to identify the presence of human blood?
5  SEMEN IDENTIFICATION

Goals

1. To become competent in the use of alternate light sources for locating semen stains.
2. To learn the physical and chemical characteristics of semen. To learn the theory and use of the various types of microscopes, e.g., stereo, compound, and phase contrast microscopes.
3. To obtain a working knowledge of factors determining the resolution of the microscope, including, but not limited to, total magnification and numerical aperture.
4. To learn proper achievement of Koehler illumination.
5. To learn the theory behind and the techniques for utilizing bright field and phase contrast microscopy.
6. To become competent in extraction techniques, staining techniques, and microscopic examination for spermatozoa.
7. To learn the theory behind the use of chemical (color) tests and immunological tests for semen.
8. To become proficient in the use of the Acid Phosphatase Test and the PSA Semiquant test by Seratec.
9. To develop an understanding of the sensitivity, specificity and limitations of the Acid Phosphatase Test and PSA Semiquant test by Seratec.
10. To be able to locate and evaluate stains on evidentiary material.

Tasks

1. Read applicable literature. See Appendix: Required Reading.
2. Read applicable protocols prior to the initiation of training assignments. Refer to the FBUMM:
   a. FB Crime-lite ML2FB
   b. FB Acid phosphatase Test
   c. FB Semen and Epithelial Cell Microscopy
   d. FB Seratec PSA Semen
   e. FB Reagents
3. Attend semen detection training lecture and demonstration
4. Prepare the acid phosphatase test reagent and perform the following tests:
   a. Serial Dilution
      i. Prepare the following dilution neat, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc. (until a negative AP test is achieved) using liquid semen an sterile water. Spot dilutions onto a bloodstain card, sterile swabs, or filter paper and allow to dry.
      ii. Test each dilution using the AP reagent.
      iii. Record the results.
      iv. Compare the results to the literature and results obtained by other trainees.
   b. Mock evidence
      i. The trainee will place six (6) liquid semen dilutions on several different types of materials. The trainee must ensure that the dilutions cover the extremes of the AP test for positivity.
         1. Cotton
         2. Denim
3. Nylon/polyester
4. Other

ii. The trainee will test approximately 10 semen stains on mock evidence for the presence of AP. The semen stains prepared should produce both strong and weak AP result.

iii. Record the results.

iv. The trainee must conduct AP testing and document the results on the following to test for specificity.
   1. Vaginal secretions
   2. Blood
   3. Urine
   4. Saliva
   5. Rectal swabs
   6. Horse-radish
   7. Mushrooms
   8. Cauliflower
   9. Other

5. Conduct presumptive semen testing using the alternate light source.
   a. Test ALS controls with appropriate wavelength and goggles as per protocol
   b. Screen mock evidence with ALS
   c. Document observations
   d. Record results

6. Obtain Christmas Tree stain and Seratec PSA cards.

7. Conduct confirmatory testing using Microscopic examination and Seratec PSA cards. When conducting Microscopic examination the trainee must:
   a. Apply proper alignment techniques necessary for phase contrast illumination when examining smears for spermatozoa.
   b. Apply proper techniques for obtaining Koehler illumination by examining spermatozoa on smears at various magnifications.
   c. Perform bright field illumination techniques by examining spermatozoa on stained smears.

8. Perform the following confirmatory tests:
   a. Serial Dilution:
      i. Using the serial dilutions prepared in the AP training module perform microscopic examination and testing with PSA cards.
      ii. Record results.
      iii. Determine the sensitivity of the assays based on results.
      iv. Compare the confirmatory result to the presumptive results.
      v. Compare the results to the literature, validation studies, and other trainee results.
   b. Analysis of Mock evidence
      i. Use the stains from the mock evidence prepared for the AP training set.
      ii. Perform microscopic examination and PSA testing.
      iii. Record the results.
      iv. Compare the results to the AP testing results.
   c. Specificity
      i. Use saliva, urine, blood, and vaginal secretions, rectal swabs, and/or other cellular material to test the specificity of the PSA cards.
ii. Using the same samples listed in #8.a.i. above, conduct microscopic examination for the presence of cellular material.

iii. Record the results.

9. Competency exam on evidence handling and serological analysis

Training Evaluation

1. Review of notes in training notebook by Technical Leader
2. Review of trainee’s completed checklist/training log.
3. Observation by Technical Leader.
4. Mock trial and practical examinations.

Technical Notes

Screening items such as clothing or bedding for the presence of semen stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (the Crime -lite ML2). Users must read the Forensic Biology Unit Protocols for the ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If proper eye protection is not worn, permanent damage to the eye may occur. The principle behind the light sources is that semen contains a component(s) which reacts to light between 450 and 455 nm wavelengths. While some sources cite flavins, other sources cite acid phosphatase as being the reactive component in semen. The reaction may either appear as a light stain against a dark background, or in some circumstances, the stain appears darker against a light background. The reaction must be interpreted with caution since other substances (such as, but not limited to, urine, saliva, makeup, detergents, and bleach) may also react to an ALS. Samples exhibiting a reaction to an ALS require further examination to detect and/or confirm the presence of semen.

When the presence of semen is suspected in a stain, the Acid Phosphatase Test, a presumptive chemical test used to screen stains for the presence of semen, is conducted. This test is based on the detection of acid phosphatase, a major component of semen. In the presence of acid phosphatase, the sodium α-naphthyl acid phosphate is hydrolyzed to α-naphthol, which diazotizes with the dye to yield a colored azo-dye. Samples giving a positive reaction to the screening test require further examination to confirm the presence of semen.

Although a positive result with the Acid Phosphatase test is strongly indicative of semen, confirmation of its presence must be established by the identification of spermatozoa or, in the absence of spermatozoa, the detection of p30 (PSA), a human seminal plasma protein. In general, the presence of semen on swabs from a Sexual Battery Evidence Collection Kit is confirmed by the finding of spermatozoa on the corresponding slides. The presence of semen in stains is confirmed by the finding of spermatozoa in an extract of the stain. If the acid phosphatase test suggests the presence of semen, but no spermatozoa are identified on the corresponding slides or in an extract of the stain, semen may be confirmed by the identification of p30 (PSA).
Microscopic Examination

Good resolving power and optimum specimen contrast are prerequisites for good microscopy. Though the optics (ocular, objectives, and sub-stage condenser) may be suitable, proper illumination is of importance. The requirement for a good illumination system is uniform intensity over the entire field of view with independent control of light intensity, size of the illuminated field of view, and angular aperture of the illuminating cone. A field diaphragm on the lamp housing usually controls the size of the illuminated field of view. The angular aperture of the illumination cone is controlled with the sub-stage iris.

The best illumination for most purposes is a special type of illumination known as Koehler illumination (named after August Koehler, 1866-1948). Here, a specific secondary source is imaged in the specimen plane. The particular secondary source in this case is the uniformly illuminated lamp lens framed by the field diaphragm.

With Koehler illumination the imaging of the lamp lens and field diaphragm in the specimen plane yields three distinct advantages: 1) the ray paths are predictable and controllable; 2) the illumination is uniform; 3) the source size - that is, the area illuminated - can be adjusted.

Procedure for Koehler Illumination

1. Using a medium to low power objective (approximately 10X), place a specimen in position and focus.
2. Close the field diaphragm.
3. Focus the image of the field diaphragm by adjusting the substage condenser.
4. Center the field diaphragm using the centering screws on the condenser.
5. Open the field diaphragm so that the rim just disappears beyond the field of view.
6. Adjust the condenser diaphragm (aperture diaphragm) to about ½ of the full aperture.

Principle of the Seratec PSA Semiquant Test

Sample is added to the sample well “S” and if PSA (p30) is present, it will react with the mobile monoclonal antihuman p30 antibody and a mobile antibody-antigen complex is thus formed. This mobile antibody-antigen complex migrates through the absorbent device toward the test area “T”. The Seratec PSA Semiquant test contains two monoclonal murine anti-PSA antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane. The upstream control region and the region of the internal standard (between control and test region) contain immobilized polyclonal goat anti-mouse antibodies. PSA (p30) antibody-dye conjugates cannot bind to the antibody in the test area “T”, but are captured by an immobilized anti-immunoglobulin antibody present in the control area “C” forming a complex. The captured pink dye particles will thus form a band in the control area “C”. A pink band in the internal standard and control “C” area indicate that the test has worked properly and proper procedures have been followed. The presence of three colored lines, one in the test area “T”, in the control area “C”, and in the internal standard area indicates a positive result. A line only in the control area “C” indicates an invalid test, where as a line in the control “C” area and internal standard area indicate a negative test.
Semen Identification Study Questions

1. Know the structure and function of the male anatomy.

2. What is semen?

3. What glands contribute to seminal fluid?

4. Know sperm morphology and function.

5. What is AP and where is it found?

6. What are the limitations of the AP test?

7. What is p30 and where is it found?

8. What is the significance of p30 and under what circumstances would you test for it?

9. What factors can lead to a diminished sperm count in the male ejaculate?

10. Describe the mechanism and the purpose of the chemicals for the AP test. What would a positive result look like and what would a positive result tell you?

11. Describe the mechanism and the purpose of the chemicals for the p30 test.

12. Compare and contrast the different methods for detecting semen stains.

13. How does an alternate light source assist in locating stains? What alternate light sources are used by Forensic Biology Unit (include filters used and wavelengths)?

14. What is the name of the stain used to stain smears for spermatozoa examination (what is the name of Christmas Tree stain A and B)? What is the purpose of each chemical?

15. Describe the appearance of stained spermatozoa using phase contrast and bright field.

16. What factors may affect the persistence of AP and sperm in a living rape victim? What, if any, differences would one expect to find with regard to the persistence of sperm in a victim of rape and murder?

17. What is the average time of persistence of AP and sperm in various body cavities (i.e. vaginal, oral, and rectal)?

18. On average what is the total volume of seminal fluid per normal ejaculate? What is considered a normal sperm count per ml of seminal fluid?

19. What, if any, is the significance of observing only sperm heads versus intact sperm on a slide?
20. Explain the difference between seminal acid phosphatase and vaginal acid phosphatase.

21. If you do not detect a positive AP result on a swab or in a stain, is it possible to identify sperm? Explain your answer.

22. How do you preserve a used condom?

23. If you have some bedding with stains and the stains test AP negative what would be your next step?

24. If you have some swabs that test AP positive and an extract of the swabs is negative for the sperm search, what is your next step?

25. How long would you expect there to be sperm in the female reproductive tract? How about in a stain on bedding?

26. You get a call from an investigator who says he’s working a case in which a victim was raped by her husband. Her previous intercourse with him was 3 days ago. What do you advise the investigator?

27. Describe Koehler Illumination and how this is achieved on the microscope.

28. What is phase contrast microscopy?

29. What is bright field microscopy?

30. What are the major differences between the stereoscope, compound microscope, and phase contrast microscope?

31. What total magnifications are used when examining specimens under low and high power and how does one arrive at the total magnification?

32. What is an objective?

33. What is an eyepiece?

34. Briefly describe field diaphragm, aperture diaphragm, and condenser.

35. What are the major parts of the compound microscope?

36. What is a venire and how is it used?

37. What is refractive index and how does it affect microscopy?

38. What is numerical aperture?
Goals

1. To familiarize the trainee with the format and FB Auto Text for report wording used in laboratory reports.
2. To familiarize the trainee with the technical and administrative review process for serological analysis.

Tasks

2. At a minimum mock case reports will be written for five cases to be provided by the trainer. The final number of reports will be determined by the Technical Leader.
3. Review a minimum of 10 previously reviewed Forensic Biology Unit case files for report wording and case file composition. The trainee must keep a log of the case files reviewed along with any pertinent notes, observations, or questions.

Training Evaluation

1. Review of trainee’s mock case files and reports.
2. Review of trainee’s completed case file review log.
3. Observation by Technical Leader.

Note: Basic report wording terminology is outlined in the FB Auto Text Report Wording form and FB Technical and Administrative Abbreviations List.
TESTIMONY AND EXPERT WITNESS QUALIFICATION

Goals

1. To become skilled in expressing results and conclusions orally in a simple, clear, concise, and technically correct manner.
2. To become familiar with legal terminology and criminal law procedures.
3. To become skilled in properly communicating with attorneys, judges, and juries.
4. To become familiar with the use of testimony aids.

Tasks

1. Read applicable literature. See Appendix: Required Reading.
2. Observe and log testimony of qualified analysts.
3. Complete Evidence Handling and Serology mock trial.

Training Evaluation

1. Review of notes and testimony log in training notebook by Technical Leader or designee.
2. Satisfactory completion of comprehensive written exam.
3. Satisfactory performance as an expert witness at mock trial.

Testimony and Expert Witness Qualification Study Questions

1. What is the difference between quality assurance and quality control?
2. How is quality control maintained in your laboratory?
3. Name 3 quality control measures you take in the laboratory?
4. Explain the following:
   a. Proof of chain of custody
   b. Opinion Testimony
   c. Voir Dire
   d. Direct examination
   e. Cross-examination
   f. Leading question
   g. Frye Rule
   h. Daubert
   i. Courtroom procedures
   j. Subpoena
   k. Subpoena Duces Tecum
   l. Objection-Overruled
   m. Objection-Sustained

5. Qualifying a serologist as a witness (Predicate Questions):
   a. Please state your name.
b. What is your occupation?

c. Where are you employed?

d. How long have you been employed with (name of agency/company)?

e. What are your specific responsibilities in your work?

f. What is the extent of your training?

g. Are you a member of any organizations related to your work?

h. Which organizations?

i. Have you authored any papers for either private circulation or publication relating to your field?

j. What subjects did you write about?

k. Were the papers you authored published?

l. If privately circulated, among whom?

m. Is your entire work devoted to your field, or do you have other duties?

n. Have you ever testified as an expert before?

o. In what jurisdiction have you testified as an expert before?

p. Approximately how many times?

q. Would you explain to the jury the nature of the work you do?

r. What is serology?

s. What is the significance of the information you obtain as a result of this type of analysis?

t. Explain tests used for analyzing blood, semen, or touch evidence.
8 Technical and Administrative Case File Review

Goals

1. This training program is designed to lead the qualified and practicing Forensic Scientist or Senior Forensic Scientist through the procedures necessary to qualify to conduct technical and administrative review of serology case files and laboratory reports. This training module is not inclusive of all the techniques and procedures that will be encountered during and after training.

2. To become familiar with the policies and procedures for conducting serology case file and laboratory report reviews.

Tasks

1. Complete a serology report writing and review mentorship with a qualified analyst. The length of the mentorship will last a minimum of four weeks. The length of the mentorship will be determined by the Technical Leader.

2. All serology reports authored by the trainee will be reviewed by an assigned mentor(s).

3. Practical exercises and observations on conducting serology technical and administrative reviews will be conducted during the assigned mentorship.

4. Review the FB Evidence Screening Case File Technical and Administrative Review worksheet with assigned mentor.

5. Review of serology case-file and laboratory reports under the supervision of the assigned mentor. The number of serology case files and laboratory reports reviewed prior to qualification will be determined by the Technical Leader.

Training Evaluation

The trainee should demonstrate a thorough understanding of serology laboratory report writing and serology case file and laboratory report technical and administrative review. This will be monitored by evaluation of the training assignments.
APPENDIX: REQUIRED READING

Blood

Microscopy

Semen


**Alternative Light Source**


**Court Room and Legal Aspects**


2. Symposium: Ethical Conflicts in the Forensic Sciences


