Department of Forensic Science

FORENSIC BIOLOGY SECTION TRAINING MANUAL

CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES

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1 INTRODUCTION

1.1 Purpose and Scope

1.1.1 The purpose of this document is to provide a uniform training program for forensic case approach and the recognition and/or identification of biological samples. It is designed to develop a person with a good scientific background into a qualified forensic examiner by providing the trainee with the knowledge and application of accepted procedures of forensic serology, as well as their legal significance and evidentiary value.

1.1.2 The 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. Most of the training will be concentrated on the methods currently used in the Virginia Department of Forensic Science Forensic Biology Section, thus allowing the trainee to become proficient 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.

1.1.3 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.

1.1.4 Oral and practical examinations and/or mock trials encompassing several topics will be staged periodically. If the case approach and identification of biological substances training will be followed by DNA training, this training program will culminate with a formal oral competency examination and satisfactory completion of a minimum of two informal mock trials using cases previously analyzed by qualified examiners prior to proceeding with the DNA training. If the trainee will begin to perform independent case approach and identification of biological substances type analysis, approximately two weeks after the formal competency examination, a formal videotaped final mock trial will be held on the assigned mock case (practical examination). The oral competency examination is used to ascertain the trainee's technical knowledge. The practical examination, analysis of a validated fabricated case, is used to ascertain the trainee's technical skills and abilities. Finally, the trainee will testify to the examinations performed on the fabricated case at the final mock trial, thus likening this test to an actual courtroom situation. Satisfactory completion of the formal oral and practical examinations and final mock trial much occur prior to the trainee being released to perform independent case approach and identification of biological substances type analysis.

1.1.5 Once the trainee begins DNA training, if the trainee has not been qualified to perform independent case approach and identification of biological substances type analysis, oral and practical examinations and/or informal mock trials related to case approach and identification of biological substances will continue throughout the DNA training to ensure that the information learned remains fresh and the skills honed. In addition, the trainee will continue to work throughout the DNA training UNDER DIRECT SUPERVISION with a qualified examiner to perform case approach and identification of biological substances type analysis.

1.1.6 Upon completion of DNA training, the trainee will undergo a comprehensive oral competency examination, followed approximately two weeks later by a formal videotaped mock trial. The oral competency examination is used to ascertain the trainee's technical knowledge of case approach, identification of biological substances, and DNA analysis, with the emphasis on DNA and how case approach and identification of biological substances integrates with DNA analysis. The practical examination, analysis of a validated fabricated case, is used to ascertain the trainee's technical skills and abilities in case approach, identification of biological substances, and DNA analysis. Finally, the trainee will testify to the examinations performed on the fabricated case at a mock trial, thus likening this test to an actual courtroom situation. Satisfactory performance in all areas (case approach, identification of biological substances, and DNA) is required prior to upgrading a trainee to a fully qualified examiner in the Forensic Biology Section.

1.2 Coordination of the Program

1.2.1 The training coordinator will be an experienced examiner. The coordinator may delegate certain duties and blocks of instruction to other qualified examiners, but will be responsible for the overall training.

1.3 Training Period

1.3.1 It is estimated that this training program can be completed in three to four months, which is to include successful completion of the formal oral examination and a minimum of two mock trials (if case approach and identification of biological substances will be part of final qualifications) or a formal oral examination and mock trial (if qualified to independently perform case approach and identification of biological substances independent of DNA). 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. The length of the training period is a matter which will be left to the discretion of the Forensic Biology Section Chief in consultation with the trainee's supervisor and training coordinator.

1.4 Location of Training

1.4.1 Whenever practical, the bulk of an individual's training will occur in the laboratory to which he/she will be assigned. If this is not possible, the training will be conducted at the most convenient laboratory. Certain phases of the instruction may be scheduled at any of the four laboratories. Such arrangements will be made through the Forensic Biology Section Chief.

1.5 Mock Trials

1.5.1 Each case a forensic examiner analyzes has the potential of involving him/her as an expert witness in courtroom testimony. The trainee must never underrate this important aspect of the work. It is the training coordinator'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.

1.5.2 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 training coordinator knows the correct answers. The fabricated case thus serves as a monitor of the trainee's proficiency in applying techniques and procedures to actual casework examinations.

1.5.3 A final mock trial will incorporate all aspects of this training program and will be held subsequent to the final practical examination of a fabricated case. THE TRAINEE WILL NOT RECEIVE THE FINAL MOCK CASE UNTIL ALL PHASES OF THIS TRAINING PROTOCOL HAVE BEEN SATISFACTORILY COMPLETED.

1.5.4 If the individual has no prior testimony experience, a minimum of 2 mock trials with attendant practical examinations are required prior to the final mock trial.

1.5.5 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. Each mock trial should serve as a constructive learning process and a good evaluation tool.

1.5.6 The scheduling of mock trials is to be done by the training coordinator as frequently as he/she deems necessary. Trials may be conducted at any of the regional laboratories, if so desired; however, the final comprehensive mock trial must be conducted in the Central Laboratory. This will be videotaped for viewing at a later date and can be used to identify weak and strong points of the trainee's testimony. The videotape will be retained for future training purposes.

1.5.7 Other related legal training will be integrated into the program on a continual basis.

1.5.8 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.

1.6 Guidelines for Comprehensive Oral Examination and Final Comprehensive Mock Trial

1.6.1 Approximately two weeks prior to the final mock trial, an informal oral examination of the trainee will be conducted by the section supervisor, and the Section Chief of the Forensic Biology Section to ascertain the technical knowledge of the individual. This will be limited to two (2) hours. Questions should be confined to technical aspects of the training and should be used to ascertain whether the goals, as set forth in each technical portion of the training program, have been achieved.

1.6.2 Immediately following the oral examination the trainee may be released while the supervisor and Section Chief of the Forensic Biology Section evaluate the trainee's performance.

1.6.3 The outcome of the oral examination evaluation will be:

1.6.3.1 Satisfactory.

1.6.3.2 Not satisfactory.

1.6.3.2.1 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

1.6.4 The final mock trial will not exceed four (4) hours. Prior to trial, the “prosecutor” and the "defense attorney" may reach an agreement as to selected items to be introduced at trial in order to remain within the set time constraints.

1.6.5 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.

1.6.6 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.

1.6.7 There may be two defense lawyers at the trial, one of whom must be a qualified examiner in the Forensic Biology Section.

1.6.8 The trial may be stopped at any time upon the request of any of the involved parties.

1.6.9 Immediately following the trial, the trainee may be released while the Department Director or his designee, the Section Chief of the Forensic Biology Section, section supervisor, and trial participants evaluate the trainee's performance.

1.6.10 The outcome of the trial evaluation will be:

1.6.10.1 Satisfactory.

1.6.10.2 Not satisfactory.

1.6.10.2.1 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

1.6.11 This evaluation may be followed by a short performance critique.

1.6.12 The training coordinator will review the videotape with the individual as soon as possible. Other comments should be gathered by the individual from trial participants/observers as soon as possible.

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

1.7 Transition from Trainee to Examiner

1.7.1 After the individual has successfully completed all training (case approach, identification of biological substances, and DNA analysis when applicable), there follows a somewhat awkward period of adjustment. The supervisor must ensure that the transition from trainee to qualified examiner takes place as smoothly as possible. A newly qualified examiner cannot function without some guidance.

1.7.2 For a period of time, all of the newly qualified examiner's reports must be reviewed prior to release by the supervisor or designee. Casework must be monitored closely for at least six (6) months.

1.7.3 The supervisor, or designee, will accompany the newly qualified examiner to court for the first few cases.

1.8 Instructions for the Training Coordinator

1.8.1 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 examiner in the Forensic Biology Section. All of the listed topics must be incorporated into the 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 THIS PROTOCOL MUST BE CLEARED WITH THE FORENSIC BIOLOGY SECTION CHIEF.

1.8.2 The training coordinator or designated examiner will document the completion of each required training task by the trainee on the designated checklist for that aspect of training. The checklist for each training topic is located at the end of each section in this training manual.

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

1.8.2.2 One copy of all completed checklists will accompany the Training Coordinator's final report to the Section Chief stating that all aspects of the training program have been completed satisfactorily.

1.8.3 The trainee will be evaluated on his/her performance during the course of the program. The training coordinator must submit monthly written evaluations of the trainee's progress to the Forensic Biology Section Chief. The monthly training report is due on each trainee within five working days of the end of the month.

1.8.4 The monthly training report must include:

1.8.4.1 A summation of the progress made during the month.

1.8.4.2 An evaluation of the trainee's notebook.

1.8.4.3 An evaluation of the progress during the month, to include:

1.8.4.3.1 Problem areas, as applicable, and their solutions or proposed solutions.

1.8.4.3.2 Trainee's strong points.

1.8.4.3.3 Trainee's weak points and suggested remedies.

1.8.4.3.4 Statement concerning trainee's overall performance.

1.8.4.3.5 Plans for the upcoming month.

1.8.5 This report will be in memorandum format, one memorandum per trainee. Each memorandum will become a part of the training history of the trainee and will be used to document the trainee's progress toward qualification. The monthly report format is located at the end of this section.

1.8.6 A review of the checklists with the trainee at the end of each month will enhance the training coordinator's ability to prepare the monthly written evaluation, and may also give the trainee a greater sense of accomplishment. The coordinator is to discuss this evaluation with the trainee and the trainee's supervisor prior to forwarding it to the Section Chief. Any comments by the trainee, coordinator, or supervisor are to be included with the report.

1.8.7 When the trainee has satisfactorily completed all training requirements (case approach, identification of biological substances, and DNA analysis when applicable), a memorandum will be issued by the Section Chief to the Department Director recommending that the person be qualified to perform the specified duties of an examiner in the section. If the trainee cannot meet the criteria expected of him/her during the period allowed for training in each of the areas, steps will be taken to effect the appropriate action.

1.8.8 The training should culminate so that the trainee has the following:

1.8.8.1 Knowledge of the principles and practices of forensic serology as these relate to the analysis of case material.

1.8.8.2 Knowledge of the theory and application of instrumentation and specialized techniques used to examine biological evidence.

1.8.8.3 The ability to perform accurate forensic analyses independently and proficiently, to accurately document the findings of all analyses in accordance with Department and Section policies and procedures, and to accurately report those findings in a Certificate of Analysis.

1.8.8.4 The ability to skillfully present and defend analytical findings in a court of law.

1.9 Instructions for the Trainee

1.9.1 The trainee is expected to keep a loose-leaf notebook on all work completed. The completed checklist for each training topic and the training coordinator's monthly reports will also be included in the notebook. This notebook will be checked monthly by the training coordinator.

1.9.2 The notebook should be organized by subject. Within each subject category, the types of tests or examinations observed and performed, notes and comments on each type of test, and the review of pertinent literature should be included. For each procedure performed, comments/notes should include the following, as appropriate: principle, procedural outline (to include the purpose of critical reagents), sensitivity, specificity, interpretation of results, possible interferences/problems, and comments, including comparison to other methods.

1.9.3 The information in the readings may overlap on some subjects. The trainee need not re-read information covered in another text. The readings in both Appendix A and Appendix B ARE REQUIRED and cover the material needed for an adequate understanding of the subject matter. Refer to Appendix A for general references for required readings. Procedures for each testing method will be found within the designated section in this manual. Refer to Appendix B for the references for each procedure.

1.9.4 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.

1.9.5 The trainee will assist with casework throughout the training, only under the direct supervision of a qualified examiner.

MONTHLY TRAINING REPORT FORMAT

(letterhead stationary)

MEMORANDUM

TO: (Name), Forensic Biology Section Chief

FROM: (Name), Training Coordinator

DATE:

SUBJECT: Training Report: (Name)

This report reviews and evaluates the forensic biology training of (Name) for the month of _____.

1. Progress during the month
2. Evaluation of trainee's notebook
3. Evaluation of progress
4. Plans for the upcoming month

cc: (Trainee)
(Regional Director)
(Supervisor, if different than the Training Coordinator)

2 SAFETY

2.1 Hazards

2.1.1 An examiner in the Forensic Biology Section must be acutely aware of the potential hazards inherent in his/her work. These hazards include, but are not limited to:

2.1.1.1 Infectious agents, such as those associated with:

- Hepatitis
- AIDS
- Sexually transmitted diseases
- Parasitic infections
- Bacterial infections

2.1.1.2 Hazardous materials, such as acids and bases.

2.2 Safety Procedures

2.2.1 Reference: Department of Forensic Science Safety Manual

2.2.1.1 All trainees are required to read and be familiar with the Department of Forensic Science Safety Manual.

2.2.2 Personal protection

2.2.2.1 Use gloves, safety glasses and other protective clothing and equipment.

2.2.2.2 Avoid production of aerosols.

2.2.2.3 No mouth pipetting.

2.2.2.4 Material Safety Data Sheets (MSDS)

2.2.2.4.1 Read and be familiar with the prescribed precautions for the handling of all chemicals used in a particular procedure before performing the procedure.

2.2.3 Biosafety practices

2.2.3.1 Follow prescribed cleaning procedures for yourself, your work areas, and equipment.

2.2.3.2 All biological materials and containers/supplies that have come in contact with biological materials and/or hazardous chemicals will be placed in biohazard bags, which will be disposed of according to approved guidelines.

2.2.3.3 All glassware for disposal will be placed in the broken glass containers, which will be disposed of according to approved guidelines.

2.2.3.4 Hazardous chemicals will be retained in appropriately labeled containers in a hood until picked up by a disposal company.

3 RECEIVING AND HANDLING PHYSICAL EVIDENCE

3.1 Goals

3.1.1 To obtain a working knowledge of factors influencing the deterioration of evidence as these relate to proper vs. improper packaging, handling, and storage.

3.1.2 To develop a thorough understanding of evidence handling procedures, including preservation of chain of custody, use of the laboratory information management system (F.A.C.E.), and intra/interlaboratory transfer of evidence.

3.1.3 To develop a knowledge of court procedures involving identification and introduction of evidence.

3.1.4 To develop a thorough understanding of the necessity for:

3.1.4.1 Detailed, comprehensive notes.

3.1.4.2 Adequate labeling of evidentiary material.

3.1.4.3 Drawings/photographs.

3.2 Tasks

3.2.1 Receive, transfer, and return evidence.

3.2.2 Assist in preservation and storage of evidence.

3.2.3 Observe and obtain instruction from qualified examiners performing routine examinations on case material.

3.2.4 Practical applications: examine, describe, and take notes on case material. **THIS MUST BE DONE UNDER THE DIRECT SUPERVISION OF A QUALIFIED EXAMINER.** It should be noted that this task will continue throughout the training period.

3.3 Training Evaluation

3.3.1 Knowledge

3.3.1.1 Evaluation of case notes by training coordinator or designee.

3.3.1.2 Review of notes in training notebook by training coordinator.

3.3.1.3 Mini-mock trials/oral and practical examinations.

3.3.1.4 Completion of trainee checklist by training coordinator.

3.3.2 Skills

3.3.2.1 The trainee should handle a sufficient number of cases to develop and exhibit an unquestionably sound technique for handling physical evidence with a wide variety of evidentiary materials. This will be monitored by continual observation by the training coordinator or designee.

3.3.2.2 Review of notes in training notebook by training coordinator.

3.3.2.3 Mini-mock trails/oral and practical examinations.

3.3.2.4 Completion of designated trainee checklist by training coordinator.

RECEIVING AND HANDLING PHYSICAL EVIDENCE STUDY QUESTIONS

1. What is a container?
2. What is F.A.C.E.?
3. What is a lock box?
4. How is evidence transferred from one laboratory to another?
5. What is the pathway that an item of evidence goes through from the time it enters DFS to the time it is returned to the agency?
6. Describe the duties of the "primary examiner". How is the "primary examiner" determined?
7. What is chain of custody?
8. How is chain of custody maintained in your laboratory?
9. How is evidence stored in your 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. What is a temporary seal and when can it be used?
14. You receive a known blood sample in a lavender top blood tube. How do you preserve this sample to ensure that no degradation occurs?
15. You receive a call from an investigator saying he's arrested the suspect in the case he submitted two weeks ago, but isn't sure what to do. What do you tell him?
16. What key pieces of information should be included on every page of your notes?
17. Introducing Physical Evidence In Court (taken from Trial Technique Predicate Questions, Second Edition, National District Attorneys Association, Alexandria, Virginia):
    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?

CHECKLIST FOR RECEIVING AND HANDLING PHYSICAL EVIDENCE

Name of Trainee:_____

1. Trainee has assisted in receiving, transferring, and returning evidence, including the use of F.A.C.E.

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

2. Trainee has assisted in preserving and storing a wide variety of evidentiary materials.

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

3. The trainee has examined, described, taken notes and conducted testing on a wide variety of different types of case material (to be completed by instructing examiner). Be specific with regard to the testing conducted and the types of case material tested. Attach additional pages as needed.

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

   _____

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

   _____

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

   _____

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

   _____

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

   _____

   Date:_____ Examiner:_____ Type of Case:_____

   Comments:_____

   _____

Date:_____ Examiner:_____ Type of Case:_____

Comments:_____

_____

4. Notebook is organized and complete.

Date:_____ Training Coordinator:_____

Comments:_____

5. Trainee has participated in a mock trial and/or practical or oral examinations. Performance was satisfactory.

Date:_____ Training Coordinator:_____

Comments:_____

6. Trainee has read and understands all applicable literature.

Date:_____ Training Coordinator:_____

Comments:_____

7. Trainee has developed and exhibited an unquestionably sound technique for handling a wide variety of physical evidence.

Date:_____ Training Coordinator:_____

Comments:_____

4 INTRODUCTION TO THE MICROSCOPE

4.1 Goals

4.1.1 To learn the theory and use of the various types of microscopes, e.g., stereo, compound, and phase contrast microscopes.

4.1.2 To learn the construction of various types of stereo and compound microscopes and the function of each component.

4.1.3 To obtain a working knowledge of factors determining the resolution of the microscope, including, but not limited to, total magnification and numerical aperture.

4.1.4 To learn proper care and maintenance of the equipment.

4.1.5 To learn proper achievement of Köehler illumination

4.1.6 To learn the theory behind and the techniques for utilizing bright field and phase contrast microscopy.

4.2 Tasks

4.2.1 Apply proper alignment techniques necessary for phase contrast illumination when examining smears for spermatozoa. Refer to centering instructions for the microscope in use.

4.2.2 Apply proper techniques for obtaining Köehler illumination by examining spermatozoa on smears at various magnifications.

4.2.3 Perform bright field illumination techniques by examining spermatozoa on stained smears.

4.2.4 Perform phase contrast illumination techniques by examining stained and unstained smears for spermatozoa.

4.2.5 Perform routine maintenance on the equipment.

4.2.6 Read applicable literature. Refer to Appendix A and Appendix B.

4.3 Training Evaluation

4.3.1 Knowledge

4.3.1.1 Review of notes in training notebook by training coordinator.

4.3.1.2 Mini-mock trials/oral and practical examinations.

4.3.1.3 Completion of checklist by training coordinator.

4.3.2 Skills

4.3.2.1 Observation by training coordinator or designee.

4.3.2.2 Satisfactory performance on training exercises.

4.3.2.3 Completion of checklist by training coordinator.

4.4 Resolving Power and Illumination of the Microscope – Technical Notes

4.4.1 Illumination

4.4.1.1 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 paramount 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.

4.4.1.2 Light intensity should be controlled for visual work by neutral density filters or by a variable voltage transformer on the light source.

4.4.1.3 A field diaphragm on the lamp housing usually controls the size of the illuminated field of view.

4.4.1.4 The angular aperture of the illumination cone is controlled with the sub-stage iris.

4.4.2 Contrast and Resolution

4.4.2.1 For good contrast, the sub-stage iris must usually be closed down slightly. This, however, cuts down the condenser aperture and decreases resolving power. It is necessary to operate with the sub-stage iris open as far as possible, consistent with image contrast, to have good resolution.

4.4.3 Köehler Illumination

4.4.3.1 The best illumination for most purposes is a special type of critical illumination known as Köehler illumination (named after August Köehler, 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.

4.4.3.2 With Köehler 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.

4.5 Procedure for Koehler Illumination (Reference 1, Appendix B)

4.5.1 Determine that the lamp is centered according to the instructions for the microscope in use.

4.5.2 Using a medium to low power objective (approximately 10X), place a specimen in position and focus.

4.5.3 Close the field diaphragm.

4.5.4 Focus the image of the field diaphragm by adjusting the substage condenser.

4.5.5 Center the field diaphragm using the centering screws on the condenser.

4.5.6 Open the field diaphragm so that the rim just disappears beyond the field of view.

4.5.7 Adjust the condenser diaphragm (aperture diaphragm) to about ½ of the full aperture.

NOTE: Resolution, contrast, and depth of field can be regulated with the condenser diaphragm. It should not be used to regulate the brightness. For this purpose, either the regulating transformer or neutral density filters should be used.

INTRODUCTION TO THE MICROSCOPE STUDY QUESTIONS

1. Describe Köehler Illumination and how this is achieved on the microscope.
2. What is phase contrast microscopy?
3. What is bright field microscopy?
4. What are the major differences between the stereoscope, compound microscope, and phase contrast microscope?
5. What total magnifications are used when examining specimens under low and high power and how does one arrive at the total magnification?
6. What is an objective?
7. What is an eyepiece?
8. Briefly describe field diaphragm, aperture diaphragm, and substage condenser.
9. What are the major parts of the compound microscope?
10. What is resolution and resolving power and how is it determined?
11. Who is credited with developing the microscope?
12. What is a micrometer and how is it used?
13. What type of light source is used on microscopes?
14. What is refractive index and how does it affect microscopy?
15. What is numerical aperture?

CHECKLIST FOR INTRODUCTION TO THE MICROSCOPE

Name of Trainee:_____

1. Trainee has satisfactorily completed the following tasks:

   Applied proper alignment techniques necessary for phase contrast illumination while examining spermatozoa smears.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Applied the proper technique for obtaining Köehler illumination.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Performed bright field illumination techniques while examining spermatozoa on stained smears.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Performed phase contrast illumination techniques while examining spermatozoa on stained and unstained smears.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Performed routine maintenance on the equipment.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee understands the theory and use of the stereo, phase contrast, and compound microscopes, including the construction, components, and proper care of each.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee exhibits a working knowledge of various resolution determining factors for the microscope.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee understands the theory behind and the practical application of the bright field and phase contrast microscopy techniques.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee has participated in a mock trial and/or practical or oral examinations. Performance was satisfactory.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

5 DETECTION OF BLOOD

5.1 Goals

5.1.1 To develop a basic understanding of the use of presumptive and confirmatory tests.

5.1.2 To develop a thorough understanding of the procedures used by the Department.

5.1.2.1 To become acquainted with the sensitivity and stability of reagents.

5.1.2.2 To determine the specificity and limitations of the various methods.

5.1.2.3 To acquire a thorough understanding of the use of controls.

5.2 Tasks

5.2.1 Prepare reagents used for the Phenolphthalein, Tetramethybenzidine, and Luminol tests.

5.2.2 Perform the Combined Phenolphthalein -Tetramethylbenzidine (PTMB) and Luminol chemical color tests on the following (record the results for the individual Phenolphthalein and Tetramethylbenzidine reactions):

5.2.2.1 Bloodstains of varying dilutions prepared in normal saline (1:10, 1:100, 1:250, 1:500, 1:750, 1:1000, 1:2000).

- 9 g Sodium chloride
- 1000 ml Distilled water
- Mix thoroughly until dissolved.

5.2.2.2 Minimum of 5 bloodstains of varying ages.

5.2.2.3 Minimum of 20 bloodstains subjected to various contaminants (including, but not limited to, super glue, fingerprint powder, ninhydrin, redwop powder - rhodamine base, bleach, soap, motor oil, luminol, and mold), and environmental conditions (heat, moisture, heat and moisture combined, decomposition).

5.2.2.4 All substances reported in the literature to give false positive reactions.

5.2.3 Observe specificity and sensitivity of all tests performed. Compare this information to that found in the literature.

5.2.4 Observe and obtain instruction from qualified examiners performing routine examinations of case material.

5.2.5 Test at least 10 unknown stains provided by training coordinator or designee.

5.2.6 Read applicable literature. Refer to Appendix A and Appendix B.

5.3 Training Evaluation

5.3.1 Knowledge

5.3.1.1 Review of notes in training notebook by training coordinator.

5.3.1.2 Mini-mock trials/oral and practical examinations.

5.3.1.3 Completion of checklist by training coordinator.

5.3.2 Skills

5.3.2.1 Observation by training coordinator or designee.

5.3.2.2 Review of notes in training notebook by training coordinator.

5.3.2.3 Mock trials/oral and practical examinations.

5.3.2.4 Completion of checklist by training coordinator.

5.4 Detection of Blood Using Catalytic Tests – Technical Notes

5.4.1 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 a benzidine derivative or phenolphthalin is present, it will oxidize the colorless reagent to form a colored product.

5.4.2 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. Therefore, the phenolphthalein test, which takes place in basic medium, and the tests using benzidine derivatives, which take place in acid medium, are not redundant. Fast positive reactions obtained with both tests on a red-brown or other appropriately colored substance can be considered very strong evidence (essentially proof for practical purposes) that the substance being tested is blood. The Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test has been routinely used by the Department for many years to indicate the presence of blood.

5.4.3 Luminol

5.4.3.1 Luminol can be oxidized by heme to a product which luminesces under darkened conditions. This test is very useful in locating "latent" bloodstains, but should only be performed after a visual search has failed to reveal suspected blood. The reagent is applied as a mist from a spray bottle over the item being analyzed.

5.4.3.2 The degree of luminescence is dependent on the substrate and will fade with time, but can be restored with an additional application of reagent mist. This may be particularly useful for weak stains that require prolonged exposure times to photograph, but care must be taken to avoid diluting the stains with unnecessary repeat spraying. If "latent" blood is suspected on a vertical surface, be prepared to photograph immediately as the spraying may cause the blood to "run" down the surface.

5.4.3.3 When appropriate, the necessary photographic equipment should be available to document any luminescence produced. If photographing, use a ruler with luminescent tape as a scale and 400 ASA film or higher.

5.4.3.4 Once possible blood is located with luminol, the Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test must be performed. Since other substances are known to react with luminol, blood is not indicated unless the PTMB Test is positive. Luminol will not interfere with this subsequent test.

5.5 Procedures for the Detection of Blood

5.5.1 COMBINED PHENOLPHTHALEIN-TETRAMETHYLBENZIDINE (PTMB) TEST (References 2, 3, 4, Appendix B)

5.5.1.1 Safety Considerations

5.5.1.1.1 Phenolphthalin - Caution! Avoid contact and inhalation!

5.5.1.1.2 Potassium hydroxide - Caution! Corrosive! Poisonous!

5.5.1.1.3 Tetramethylbenzidine - Caution! Harmful if swallowed, inhaled or absorbed through skin! Emits toxic fumes under fire conditions!

5.5.1.1.4 Glacial acetic acid - Caution! Corrosive! Flammable!

5.5.1.1.5 Ethanol - Caution! Flammable! Poisonous!

5.5.1.1.6 Oxidized zinc - Caution! Danger of spontaneous combustion if allowed to dry!

5.5.1.2 Materials and Equipment

5.5.1.2.1 Dropper bottles

5.5.1.2.2 Cotton swabs

5.5.1.2.3 Test tubes, microtiter plates, or filter paper

5.5.1.2.4 100 ml graduated cylinder

5.5.1.2.5 Weigh boats or weigh paper

5.5.1.2.6 Balance

5.5.1.2.7 Spatula

5.5.1.2.8 Scissors

5.5.1.2.9 Tweezers

5.5.1.3 Stock Solutions

5.5.1.3.1 Phenolphthalin Stock Solution

- 1 g Phenolphthalin
- 25 g Potassium Hydroxide (KOH)
- 100 ml Distilled water
- The above ingredients are mixed until thoroughly dissolved.

5.5.1.3.1.1 Storage

5.5.1.3.1.1.1 This colorless solution is stored under refrigeration over fresh granular zinc to keep it in the reduced form. The oxidized zinc in the bottle should not be allowed to dry (see 5.5.1.1 Safety Considerations and 5.5.1.3.1.3.1 Disposal).

5.5.1.3.1.2 Labeling

5.5.1.3.1.2.1 Label the bottle as Phenolphthalin Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).
Example: Phenolphthalin Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

5.5.1.3.1.2.2 There is no expiration date (see 5.5.1.5 Minimum Standards and Controls).

5.5.1.3.1.3 Disposal

5.5.1.3.1.3.1 When the reduced phenolphthalin stock solution is depleted, cover the zinc in the bottom of the bottle completely with a solution of potassium hydroxide in distilled water (25 g KOH/100 ml dH_20).

5.5.1.3.1.3.2 Label the bottle with the contents and "For Disposal" and refrigerate. Notify the Safety Officer (Eastern, Northern, and Western Laboratories) or the Department Safety Coordinator (Central Laboratory) that the zinc is ready to be disposed of in accordance with Department procedures.

5.5.1.3.2 Tetramethylbenzidine (TMB) Stock Solution

- 10 mg Tetramethylbenzidine (TMB)
- 30 ml Glacial acetic acid
- Mix the above ingredients until thoroughly dissolved.

5.5.1.3.2.1 Storage

5.5.1.3.2.1.1 The TMB stock solution may be stored under refrigeration or at room temperature.

5.5.1.3.2.2 Labeling

5.5.1.3.2.2.1 Label the bottle as TMB Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).
Example: TMB Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

5.5.1.3.2.2.2 There is no expiration date (see 5.5.1.5 Minimum Standards and Controls).

5.5.1.3.2.3 Disposal

5.5.1.3.2.3.1 Dispose of the TMB stock solution and other materials contaminated with this solution as hazardous waste in accordance with Department procedures.

5.5.1.4 Working Solutions

- Distilled water
- Ethanol
- 3% Hydrogen peroxide
- 1:5 dilution of phenolphthalin stock solution in distilled water (1 part of the phenolphthalin stock solution diluted with 4 parts of distilled water)
- TMB stock solution

5.5.1.4.1 Storage

5.5.1.4.1.1 All bottles of working solutions are stable at room temperature.

5.5.1.4.2 Labeling

5.5.1.4.2.1 Bottles containing working solutions of ethanol and 3% hydrogen peroxide will be labeled with the contents and the lot number.

5.5.1.4.2.2 The bottle containing the 1:5 dilution of phenolphthalin stock solution must be labeled appropriately with the lot number of the stock solution, the date of the dilution, and the initials of the person making the dilution.

5.5.1.4.2.3 There is no expiration date for the working solutions (see 5.5.1.5 Minimum Standards and Controls).

5.5.1.5 Minimum Standards and Controls

5.5.1.5.1 On the day of use a positive reagent control (known bloodstain) and a negative reagent control (distilled water) must be tested to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

5.5.1.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

5.5.1.6 Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test Procedure

5.5.1.6.1 Gently rub a suspected stain with a cotton swab which has been moistened with distilled water or place a small cutting of the stain in a small test tube or microtiter plate, or on filter paper and moisten with distilled water if desired.

5.5.1.6.2 Add one drop of ethanol.

5.5.1.6.3 Add one drop of 1:5 dilution of phenolphthalin (i.e., the working solution of phenolphthalin).

5.5.1.6.4 Add one drop of 3% hydrogen peroxide.

5.5.1.6.5 Note any color change. An immediate pink color is expected if blood is present.

5.5.1.6.6 Add one drop of tetramethylbenzidine stock solution.

5.5.1.6.7 Note any color change. An immediate blue-green color is expected if blood is present.

5.5.1.6.8 Interpretation

5.5.1.6.8.1	Positive Reaction =	Immediate pink color at 5.5.1.6.5, followed by immediate blue-green color at 5.5.1.6.7
5.5.1.6.8.2	Negative Reaction =	No color change at 5.5.1.6.5, followed by no color change at 5.5.1.6.7
5.5.1.6.8.3	Inconclusive Reaction =	Development of color combinations other than those specified for a positive reaction, including one test positive and the other test negative.

5.5.1.6.9 Reporting Results

5.5.1.6.9.1 Report positive test results as "blood was indicated…"

5.5.1.6.9.2 Report negative test results as "no blood was detected..."

5.5.1.6.9.3 Report inconclusive test results as "tests for blood were inconclusive…"

5.5.2 Luminol Test (Reference 5, Appendix B)

5.5.2.1 Safety Considerations

5.5.2.1.1 Sodium perborate - Caution! Harmful if swallowed, inhaled or absorbed through skin!

5.5.2.1.2 Aminophthalhydrazide (luminol) - Caution! Irritant! Emits toxic fumes under fire conditions!

5.5.2.2 Materials and Equipment

5.5.2.2.1 Spray bottle (must contain no metal parts as the luminol reacts with some metals)

5.5.2.2.2 50 ml graduated cylinder

5.5.2.2.3 Balance

5.5.2.2.4 Weigh boats or weigh paper

5.5.2.2.5 Spatula

5.5.2.2.6 Ziploc bags (optional)

5.5.2.3 Stock Solutions

5.5.2.3.1 Solution A

- 0.7 g Sodium perborate
- 50.0 ml Distilled water
- Mix above ingredients until thoroughly dissolved. USE IMMEDIATELY! DO NOT STORE.

5.5.2.3.2 Solution B

- 0.1 g Aminophthalhydrazide (luminol)
- 5.0 g Sodium carbonate
- 50.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved. USE IMMEDIATELY! DO NOT STORE.

NOTE: The dry chemicals in Solutions A and B can be weighed out and placed in appropriately labeled zip lock bags and stored in the dark at room temperature. Each bag must be labeled with the date prepared, the expiration date, the initials of the person who prepared each package, and the amount of distilled water to be added. Water can be added when needed.

5.5.2.4 Minimum Standards and Controls

5.5.2.4.1 Test a positive reagent control (known bloodstain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

5.5.2.4.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

5.5.2.5 Luminol Procedure

5.5.2.5.1 WHEN THE TEST IS READY TO BE CONDUCTED, mix equal parts of solutions A and B and place in a sprayer bottle.

5.5.2.5.2 Under darkened conditions, immediately after mixing equal parts of solutions A and B, spray the positive and negative controls to ensure that the reagents are working properly. If both controls give the expected results, proceed with spraying the area of interest. Document results in the case file.

5.5.2.5.3 Areas containing blood will luminesce immediately and maintain a sustained glow.

5.5.2.5.4 Mark luminescent areas for subsequent testing with the Combined Phenolphthalein-Tetramethylbenzidine Test.

5.5.2.5.5 Interpretation

5.5.2.5.5.1	Positive Reaction =	Immediate luminescence
5.5.2.5.5.2	Negative Reaction =	No luminescence

5.5.2.5.5.3	Inconclusive Reaction =	Slow and/or weak luminescence

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. What is the PTMB test?
4. When is the PTMB test performed?
5. What is the mechanism behind the PTMB test?
6. What is the purpose of each chemical used for the PTMB testing?
7. If an oxidizer, such as potassium permanganate, was tested with the chemicals used for PTMB testing, what reaction would be expected and why?
8. What is the benefit of using the combined PTMB chemical test?
9. Which PTMB reagent works best in the acidic environment and which works best in the basic environment?
10. What does a positive PTMB result tell you?
11. What would you do if your P test was positive but the TMB test was negative?
12. What action would you take if your negative control was positive?
13. What is the mechanism for luminol?
14. When is luminol used?
15. What does a positive luminol reaction look like and what does it tell you?
16. You get a call from an investigator requesting luminol. How do you handle it? What are the essential questions you need to ask? How do you instruct him to use it?
17. You get a call from an investigator asking if luminol can be used to examine the back yard where it is believed that a husband shot his wife two weeks ago. What do you tell him?
18. You get a call from a patrol officer saying that he is processing the scene of a B&E where he sees blood on the broken window. He's never done this before. How do you advise him to proceed?
19. You get a "supercan" trashcan for a case in which the victim's body was found in the supercan itself. What do you do with it?
20. What is the purpose a positive control?
21. Name 2 presumptive tests for blood and 2 confirmatory tests for blood not used by the Department.

CHECKLIST FOR THE DETECTION OF BLOOD

Name of Trainee:_____

1. Preparation of the Combined Phenolphthalein-Tetramethylbenzidine and Luminol test reagents.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Completion of the Combined Phenolphthalein-Tetramethylbenzidine and Luminol tests for:

   Bloodstain dilutions (1:10, 1:100, 1:250, 1:500, 1:750, 1:1000, 1:2000).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Aged bloodstains (5 minimum).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Bloodstains subjected to various contaminants (20 minimum).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Substances reported to give false positive reactions.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Accurately tested at least 10 unknown stains (provided by training coordinator or designee).

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee has developed a thorough understanding of the theory behind and practical application of the various color tests for blood, including the sensitivity and specificity of the reagents, specificity and limitations of the various methods used, and the purpose and use of controls.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee has developed a basic understanding of the use of presumptive and confirmatory tests and is familiar with tests in each category.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee has participated in a mock trial and/or practical or oral examinations. Performance was satisfactory.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

6 DETERMINATION OF SPECIES ORIGIN

6.1 Goals

6.1.1 To acquire a basic understanding of immunology, including the theory and procedures for species origin determination.

6.1.2 To acquire a thorough understanding of the use of controls.

6.1.3 To become acquainted with the specificity, sensitivity, and limitations of the Ouchterlony double diffusion method.

6.2 Tasks

6.2.1 Determine the sensitivity of anti-human precipitin serum by testing various dilutions in Normal Saline (neat, 1:5, 1:10, 1:20, 1:50, 1:100) of known human blood and bloodstains using Ouchterlony double diffusion.

6.2.1.1 Normal Saline (0.9%):

- 9 g Sodium chloride
- 1000 ml Distilled water
- Mix thoroughly until dissolved.

6.2.2 Test at least 15 bloodstains subjected to various environmental conditions (heat, moisture, heat and moisture combined), and decomposition using Ouchterlony double diffusion.

6.2.3 Test at least 15 bloodstains exposed to various contaminants (including, but not limited to superglue, fingerprint powder, ninhydrin, redwop power - rhodamine base, bleach, soap, motor oil, luminol, and mold) using Ouchterlony double diffusion.

6.2.4 Test the precipitating antisera in the routine species collection addressed in 6.4.6 for cross-reactivity using Ouchterlony double diffusion. Observe specificity.

6.2.5 Stain Ouchterlony plates using Coomassie Brilliant Blue R250. Note enhancement of weak reactions.

6.2.6 Instruction by and observation of qualified examiners performing routine examinations of case material.

6.2.7 Test at least 10 unknown stains provided by training coordinator or designee.

6.2.8 Read applicable literature. Refer to Appendix A and Appendix B.

6.3 Training Evaluation

6.3.1 Knowledge

6.3.1.1 Review of notes in training notebook by training coordinator.

6.3.1.2 Mini-mock trials/oral and practical examinations.

6.3.1.3 Completion of checklist by training coordinator.

6.3.2 Skills

6.3.2.1 Observation by training coordinator or designee.

6.3.2.2 Review of notes in training notebook by training coordinator.

6.3.2.3 Mini-mock trials/oral and practical examinations.

6.3.2.4 Completion of checklist by training coordinator.

6.4 Species Identification – Technical Notes

6.4.1 The procedure described in this section requires the use of precipitating antiserum to determine species origin. This procedure is an immunological procedure for the identification of animal or human protein. The identification is made by comparing the reaction of the unknown protein (in the case sample) against a known antiserum with the reaction of a known protein against a known antiserum.

6.4.2 A positive control (a known sample of normal serum or blood against which the antiserum is directed) and a substrate control (when available) must be run on each plate when the testing is performed on a case. If a substrate control is not available, distilled water will be used. The substrate control monitors for contaminating protein activity (which could cause a false positive reaction) in the unstained portion of the substrate as well as for contaminating protein activity in the reagent (distilled water) used for the extraction.

6.4.3 Alternatively, a human DNA quantitation method may be used to determine that a sample is of human origin.

6.4.4 Quality Control of Antiserums

6.4.4.1 Before using any new lot number of precipitating antiserum for testing casework samples, the specificity must be tested and appropriately documented in the laboratory's quality control (QC) records. Routine quality control testing will be performed at the time the antiserum is reconstituted, or if the antiserum is received in liquid form, within one week of receipt.

6.4.4.2 Anti-human serum, not anti-human hemoglobin, will be used with the procedure in this section for determining whether a sample is of human origin. Anti-human serum, as well as all animal antiserums in the "Routine Species Collection" specified below, must be tested against all available species (normal or whole serums and known bloods) in the "Routine Species Collection".

6.4.4.3 A positive control, a host control (typically normal rabbit serum or normal goat serum), and a negative control (distilled water) must be included in the specificity testing. The host control (representing the animal in which the antiserum was prepared) is used to demonstrate that the antiserum is not reacting to any proteins in the animal in which it was made.

6.4.4.4 The quality control documentation will include:

6.4.4.4.1 A diagrammatic representation of the placement of samples in the wells of the rosette on the Ouchterlony plate with the subsequent observations (i.e., precipitin lines).

6.4.4.4.2 Date of the testing.

6.4.4.4.3 Initials of the person conducting the testing.

6.4.4.4.4 Lot number, date of receipt, and manufacturer of the antiserum being tested.

6.4.4.4.5 Lot number, date of receipt, and manufacturer of the normal serums being used for the testing.

6.4.4.4.6 Results of the testing.

6.4.4.5 Once the appropriate testing has been performed on a particular lot number of antiserum, it need not be repeated for each case. If another vial of the same lot number is received on a different date, the QC testing described above must be repeated.

6.4.5 Quality Control of Normal (Whole) Serums

6.4.5.1 Before using any new lot number of normal serum for testing casework samples, the specificity must be tested and appropriately documented in the laboratory's quality control (QC) records. Routine quality control testing will be performed at the time the normal serum is reconstituted, or if the normal serum is received in liquid form, within one week of receipt.

6.4.5.2 All normal serums in the "Routine Species Collection" must be tested against all available antiserums in the "Routine Species Collection".

6.4.5.3 A positive control must be included in the specificity testing. Although normal human serum may be purchased, a straw colored dilution of known human blood may be used instead. Similarly, the use of known blood from other species may replace the purchase and use of normal serums from the species.

6.4.5.4 It is not necessary to conduct quality control testing on the known bloods. Label known bloods with the species name and date of preparation/initials of person preparing the sample. Store known bloods in the freezer.

6.4.5.5 The quality control documentation will include:

6.4.5.5.1 A diagrammatic representation of the placement of samples in the wells of the rosette on the Ouchterlony plate with the subsequent observations (i.e., precipitin lines).

6.4.5.5.2 Date of the testing.

6.4.5.5.3 Initials of the person conducting the testing.

6.4.5.5.4 Lot number, date of receipt, and manufacturer of the normal serum being tested.

6.4.5.5.5 Lot number, date of receipt, and manufacturer of the antiserums being used for the testing.

6.4.5.5.6 Results of the testing.

6.4.5.6 Once the appropriate testing has been performed on a particular lot number of normal serum, it need not be repeated for each case. If another vial of the same lot number is received on a different date, the QC testing described above must be repeated.

6.4.6 Routine Species Collection

6.4.6.1 The following will be maintained in each laboratory as the "Routine Species Collection" and will undergo QC testing for specificity as outlined above:

6.4.6.1.1 Bovine antiserum and normal bovine serum or known blood

6.4.6.1.2 Swine antiserum and normal swine serum or known blood

6.4.6.1.3 Cat antiserum and normal cat serum or known blood

6.4.6.1.4 Dog antiserum and normal dog serum or known blood

6.4.6.1.5 Rabbit antiserum and normal rabbit serum or known blood

6.4.6.1.6 Sheep antiserum and normal sheep serum or known blood

6.4.6.1.7 Deer antiserum and normal deer serum or known blood

6.4.6.1.8 Human antiserum and normal human serum or known blood

6.4.6.1.9 Normal goat serum or known blood (goat antiserum is unavailable)

NOTE: It is recommended that the antiserums and normal serums listed above be reconstituted and QC tested at the time of receipt to ensure ready availability.

6.4.6.2 Other antiserums/normal serums, such as bear, rodent, fowl, horse, etc. should also be maintained for use in special cases and must also undergo QC testing as specified above. However, since these are used only in special cases, it is recommended that they not be reconstituted, aliquoted, and QC tested until it is determined that there is a specific need to do so.

6.4.7 Storage of Antiserum/Normal Serum

6.4.7.1 Small aliquots of the antiserum/normal serum will be prepared for routine use and frozen within one week of reconstitution (when antiserum is lyophilized) or upon receipt (when antiserum is liquid). All frozen aliquots have an indefinite expiration date.

6.4.7.2 A thawed aliquot may be stored refrigerated for up to 1 month. If the aliquot is to be maintained in this manner, the expiration date must be clearly marked on the vial. Otherwise, the aliquot will be immediately discarded following its use.

6.4.8 Labeling of Antiserum/Normal Serum

6.4.8.1 Labels on each aliquot will include:

6.4.8.1.1 The manufacturer

6.4.8.1.2 Type of antiserum or normal serum

6.4.8.1.3 Lot number

6.4.8.1.4 Date reconstituted/date frozen

6.4.8.1.5 Initials of the person preparing the aliquot

6.4.9 Ouchterlony (Double Diffusion) Test (Reference 6, pp. 221-241, Appendix B)

6.4.9.1 Equipment

6.4.9.1.1 Punch

6.4.9.1.2 Aspirator

6.4.9.1.3 100 ml and 500 ml graduated cylinders

6.4.9.1.4 Balance

6.4.9.1.5 Spatula

6.4.9.1.6 Scissors

6.4.9.1.7 Tweezers

6.4.9.1.8 Hot plate or oven (37° C)

6.4.9.1.9 Incubator (optional)

6.4.9.1.10 Magnetic stir plate

6.4.9.1.11 Refrigerator (optional)

6.4.9.2 Materials

6.4.9.2.1 Petri dishes, slides, or comparable containers

6.4.9.2.2 Test tubes

6.4.9.2.3 Weigh boat or weigh paper

6.4.9.2.4 Moisture chamber

6.4.9.2.5 Disposable pipets

6.4.9.2.6 Capillary tubes

6.4.9.3 Reagents

6.4.9.3.1 Normal saline (0.9%)

6.4.9.3.2 Agarose gel (1%)

6.4.9.3.3 Distilled water

6.4.9.3.4 Antiserum

6.4.9.3.5 Normal serum or known blood (positive control)

6.4.9.4 Agarose Gel Preparation

6.4.9.4.1 Normal saline (0.9% NaCl):

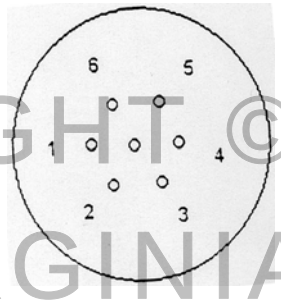
- 9 g Sodium chloride
- 1000 ml Distilled water
- Mix thoroughly until dissolved.

6.4.9.4.2 Agarose gel (1%):

- 1 g Type I agarose
- 100 ml Normal saline (0.9% NaCl)
- Heat until agarose is dissolved. Allow to cool slightly.

6.4.9.4.3 Pore the agarose into a petri dish, onto a slide, or into a comparable container to a thickness of 2-3 mm and allow to cool.

6.4.9.4.4 Cut wells in a rosette pattern (refer to the diagram below) into the gel using a punch or disposable pipet connected to an aspirator.



6.4.9.5 Storage and Labeling

6.4.9.5.1 When a batch of plates is prepared, the plates should be numbered consecutively and placed in a moisture chamber in the refrigerator. Label the moisture chamber with the lot number of the batch (date of preparation/initials of person preparing the plates).

6.4.9.5.2 There is no expiration date (see 6.4.9.6 Minimum Standards and Controls).

6.4.9.6 Minimum Standards and Controls

6.4.9.6.1 A positive control (known sample against which the antiserum is directed) and a substrate control (or if not available, distilled water) must be tested on each plate, unless the stain is on a cotton swab. It is not necessary to test submitted control swabs.

6.4.9.7 Ouchterlony Double Diffusion Procedure

6.4.9.7.1 To prepare an extract of the stain, place a small cutting of the stain in distilled water until a straw color is obtained. A small piece of stained material, which is moistened with distilled water, can be used in lieu of an extract. Treat the substrate control in the same manner as the stain.

6.4.9.7.2 Add antiserum in the center well of the Ouchterlony plate with a disposable pipet or capillary tube.

6.4.9.7.3 Add appropriate extracts/pieces of stained material, the positive control, and negative control(s) to the surrounding wells. Do not overfill the wells. Avoid getting bubbles in the wells. Document the placement of samples in the wells of the rosettes on the Ouchterlony work sheet found near the end of this chapter.

6.4.9.7.4 Record the lot numbers of antiserums and normal serums used for the testing procedure.

NOTE: Alternatively, known normal serum/known blood extract may be placed in the center well with appropriate antiserums in the surrounding wells or the stain extract/piece of stained material may be placed in the center well with appropriate antiserums in the surrounding wells.

6.4.9.7.5 Incubate the plate in a moisture chamber at 37° C for 3-4 hours. Alternatively, it may be left overnight at room temperature or 4° C.

6.4.9.7.6 Record observations (precipitin lines) on the diagram, and interpret and record the results.

6.4.9.7.7 All controls must give the expected results before a conclusion can be reached on an unknown sample, i.e., white precipitin lines must be observed between the antiserum and positive control (known serum) and no precipitin lines should be observed between the antiserum and the negative control.

6.4.9.7.8 Interpretation

6.4.9.7.8.1	Positive Result =	White precipitin lines between the antiserum well and the sample well
6.4.9.7.8.2	Negative Result =	No precipitin lines between the antiserum well and the sample well
6.4.9.7.8.3	Inconclusive Result =	Questionable precipitin lines between the antiserum well and the sample well, OR precipitin lines between the antiserum well and the negative control well(s), OR no precipitin line between the antiserum well and the positive control well. If sufficient sample remains, an inconclusive result should be repeated.

NOTE: The prozone phenomenon can result in a soluble antigen-antibody complex due to too many antibodies present to form a complete lattice (Reference 8, Appendix B). Because of this phenomenon, weak precipitin lines may be observed initially, but disappear upon staining with Coomassie Brilliant Blue R250. This is considered an inconclusive result. Therefore, it is recommended that the testing results be recorded PRIOR TO staining as well as after staining.

6.4.9.7.9 Staining the plate with Coomassie Brilliant Blue R250 may be necessary to visualize weak reactions. Refer to 6.4.10 for the Coomassie Brilliant Blue R250 staining procedure.

6.4.9.7.10 Reporting Results

6.4.9.7.10.1 Report positive test results as "(species tested according to the label on the antiserum) protein was detected…"

6.4.9.7.10.2 Report negative test results as "no (species tested according to the label on the antiserum) protein was detected…"

6.4.9.7.10.3 Report inconclusive test results as "the test for (species tested according to the label on the bottle) protein was inconclusive…"

6.4.10 Coomassie Brilliant Blue R250 Staining Procedure (Reference 13, Appendix B)

6.4.10.1 Safety Considerations

6.4.10.1.1 Coomassie Brilliant Blue R250 - Caution! Avoid contact and inhalation! Emits toxic fumes under fire conditions! Container explosion may occur under fire conditions!

6.4.10.1.2 Methanol - Caution! Irritant! Dangerous when exposed to heat or flame!

6.4.10.1.3 Glacial acetic acid - Caution! Corrosive! Flammable!

6.4.10.2 Equipment

6.4.10.2.1 Weight

6.4.10.2.2 Oven or Incubator (40-60°C)

6.4.10.2.3 Rotator (optional)

6.4.10.2.4 10 ml, 50 ml, and 500 ml graduated cylinders

6.4.10.2.5 Balance

6.4.10.2.6 Spatula

6.4.10.2.7 Trays for staining and destaining

6.4.10.3 Materials

6.4.10.3.1 Gel bond, glass plate, or other support medium

6.4.10.3.2 Weigh boats or weigh paper

6.4.10.3.3 Whatman #1 filter paper

6.4.10.3.4 Paper towels

6.4.10.4 Reagents

6.4.10.4.1 Staining Solution

6.4.10.4.2 Destaining Solution

6.4.10.4.3 Normal saline (0.9% NaCl) – refer to 6.4.10.8.1

6.4.10.4.4 Distilled water

6.4.10.5 Preparation of Stain and Destain Solutions

6.4.10.5.1 Staining Solution

- 0.1 g Coomassie Brilliant Blue R250
- 45.0 ml Methanol
- 10.0 ml Glacial acetic acid
- 45.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved.

6.4.10.5.2 Destaining Solution

- 45.0 ml Methanol
- 10.0 ml Glacial acetic acid
- 45.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved.

6.4.10.6 Storage

6.4.10.6.1 The Staining and Destaining Solutions are stable at room temperature.

6.4.10.7 Labeling

6.4.10.7.1 Label as Staining or Destaining Solution with the lot number (date of preparation followed by the initials of the person preparing the solution).

6.4.10.7.2 There is no expiration date.

6.4.10.8 COOMASSIE BRILLIANT BLUE R250 STAINING PROCEDURE

6.4.10.8.1 Wash the plate overnight in normal saline solution (0.9% NaCl - 9 g NaCl in 1000 ml distilled water) to remove unprecipitated proteins.

6.4.10.8.1.1 If pieces of stained material were used in lieu of extracts, remove these prior to washing the plate.

6.4.10.8.1.2 The gel may detach from the plate during the washing process. Mark the orientation of the gel to ensure that it can be re-oriented properly after the washing has been completed.

6.4.10.8.1.3 Alternatively, the gel may be removed from the plate before beginning the washing process. If this is done, mark the gel to ensure that it can be re-oriented properly after the washing has been completed.

6.4.10.8.2 The next day wash the gel for approximately fifteen minutes in distilled water. Rinse the gel and repeat the wash. Ensuring proper orientation, place the gel (face up) on the hydrophilic side of a piece of gel bond or on some other support medium such as a glass plate.

6.4.10.8.3 Cover the gel with a piece of Whatman #1 filter paper moistened with distilled water. Add a layer of paper towels on top of the filter paper and press with a weight for approximately 30 minutes. Remove paper towels and filter paper and dry the gel in a 40-60° C oven.

6.4.10.8.4 Place the gel in the staining solution and allow it to soak for 1 to 10 minutes. This may be done on a rotator. Intermittently check staining progress to prevent over staining.

6.4.10.8.5 Place the gel in the destaining solution until the background is clear or until no more dye leaches from the gel. This may be done on a rotator. Change the destaining solution and destain further if desired.

6.4.10.8.6 Record observations (precipitin lines) on the diagram, and interpret and record the results. All controls must give the expected results before a conclusion can be reached on an unknown sample, i.e., blue precipitin lines must be observed between the antiserum and positive control (known serum) and no precipitin lines should be observed between the antiserum and the negative control.

6.4.10.8.7 Interpretation

6.4.10.8.7.1	Positive Result =	Blue precipitin lines between the antiserum well and the sample well.
6.4.10.8.7.2	Negative Result =	No precipitin lines between the antiserum well and the sample well.

6.4.10.8.7.3 Inconclusive Result = Questionable precipitin lines between the antiserum well and the sample well, OR precipitin lines between the antiserum well and the negative control well(s), OR no precipitin line observed between the antiserum well and the positive control well. If sufficient sample remains, an inconclusive result should be repeated.

6.4.10.8.8 Reporting Results

6.4.10.8.8.1 Refer to 6.4.9.7.10.

DETERMINATION OF SPECIES ORIGIN STUDY QUESTIONS

1. What is an antigen?
2. What are the conditions of antigenicity?
3. What is an antibody?
4. How is antiserum made? How is normal serum made?

5. What is meant by cross-reactivity?
6. What are the advantages of Ouchterlony double diffusion? Are there disadvantages?
7. Explain Ouchterlony double diffusion and the purpose of each component/control.
8. What does a positive result look like and what does it tell you?
9. When using the Coomassie Blue stain, what is the dye staining? Why is this used?
10. What is Prozone and Postzone?
11. What would you do if you had a very small possible bloodstain and the investigator wanted to know if it was human blood, but species testing would consume the stain?
12. Why is specificity testing of antiserums and normal serums required prior to using these for testing case material?

TRAINEE CHECKLIST FOR THE DETERMINATION OF SPECIES ORIGIN

Name of Trainee:_____

1. Completion of the following tasks using Ouchterlony double diffusion:

   Tested known human blood and bloodstains of varying dilutions (neat, 1:5, 1:10, 1:20, 1:50, 1:100).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Tested bloodstains subjected to various environmental conditions (15 minimum).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Tested bloodstains exposed to various contaminants (15 minimum).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Tested the precipitating antisera in the routine species collection addressed in 6.4.6 for cross- reactivity.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Stained Ouchterlony plates using Coomassie Brialliant Blue R250.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Accurately tested at least 10 unknown stains (provided by training coordinator or designee).

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee has developed a thorough understanding of the theory behind species origin determination, including basic immunology, specificity, sensitivity, and limitations of all methods, as well as the purpose and use of controls. An unquestionably sound technique has been developed for the use of Ouchterlony double diffusion.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee has participated in a mock trial and/or practical or oral examinations. Performance was satisfactory.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

7 SEMEN IDENTIFICATION

7.1 Goals

7.1.1 To become proficient in the use of alternate light sources for locating semen stains.

7.1.2 To learn the physical and chemical characteristics of semen (animal and human).

7.1.3 To become proficient in extraction techniques, staining techniques, and microscopic examination for spermatozoa.

7.1.4 To learn the theory behind the use of chemical (color) tests and immunological tests for semen.

7.1.5 To become proficient in the use of the Acid Phosphatase Test and the p30 test by OneStep ABAcard®, including the use of controls and possible sources of error.

7.1.6 To develop an understanding of the sensitivity, specificity and limitations of the Acid Phosphatase Test (qualitative and quantitative) and p30 test by OneStep ABAcard®.

7.1.7 To be able to locate and evaluate stains on evidentiary material.

7.1.8 To become proficient in techniques used to prevent cross-contamination of seminal fluid/spermatozoa between samples.

7.2 Tasks

7.2.1 Examine and compare at least 20 samples of known physiological fluids (including, but not limited to, different semen dilutions prepared in distilled water, blood, saliva, perspiration, and mixtures) and substances known to react to an alternate light source (including, but not limited to, milk, yogurt, lotion, and "bleach alternative" detergent) on different substrates with the aid of all alternate light sources available in the section.

7.2.2 Examine several stained and unstained smears for spermatozoa using phase contrast microscopy and compare results.

7.2.3 Perform presumptive and confirmatory tests, as appropriate, on a minimum of 50 known semen samples of varying ages, on various substrates, including mixtures and dilutions (neat to 1:100), and stains subjected to various contaminants and environmental conditions.

7.2.4 Examine and compare 20 different prepared slides of animal spermatozoa in the reference collection.

7.2.5 Perform presumptive and confirmatory tests, as appropriate, on at least 20 samples of various known physiological fluids, including different semen dilutions, mixtures, and aspermic semen samples.

7.2.6 Test a minimum of 12 samples of varying dilutions of semen using the OneStep ABAcard® p30 Test to determine the sensitivity of the p30 test. Compare results.

7.2.7 Using presumptive and confirmatory tests, as appropriate, examine a series of unknown samples (25 minimum) for spermatozoa identification as provided by the training coordinator or designee. These samples should consist of samples of varying dilutions of spermatozoa as well as samples with no spermatozoa. Use appropriate cleaning techniques between samples to ensure that no cross-contamination has occurred.

7.2.8 Observe and obtain instruction from qualified examiners performing routine examinations of case material.

7.2.9 Read applicable literature. Refer to Appendix A and Appendix B.

7.3 Training Evaluation

7.3.1 Knowledge

7.3.1.1 Review of notes in training notebook by training coordinator.

7.3.1.2 Mini-mock trials/oral and practical examinations.

7.3.1.3 Completion of checklist by training coordinator.

7.3.2 Skills

7.3.2.1 Observation by training coordinator or designee.

7.3.2.2 Observation by training coordinator or designee.

7.3.2.3 Review of notes in training notebook by training coordinator.

7.3.2.4 Mini-mock trials/oral and practical examinations.

7.3.2.5 Completion of checklist by training coordinator.

7.4 Technical Notes

7.4.1 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 (sometimes referred to as a "Wood's Lamp" by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each 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, yogurt, cleaners, bleach alternatives such as UV dyes) 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.

7.4.2 When the presence of semen is suspected in a stain, the Acid Phosphatase Test, a preliminary chemical test used to screen stains for the presence of semen, is conducted initially. 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.

7.4.3 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, a human seminal plasma protein. In general, the presence of semen on swabs from a Physical Evidence Recovery Kit is confirmed by the finding of spermatozoa on the correspondingly labeled smears. Acid Phosphatase testing is optional when the correspondingly labeled smears are positive for spermatozoa. 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 correspondingly labeled smears or in an extract of the stain, semen may be confirmed by the identification of p30.

7.5 Acid Phosphatase Test (Reference 6, pp. 162-163, Appendix B)

7.5.1 Safety Considerations

7.5.1.1 Glacial acetic acid - Caution! Corrosive! Flammable!

7.5.1.2 Sodium acetate - Caution! Irritant!

7.5.1.3 Sodium α-naphthyl acid phosphate - Caution! Irritant! Emits toxic fumes under fire conditions!

7.5.1.4 o-Dianisidine (Naphthanil diazo blue B) - Caution! Highly toxic! Emits toxic fumes under fire conditions!

7.5.1.5 Naphthanil diazo red - Caution! Avoid contact and inhalation! Emits toxic fumes under fire conditions!

7.5.2 Equipment

7.5.2.1 5 ml and 500 ml Graduated cylinders

7.5.2.2 Balance

7.5.2.3 Spatula

7.5.2.4 Scissors

7.5.2.5 Tweezers

7.5.3 Materials

7.5.3.1 Filter paper or microtiter plate (optional)

7.5.3.2 Weigh boats or weigh paper

7.5.3.3 Cotton swabs

7.5.3.4 Test tubes or bottles

7.5.3.5 Disposable transfer pipets or droppers

7.5.4 Working Solutions

7.5.4.1 Acid Phosphatase (AP) Buffer

- 2.5 ml Glacial acetic acid
- 10.0 g Sodium acetate (anhydrous)
- 450.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved.

7.5.4.1.1 Storage

7.5.4.1.1.1 The AP Buffer is stable at room temperature.

7.5.4.1.2 Labeling

7.5.4.1.2.1 Label the bottle as AP Buffer with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).
Example: AP Buffer Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

7.5.4.1.2.2 There is no expiration date (see 7.5.5 Minimum Standards and Controls).

7.5.4.2 Sodium α-Naphthyl Acid Phosphate Solution

7.5.4.2.1 Add a small amount (approximately 4 mg) of sodium α-naphthyl acid phosphate to approximately 3 ml of Acid Phosphatase buffer in an appropriately labeled 10 X 75 mm test tube or bottle.

7.5.4.2.2 Discard the solution at the end of the day.

7.5.4.3 Dye Solution

7.5.4.3.1 Add a small amount (approximately 4 mg) of o-dianisidine or naphthanil diazo red to approximately 3 ml of Acid Phosphatase buffer in an appropriately labeled 10 X 75 mm test tube or bottle.

7.5.4.3.2 Discard the solution at the end of the day.

7.5.4.4 Distilled water

7.5.5 Minimum Standards and Controls

7.5.5.1 Test a positive reagent control (known semen stain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

7.5.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

7.5.5.3 If the results of the test are positive, a substrate control (if available) must also be tested, unless the stain is on a cotton swab, and the results of the testing documented in the case file. It is not necessary to test submitted control swabs.

7.5.6 Acid Phosphatase (AP) Test Procedure

7.5.6.1 Moisten filter paper/swab with distilled water. (Do not use AP buffer solution, as this will contaminate the stained area.) Press the filter paper against the suspected stain or gently rub the stained area with the moistened swab. Alternatively, a small piece of the stain/swab can be placed on filter paper, in a small test tube, or in a microtiter plate. Treat the substrate control in the same manner.

7.5.6.2 Add 1-2 drops of sodium α-naphthyl acid phosphate solution.

7.5.6.3 Add 1-2 drops of dye solution.

7.5.6.4 The development of a blue/purple color with o-dianisidine or an orange/red color with naphthanil diazo red within 10 to 15 seconds is indicative of acid phosphatase levels in the semen range. Although the development of a pink/peach color may be observed with o-dianisidine, this is not indicative of seminal acid phosphatase and therefore, is not considered a positive reaction.

7.5.6.5 The presence of semen in all samples exhibiting an inconclusive result or a positive result must be confirmed by identifying spermatozoa or, in the absence of spermatozoa, p30.

7.5.6.6 Interpretation

7.5.6.6.1	Positive Reaction =	Blue/purple color with o-dianisidine within 10 to 15 seconds OR Orange/red color with naphthanil diazo red within 10 to 15 seconds
7.5.6.6.2	Negative Reaction =	No color development, slight/slow color development
7.5.6.6.3	Inconclusive Reaction =	Slow moderate to strong color development

7.5.6.7 Refer to section 7.11 for reporting results.

7.6 Extraction of Spermatozoa from a Substrate

7.6.1 Equipment

7.6.1.1 Rotator, vortex, sonicator,or centrifuge (depending on extraction method used)

7.6.1.2 Scissors

7.6.1.3 Tweezers

7.6.1.4 Dissecting needle (optional)

7.6.2 Materials

7.6.2.1 Microscope slides

7.6.2.2 Test tubes

7.6.3 Reagents

7.6.3.1 Distilled water

7.6.4 Extraction Methods

7.6.4.1 Cut a small portion of a stain and soak in a test tube overnight in distilled water.

7.6.4.2 Soak a small portion of a stain in distilled water and rotate overnight.

7.6.4.3 Soak a small portion of a stain in distilled water and sonicate for 10 seconds, followed by a 30 second sonication.

7.6.4.4 Tease fibers apart and soak in a small amount of distilled water.

7.6.4.5 Soak a small portion of a stain in distilled water and vortex.

7.6.4.6 Soak a small portion of a stain in distilled water on a microscope slide, stain side down (may be followed by mastication).

7.6.4.7 Cut the stain into small pieces, place the pieces on a microscope slide, and soak in a small amount of distilled water (may be followed by mastication).

7.6.4.8 For the OneStep ABAcard® p30 Test extraction method, refer to 7.11.6.1 through 7.11.6.7.

NOTES: Always soak the material first; prolong the soaking for difficult stains.
Use the sonicator on low (high setting will disintegrate spermatozoa).
To concentrate an extract, after soaking a small portion of a stain or swab, centrifuge and make a smear of the sediment.
DNA extracts can also be used to search for spermatozoa.

7.7 Kernechtrot-Picoindigocarmine Stain (Christmas Tree Stain) (Reference 7, p. 141, Appendix B)

7.7.1 Kernechtrot-Picroindigocarmine (KPIC) differential biological stain is used to assist in the identification of spermatozoa. The solutions for this procedure can either be purchased (SERI) or prepared in-house.

7.7.2 Safety Considerations

7.7.2.1 Aluminum sulfate - Caution! Harmful by inhalation, in contact with skin, and if swallowed! Emits toxic fumes under fire conditions!

7.7.2.2 Nuclear fast red - Caution! Irritant! Emits toxic fumes under fire conditions!

7.7.2.3 Saturated picric acid solution - Caution! Toxic! Explosive when dry! Emits toxic fumes under fire conditions!

7.7.2.4 Indigocarmine dye - Caution! Harmful if swallowed! Emits toxic fumes under fire conditions!

7.7.3 Equipment

7.7.3.1 Flame or heat block (37° C)

7.7.4 Materials

7.7.4.1 Fixative (optional)

7.7.5 Reagents

7.7.5.1 Kernechtrot staining solution (KS)

7.7.5.2 Picroindigocarmine staining solution (PICS)

7.7.5.3 Distilled water

7.7.5.4 95% ethanol or methanol

7.7.6 Stock Solutions (In-house Preparation)

7.7.6.1 Equipment

7.7.6.1.1 Filtration apparatus

7.7.6.1.2 500 ml glass beakers
7.7.6.1.3 Balance

7.7.6.1.4 Spatula

7.7.6.1.5 Glass rod

7.7.6.1.6 Plastic bottles

7.7.6.2 Materials

7.7.6.2.1 Filter paper

7.7.6.2.2 Weigh boats or weigh paper

7.7.6.3 Reagents

7.7.6.3.1 Aluminum sulfate

7.7.6.3.2 Nuclear Fast Red

7.7.6.3.3 Distilled water

7.7.6.3.4 Picroindigocarmine dye

7.7.6.3.5 Saturated picric acid solution **(Purchase saturated solution. DO NOT PURCHASE DRY PRODUCT! See Safety Considerations, 7.7.2.3.)**

7.7.6.4 Kernechtrot Solution (KS)

- In a beaker dissolve 5 g of aluminum sulfate in 100 ml of hot distilled water.
- Immediately add 0.1 g of Nuclear Fast Red and stir with a glass rod.
- Allow to cool and filter through filter paper.

7.7.6.4.1 Storage

7.7.6.4.1.1 The Kernechtrot Solution is stable at room temperature for up to 6 months, but may need to be refiltered after standing.

7.7.6.4.2 Labeling

7.7.6.4.2.1 Label the bottle as KS with the expiration date and a lot number (the date of preparation followed by the initials of person preparing the solution).
Example: KS Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

7.7.6.5 Picroindigocarmine Solution (PICS)

- Dissolve 1 g of Indigocarmine dye in 300 ml of a commercially purchased saturated solution of picric acid.
- Filter and store.

7.7.6.5.1 Storage

7.7.6.5.1.1 The Picroindigocarmine Solution is stable at room temperature for up to 6 months, but may need to be refiltered after standing.

7.7.6.5.2 Labeling

7.7.6.5.2.1 Label the bottle as PICS with an expiration date and a lot number (the date of preparation followed by the initials of person preparing the solution).
Example: PICS Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

7.7.7 SERI Christmas Tree Stain (R540) Kit

7.7.7.1 Contents

7.7.7.1.1 Solution A (Kernechtrot Solution - KS) - 30 ml

7.7.7.1.2 Solution B (Picroindigocarmine Solution - PICS) - 30 ml

7.7.7.1.3 Directions for use.

7.7.7.2 Store under refrigeration in bottles provided.

7.7.7.3 Shelf life: 6 months

7.7.8 KPICS/Christmas Tree Staining Procedure

7.7.8.1 Prepare a thin smear of an extract of a suspected semen stain and allow to dry, or examine a smear from the Physical Evidence Recovery Kit (PERK). Fix the smear with a quick flame or fixative, or by placing it on a 37° C heat block overnight.

7.7.8.2 Add a sufficient amount (2-5 drops) of KS (red reagent) to cover the stained portion of the microscope slide.

7.7.8.3 Let the slide stand at room temperature for at least 15 minutes.

7.7.8.4 Wash KS off of the slide with a gentle stream of distilled water and drain the slide.

7.7.8.5 Add a sufficient amount (2-5 drops) of PICS (green reagent) to cover the stained portion of the slide.

7.7.8.6 Allow PICS to stain the smear for 5-15 seconds.

7.7.8.7 Wash PICS off of the slide with 95% ethanol or methanol.

7.7.8.8 Dry the slide at room temperature.

7.8 Microscopic Examination of Stained Slides for Spermatozoa

7.8.1 Equipment

7.8.1.1 Microscope (with approximately 200X – 400X total magnification, with or without phase capability)

7.8.2 Materials

7.8.2.1 Distilled water, xylene substitute, or other appropriate mounting medium

7.8.2.2 Coverslips

7.8.3 Procedure for the Microscopic Examination of Stained Slides for Spermatozoa

7.8.3.1 Quickly scan at approximately 200X total magnification. Confirm at approximately 400X total magnification.

7.8.3.1.1 With phase microscopy: Spermatozoa heads are neon-like pink/red with darker pink/purple acrosomal caps and green tails. Epithelial cells and most bacteria stain green with some of the nuclei pink/red; however, these are shaped differently than spermatozoa. Yeast cells take on the same color as spermatozoa, but are shaped differently.

7.8.3.1.2 Without phase microscopy: Spermatozoa heads are neon-like pink/red with pale pink (almost colorless) acrosomal caps, blue-green neck/midpieces, and green tails. Epithelial cells appear bright blue with red to purple nuclei.

7.8.3.2 Document the approximate number of spermatozoa and spermatozoa heads on the smear per hpf (approximately 400X total magnification), per lpf (approximately 200X total magnification), per length of slide, or per slide, as appropriate. **If only 1 spermatozoon or spermatozoon head is observed, there must be documented confirmation of its presence by a second qualified examiner.**

7.8.3.3 Place all smears submitted in the PERK back into the PERK. Properly label and return all other spermatozoa positive smears with the evidence. **Note: If a stain is consumed in the preparation of a smear, properly label and return the smear even when no spermatozoa are identified.**

7.8.4 Refer to section 7.11 for reporting results.

7.9 Microscopic Examination of Unstained Slides for Spermatozoa

7.9.1 Unstained smears may be examined using phase contrast microscopy.

7.9.2 Equipment

7.9.2.1 Microscope (approximately 200X – 400X total magnification) with phase capability

7.9.3 Materials

7.9.3.1 Microscope slides

7.9.3.2 Coverslips

7.9.3.3 Applicator sticks

7.9.4 Reagents

7.9.4.1 Distilled water

7.9.5 Procedure for the Microscopic Examination of Unstained Slides for Spermatozoa

7.9.5.1 Place a small amount of an extract of a suspected semen stain on a microscope slide and cover with a coverslip, or add a drop of distilled water to a smear from the PERK, mix the water and the material on the smear, and cover with a coverslip.

7.9.5.2 Scan quickly with phase at approximately 200X total magnification. Confirm with phase at approximately 400X total magnification.

7.9.5.3 When the coverslip is touched gently, the spermatozoa and/or spermatozoa heads will roll, exhibiting their characteristic 3-dimensional shape. Use the distinctive size and morphology to identify the spermatozoa/spermatozoa heads.

7.9.5.4 Document the approximate number of spermatozoa and spermatozoa heads on the smear per hpf (approximately 400X total magnification), per lpf (approximately 200X total magnification), per length of slide, or per slide, as appropriate. **If only 1 spermatozoon or spermatozoon head is observed, there must be documented confirmation of its presence by a second qualified examiner.**

7.9.5.5 Place all smears submitted in the PERK back into the PERK. Properly label and return all other spermatozoa positive smears with the evidence. **Note: If a stain is consumed in the preparation of a smear, properly label and return the smear even when no spermatozoa are identified.**

7.9.6 Refer to section 7.11 for reporting results.

7.10 ABAcard® OneStep p30 DETECTION TEST

7.10.1 Technical Notes

7.10.1.1 This test for the prostate protein p30 was characterized by Hochmeister, et al. in a paper entitled "Evaluation of Prostate-Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid" (reference 9, Appendix B) as an immunochromatographic PSA membrane test. A stain can be extracted for microscopic sperm identification using the sample preparation procedure set forth in section 7.11.6. If no spermatozoa are identified, the analyst can then proceed to identify semen with the OneStep ABAcard® Test.

7.10.1.2 Principle of the ABAcard® OneStep p30 Detection Test

Sample is added to the sample well "S" and if 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". In the test area "T", a polyclonal antihuman p30 antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the p30 concentration in the sample exceeds 4 ng/ml the pink dye particles will form a pink colored band in the test area "T" indicating a positive test result. As an internal positive control, p30 antibody-dye conjugates cannot bind to the antibody in the test area "T", but are captured by an immobilized anti-immunoglobin 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" indicating that the test has worked properly and proper procedures have been followed. The presence of two colored lines, one in the test area "T" and the other in the control area "C", indicates a positive result, while a line only in the control area "C" would indicate a negative result.

7.10.2 Quality Control

7.10.2.1 Before using a new lot number of the ABAcard® OneStep p30 Detection Test, its specificity must be tested and appropriately documented in the laboratory's quality control records. It is also desirable to test dilutions of semen to determine the sensitivity of the test.

7.10.2.2 The ABAcards® ("test devices") must be tested against human blood, vaginal fluid, saliva, feces, urine, a positive control (semen), and a negative control (distilled water) to ensure that the test is semen specific.

7.10.2.2.1 Samples of human blood, vaginal fluid, saliva, feces, urine, and semen will be prepared in-house.

7.10.2.2.2 Label the known samples with the name of the substance (i.e., human semen, etc.) and the lot number (the date of preparation followed by the initials of the person preparing the sample).

Example: human semen Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

7.10.2.2.3 Store known samples in the freezer.

7.10.2.3 The quality control documentation will include:

7.10.2.3.1 The lot number, receipt date, expiration date, and manufacturer of the ABAcard® OneStep p30 Detection Test.

7.10.2.3.2 The date of testing.

7.10.2.3.3 Initials of the person conducting the testing.

7.10.2.3.4 Results of the testing.

7.10.2.4 Once the appropriate tests have been performed on a lot number of the ABAcard® OneStep p30 Detection Test, they need not be repeated for each case. If another shipment of the same lot number is received on a different date, the QC testing described above must be repeated.

7.10.3 "High Dose Hook Effect"

7.10.3.1 The "High Dose Hook Effect" is a false negative result that is obtained in the presence of high concentrations of p30 (usually undiluted semen). This effect results from large amounts of human p30 binding to the antibody to form an antigen-antibody complex and free p30 migrating toward the test area "T". The antibody in the test area "T" is blocked by this free p30. Therefore, the mobile antigen-antibody complex cannot bind to the antibody. As a result no pink line will form in the test area "T". To confirm the presence of "High Dose Hook Effect", repeat the test using a 10-10,000 fold dilution of the sample.

7.10.4 Stability, Storage and Shelf Life

7.10.4.1 The OneStep ABAcard® p30 Detection Test should be stored below 82° F (28° C).

7.10.4.2 The test can be stored in the sealed pouch below 82° F (28° C) until the expiration date as printed on the sealed test pouch.

7.10.4.3 DO NOT FREEZE.

7.10.4.4 Do not use the test after the expiration date.

7.10.5 p30 BY OneStep ABAcard® (References 9, 10, 11, Appendix B) Test Kit

7.10.5.1 Reagents and Materials Provided

7.10.5.1.1 Test Device (25 pieces, each individually sealed in a test pouch) - one device needed per sample tested

7.10.5.1.2 A dropper and a desiccant sealed inside each of the test pouches

7.10.5.1.3 Test Instructions

7.10.5.2 Equipment Required But Not Provided

7.10.5.2.1 Microcentrifuge

7.10.5.2.2 Timer

7.10.5.2.3 Scissors

7.10.5.2.4 Tweezers

7.10.5.2.5 Microcentrifuge tube rack

7.10.5.2.6 Microcentrifuge tube rack

7.10.5.2.7 Dissecting needle

7.10.5.3 Materials Required But Not Provided

7.10.5.3.1 Microcentrifuge tubes

7.10.5.3.2 Microcentrifuge tube lids

7.10.5.3.3 Pipette tips

7.10.5.4 Reagents Required But Not Provided

7.10.5.4.1 Known semen sample

7.10.5.4.2 Reagent blank

7.10.5.4.3 Distilled water

7.10.5.5 Minimum Standards and Controls

7.10.5.5.1 On the day of use a positive reagent control (known seminal fluid) and a negative reagent control (distilled water) must be tested to ensure that the reagents and test device are working properly. The results of this testing must be documented in the case file.

7.10.5.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

7.10.5.5.3 A substrate control (when available) must also be tested, unless the stain is on a cotton swab, and the results of the testing documented in the case file. It is not necessary to test submitted control swabs.

7.10.5.6 p30 BY OneStep ABAcard® Procedure

7.10.5.6.1 Cut a portion of the stain into small pieces (size based upon the substrate and the intensity of the acid phosphatase test) and place into a labeled microcentrifuge tube.

7.10.5.6.2 Add 200 µl of distilled water (250 µl if a sperm search is also being conducted) and cap the tube.

7.10.5.6.3 Allow the sample to extract at room temperature for a minimum of 2 hours. Extraction can be done overnight if desired.

7.10.5.6.4 Punch holes in the lid of the tube.

7.10.5.6.5 Place the cuttings into the lid.

7.10.5.6.6 Centrifuge for 5 minutes at approximately 10,000 rpm to recover the liquid.

7.10.5.6.7 If a microscopic sperm search is to be conducted, remove approximately 220 µl of the extract and place into a new labeled microcentrifuge tube. This aliquot will be used for the test procedure and may be stored between 2-8°C or frozen if not used immediately. The remaining extract and pellet can be used for the sperm search.

7.10.5.6.8 **Allow the sample to warm to room temperature** if it has been refrigerated or frozen.

7.10.5.6.9 Remove the device and dropper from the sealed pouch.

7.10.5.6.10 Add approximately 200 µl (or 8 drops with the dropper) of the sample to the sample well "S" on a labeled test device.

7.10.5.6.11 Record result at 10 minutes. A positive result can be seen as early as 1 minute. For negative results, one must wait for the full 10 minutes. All control samples must give the expected results before the result on an unknown sample can be called, i.e., the substrate control is negative, the reagent blank is negative, and the known semen sample is positive. A diagrammatic representation of the results is located below.

7.10.5.6.12 Interpretation

7.10.5.6.12.1	Positive Result =	2 pink lines, one in the test area "T" and one in the control area "C" p30 level is at or above 4ng/ml
7.10.5.6.12.2	Negative Result =	1pink line in the control area "C" No p30 is present above 4ng/ml OR presence of "High Dose Hook Effect".
7.10.5.6.12.3	Invalid Result =	No pink line in the control area "C" The test is inconclusive. Repeat the test.

7.10.5.6.12.4 Refer to the diagrammatic representation of the results on the next page.

***OneStep* ABAcard® p30 TEST RESULTS DIAGRAMMATIC REPRESENTATION**

Note: OneStep ABAcard® p30 Test results diagrammatic representation is taken from Abacus Diagnostics, OneStep-ABAcard® p30 Test For Identification of Semen, Technical Information Sheet (Revised 10/98).

7.11 Reporting Results

7.11.1 Report the results of semen testing using the statements which follow:

7.11.1.1 Positive findings

7.11.1.1.1 "Spermatozoa were identified …"

7.11.1.1.2 "A spermatozoon was identified …"

7.11.1.1.3 "Seminal fluid, but no spermatozoa, was identified …"

7.11.1.2 Negative findings

7.11.1.2.1 "No spermatozoa or seminal fluid was detected…"

7.11.1.2.2 "No seminal fluid was detected…" (This wording will also be used if only a visual exam, with or without ALS or AP, was conducted.)

7.11.1.3 Inconclusive findings

7.11.1.3.1 "Tests for seminal fluid were inconclusive…"

7.11.1.3.2 "Tests for seminal fluid were inconclusive and the stain was insufficient for further body fluid identification testing…"

SEMEN IDENTIFICATION STUDY QUESTIONS

1. What is semen?
2. What glands contribute to seminal fluid?
3. What is p30 and where is it found?
4. What is the significance of p30 and under what circumstances would you test for it?

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

6. 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?

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

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

9. How does an alternate light source assist in locating stains? What alternate light sources are used by DFS (include filters used and wavelengths)?

10. What is the name of the stain used to stain smears for spermatozoa examination? What is the purpose of each chemical?

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

12. Describe the morphology of a spermatozoon.

13. What factors may affect the persistence of 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?

14. 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?

15. What, if any, is the significance of observing only sperm heads versus intact sperm on a slide?

16. Explain the difference between seminal acid phosphatase and vaginal acid phosphatase.

17. 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.

18. You get a call from an investigator saying he has a girl who is pregnant due to a rape that occurred about 6 weeks ago. She wants to get an abortion now. What do you advise the investigator?

19. How do you preserve a used condom?

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

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

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

23. 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?

CHECKLIST FOR SEMEN IDENTIFICATION

Name of Trainee:_____

1. Examination of several stained and unstained smears for spermatozoa using phase contrast microscopy. Results compared.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Completion of presumptive and confirmatory testing on 50 (minimum) known semen samples in the following categories:

   Semen of varying ages (approximately 10)

   Date:_____ Training Coordinator:_____

   Comments:_____

   Semen on varying substrates (approximately 10)

   Date:_____ Training Coordinator:_____

   Comments:_____

   Mixture samples (approximately 10)

   Date:_____ Training Coordinator:_____

   Comments:_____

   Diluted Semen (neat to 1:100) (approximately 10)

   Date:_____ Training Coordinator:_____

   Comments:_____

   Semen stains subjected to various contaminants and environmental conditions (approximately 10)

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Examination and comparison of 20 different animal semen samples.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Completion of presumptive and confirmatory testing on various known physiological fluids, including different semen dilutions, mixtures, and aspermic semen samples (20 minimum).

Date:_____ Training Coordinator:_____

Comments:_____

5. Determined the sensitivity of p30 by testing various dilutions of semen using the p30 By OneStep ABA Card® (12 minimum).

Date:_____ Training Coordinator:_____

Comments:_____

6. Accurately examined a series of unknown samples of varying dilutions of seminal fluid including samples with no spermatozoa, using presumptive and confirmatory tests, as appropriate (25 minimum).

Date:_____ Training Coordinator:_____

Comments:_____

7. Trainee has developed a thorough understanding of the theory behind the acid phosphatase test and the p30 By OneStep ABA Card® test for semen, including the use of controls, sources of error, and the specificity and limitations of the tests.

Date:_____ Training Coordinator:_____

Comments:_____

8. Trainee has become proficient in the use of the acid phosphatase test and the p30 By OneStep ABA Card® test for semen, as well as in the extraction, staining and microscopic examination of spermatozoa.

Date:_____ Training Coordinator:_____

Comments:_____

9. Trainee has become proficient in the use of alternate light sources for locating semen stains.

Date:_____ Training Coordinator:_____

Comments:_____

10. Trainee has become proficient in techniques used to prevent cross-contamination between samples.

Date:_____ Training Coordinator:_____

Comments:_____

11. Trainee's notebook is organized and complete.

Date:_____ Training Coordinator:_____

Comments:_____

12. Trainee has participated in a mock trial and/or practical or oral examinations. Performance was satisfactory.

Date:_____ Training Coordinator:_____

Comments:_____

13. Trainee has read and understands all applicable literature.

Date:_____ Training Coordinator:_____

Comments:_____

8 OTHER BIOLOGICAL SAMPLES

8.1 Goals

8.1.1 To become familiar with the physical and chemical characteristics of vaginal fluid.

8.1.2 To learn the theory behind the procedures for the detection of urine and feces, including the specificity and limitations of the tests as well as the use of controls.

8.1.3 To become acquainted with the limitations of the Ouchterlony double diffusion test to determine species origin on vaginal, urine, fecal, and tissue samples.

8.1.4 To become acquainted with the limitations and specificity of the chemical tests for the presence of amylase in saliva.

8.2 Tasks

8.2.1 Test several human and animal urine stains of varying dilutions and sizes using the urease test.

8.2.2 Test physiological fluid stains (blood, semen, vaginal fluid, saliva, and feces) using the urease test.

8.2.3 Test several human and animal fecal stains of varying sizes using Edelman's Test.

8.2.4 Test several human vaginal, urine, feces, and tissue samples using Ouchterlony double diffusion.

8.2.5 Compare results. Refer to section 6.4.9, Ouchterlony double diffusion, for the procedure.

8.2.6 Observe and obtain instruction from qualified examiners performing routine examinations of case material.

8.2.7 Read applicable literature. Refer to Appendix A and Appendix B.

8.3 Training Evaluation

8.3.1 Knowledge

8.3.1.1 Review of notes in training notebook by training coordinator.

8.3.1.2 Mini-mock trials/oral and practical examinations.

8.3.1.3 Completion of checklist by training coordinator.

8.3.2 Skills

8.3.2.1 Observation by training coordinator or designee.

8.3.2.2 Review of notes in training notebook by training coordinator.

8.3.2.3 Mini-mock trials/oral and practical examinations.

8.3.2.4 Completion of checklist by training coordinator.

8.4 Detection of Urine – Technical Notes

8.4.1 Screening items such as clothing or bedding for the presence of urine stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a "Wood's Lamp" by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime

Scope MCS400, to name a few. Users must read the directions accompanying each 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 urine contains a component (urea) which reacts to light between 450 and 455 nm wavelengths. The reaction appears as a light stain against a dark background. The reaction must be interpreted with caution since other substances (such as, but not limited to, semen, saliva, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Samples exhibiting a reaction to an ALS require further examination to detect the presence of urine.

8.4.2 The urease test is a presumptive test for the presence of urine and is based on the fact that urea is found in high concentration in urine. Although there are many different presumptive tests for the presence of urine, there are no confirmatory tests available for the identification of urine in a dried stain. The urease reagent reacts with urea, releasing ammonia from the stain, which turns red litmus paper to a blue color.

8.5 Urease Test (Reference 6, pp. 191-193, Appendix B)

8.5.1 Equipment

8.5.1.1 Scissors

8.5.1.2 Tweezers

8.5.1.3 Scalpel or other sharp instrument (to cut cork)

8.5.1.4 Heat block (37° C)

8.5.2 Materials

8.5.2.1 Test tubes (10 X 75 mm)

8.5.2.2 Corks

8.5.2.3 Disposable pipets

8.5.3 Reagents

8.5.3.1 Distilled water

8.5.3.2 Urease reagent

8.5.3.2.1 Store at 2-8° C.

8.5.3.2.2 According to the manufacturer (Sigma), reagents stored at 2-8° C will have a shelf life of 2 years from the manufacturer's quality control date.

8.5.3.3 Red litmus paper

8.5.3.4 Positive control (known urine)

8.5.4 Minimum Standards and Controls

8.5.4.1 A positive reagent control (known urine stain) and a substrate control (or if not available, distilled water) must be tested and results documented in the case file. If the stain is on a cotton swab, it is not necessary to test a substrate control. It is not necessary to test submitted control swabs.

8.5.5 Urease Test Procedure

8.5.5.1 Cut an approximate 2 cm² piece of a suspected urine stain and the appropriate controls into small pieces. Place the cuttings into appropriately labeled 10 X 75 mm test tubes.

8.5.5.2 Add 3-4 drops of distilled water and 6-7 drops of urease reagent to each tube.

8.5.5.3 Cut a slit into the small end of a cork and insert a strip of red litmus paper into this slit.

8.5.5.4 Place the cork (with red litmus paper) into each test tube. Do not allow the litmus paper to touch the liquid.

8.5.5.5 Incubate the samples in a 37° C heat block for 30 minutes.

8.5.5.6 Observe any change in the color of the litmus paper. Document results in the case file.

8.5.5.7 All controls must give the expected results before a conclusion can be reached on an unknown sample. When all controls work properly and a positive reaction is observed for the unknown sample, urine is indicated to be present.

8.5.5.8 Interpretation

8.5.5.8.1	Positive Reaction =	Red litmus paper turns blue
8.5.5.8.2	Negative Reaction =	No color change to red litmus paper
8.5.5.8.3	Inconclusive Reaction =	No color change of the positive control to the red litmus paper and/or substrate control turns red litmus paper blue

8.5.5.9 Reporting Results

8.5.5.9.1 Report positive test results as “Urine was indicated…”

8.5.5.9.2 Report negative test results as “No urine was detected…”

8.5.5.9.3 Report inconclusive test results as “The test for urine was inconclusive…”

8.6 Detection of Fecal Material – Technical Notes

8.6.1 Edelman’s test is a presumptive test for the presence of fecal material and is based on the detection of urobilinogen found in high concentration in feces. Urobilinogen is formed in the intestine by the reduction of bilirubin. Urobilinogen is oxidized to urobilin, which is soluble in alcohol. In the presence of neutral alcoholic salts, a green fluorescent complex is formed between urobilin from human or Carnivore feces and zinc. Due to the presence of chlorophyll in Herbivore (ruminants, such as cattle, sheep, and deer) feces, fluorescence will be orange-pink. Although there are other presumptive tests to indicate the presence of fecal material, there are no confirmatory tests available for the identification of fecal material.

8.7 Edelman’s Test (Reference 12, pp. 4-7, Appendix B)

8.7.1 Safety Considerations

8.7.1.1 Mercuric chloride - Caution! Very toxic if inhaled or swallowed, or if in contact with skin! Poisonous! Dangerous! May be fatal!

8.7.1.2 Zinc chloride - Caution! Corrosive!

8.7.1.3 Amyl alcohol (isopentyl alcohol) - Caution! Harmful if swallowed or inhaled! Irritant! Combustible!

8.7.2 Equipment

8.7.2.1 Scissors

8.7.2.2 Tweezers

8.7.2.3 Centrifuge

8.7.2.4 Long wavelength ultraviolet light source

8.7.2.5 Vortex

8.7.3 Materials

8.7.3.1 Disposable pipets

8.7.3.2 Test tubes and/or microcentrifuge tubes

8.7.4 Reagents

8.7.4.1 10% Saturated mercuric chloride solution (1 g in 10 ml of 95% ethanol)

8.7.4.2 10% Saturated zinc chloride solution (1 g in 10 ml of 95% ethanol)

8.7.4.3 Amyl alcohol (isopentyl alcohol)

8.7.4.4 Distilled water

8.7.4.5 Positive control (known feces)

8.7.5 Storage

8.7.5.1 The 10% saturated solutions of mercuric chloride and zinc chloride are stable at room temperature.

8.7.6 Labeling

8.7.6.1 Label each bottle with the contents and lot number (date of preparation followed by the initials of person preparing the solution).

Example: 10% saturated zinc chloride solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

8.7.6.2 There is no expiration date (see 8.7.7 Minimum Standards and Controls).

8.7.7 Minimum Standards and Controls

8.7.7.1 A positive reagent control (known fecal stain), and a substrate control (when available) must be tested and results documented in the case file. Distilled water will be used as a negative control. If the stain is on a cotton swab, it is not necessary to test a substrate control. It is not necessary to test submitted control swabs.

8.7.8 Edelman's Test Procedure

8.7.8.1 Place an approximate ½ cm² piece of suspected fecal stain and controls in appropriately labeled test tubes or microcentrifuge tubes and extract in a minimum of 3 drops of distilled water at room temperature for at least 15 minutes.

8.7.8.2 Remove the material and add a minimum of 3 drops of 10% saturated zinc chloride solution to the extract.

8.7.8.3 Add 5 drops of amyl alcohol (isopentyl alcohol) to the extract and vortex.

8.7.8.4 Centrifuge for 5 minutes. Pipet the supernatant layer into an appropriately labeled test tube.

8.7.8.5 Add 3 drops of 10% saturated mercuric chloride solution.

8.7.8.6 Observe color changes in both white and ultraviolet light. Document results. If urobilin is present the solution may become rose-pink, but will show a crab apple green fluorescence under long wave ultraviolet light.

8.7.8.7 All controls must give the expected results before a conclusion can be reached on an unknown sample. When all controls work properly and a positive reaction is observed for the unknown sample, feces is indicated to be present.

8.7.8.8 Interpretation

8.7.8.8.1	Positive Reaction =	Crab apple green fluorescence under long wave ultraviolet light
8.7.8.8.2	Negative Reaction =	No green fluorescence under long wave ultraviolet light
8.7.8.8.3	Inconclusive Reaction =	No green fluorescence of the positive control under long wave ultraviolet light and/or substrate control exhibits crab apple green fluorescence under long wave ultraviolet light

8.7.8.9 Reporting Results

8.7.8.9.1 Report positive results as "Fecal material was indicated…"

8.7.8.9.2 Report positive results as "Fecal material was indicated…"

8.7.8.9.3 Report inconclusive results as "The test for fecal material was inconclusive…"

8.8 Detection of Saliva – Technical Notes

8.8.1 Screening items such as masks or clothing for the presence of saliva stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a "Wood's Lamp" by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each 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 biological fluids may react to light between 450 and 455 nm wavelengths. The reaction may either appear as a faint 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, semen, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Since the Department does not conduct presumptive testing for the presence of saliva, samples exhibiting a reaction to an ALS may require DNA analysis.

OTHER BIOLOGICALS STUDY QUESTIONS

1 Describe the mechanism for the Urease test, including chemicals used and why.

2 Describe the mechanism for Edelman's test including chemicals used and why.

3 Are there any false positives for the Urease test? Edelman's test?

4 When would you test evidence for urine? Feces?

5 In what cases does the finding of urine become important? Feces?

6 What are some other methods used for the detection of urine and feces?

7 Name at least one method that can be used to indicate the presence of saliva. Why doesn't DFS test for saliva?

8 Is there a test to indicate the presence of vaginal fluid? If there is such a test, why doesn't DFS use the test?

9 You receive a piece of bone and a piece of tissue from a decomposed body. How do you preserve these samples for possible future testing?

10 You get a call from an investigator saying he has what appears to be a piece of scalp tissue on broken glass at a felonious assault scene. What do you tell him to do with regard to packaging it and submitting it to the lab?

CHECKLIST FOR OTHER BIOLOGICAL SAMPLES

Name of Trainee:_____

1. Tested several human and animal urine stains of varying dilutions and sizes using the UreaseTest.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Tested physiological fluid stains (blood, semen, vaginal fluid, saliva, and feces) using the Urease Test.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Tested several human and animal fecal stains of varying sizes using Edelman's Test.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Tested several human vaginal, urine, feces and tissue samples using Ouchterlony double diffusion.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Trainee has learned the theory behind the techniques used for the detection of urine and feces, including specificity and limitations of the tests as well as the use of controls.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee has learned the limitations of the Ouchterlony double diffusion test to determine species origin on vaginal fluid, urine, fecal, and tissue samples.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee's notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

8. Trainee has participated in a mock trial and/or a practical or oral examination. Performance was satisfactory.

   Date:_____ Training Coordinator:_____

   Comments:_____

9. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

9 RELATED PROCEDURES

9.1 Goals

9.1.1 To acquire a basic knowledge of bloodstain patterns and surface deposition of stains, including how and when to group stains together for testing.

9.1.2 To acquire a thorough understanding of the design and use of Victim and Suspect Physical Evidence Recovery Kits and Blood or Buccal Swabs and Hair Samples Kit.

9.1.3 To acquire proficiency in the recovery and packaging of hairs and fibers and an understanding of when hair/fiber examinations are conducted.

9.1.4 To acquire a basic understanding of other forensic disciplines in order to recognize and preserve potential evidence related to these areas.

9.1.5 To acquire proficiency in the recovery of body fluids from porous and nonporous surfaces.

9.2 Tasks

9.2.1 Instruction from and observation of qualified examiners performing bloodstain pattern examinations, including surface of stain deposition, and how and when to group stains together for testing.

9.2.1.1 Although there is the potential for some investigative information to be gleaned from the nature of bloodstain deposits (drop, smear, splatter), it is well advised that any examiner in the Forensic Biology Section exercise extreme caution in establishing definitive opinions regarding the ballistics of such patterns. Definitive opinions should be reserved for those individuals with considerable expertise in this area.

9.2.2 Read applicable literature. Refer to Appendix A.

9.2.3 Instruction from and observation of qualified examiners performing examinations of Victim and Suspect Physical Evidence Recovery Kits, and Blood or Buccal Swabs and Hair Samples Kits.

9.2.4 Instruction from and observation of qualified examiners recovering/packaging hairs and fibers using various recovery techniques such as scraping and using tweezers and post-it notes.

9.2.5 Instruction from and observation of qualified examiners in the recovery of body fluids from porous and nonporous surfaces.

9.2.6 Instruction from and observation of qualified examiners in other forensic disciplines, particularly the Trace Evidence, Firearms, and Latent Fingerprint Sections.

9.2.6.1 Arrangements will be made by the Training Coordinator.

9.2.6.2 This period of instruction should be brief and confined to instruction and observation of items of evidence involved with examinations in the Forensic Biology Section.

9.3 Training Evaluation

9.3.1 Knowledge

9.3.1.1 Review of notes in training notebook by training coordinator.

9.3.1.2 Mini-mock trials/oral and practical examinations.

9.3.1.3 Completion of checklist by coordinator.

9.3.2 Skills

9.3.2.1 Observation of skills by training coordinator or designee.

9.3.2.1.1 The trainee should examine a sufficient number of cases to develop and exhibit an unquestionably sound technique for grouping stains, determining the surface of stain deposition, recovering and packaging hairs and fibers, recovering body fluids from porous and nonporous surfaces, and examining Victim and Suspect Physical Evidence Recovery Kits and Blood or Buccal Swabs and Hair Samples Kits.

9.3.2.1.2 The trainee should be able to easily recognize potentially valuable evidence involving other forensic disciplines and know how to handle the associated items so that the evidence is not compromised.

9.3.2.2 Review of notes in training notebook by training coordinator.

9.3.2.3 Mini-mock trials/oral and practical examinations.

9.3.2.4 Completion of checklist by coordinator.

RELATED PROCEDURES STUDY QUESTIONS

1. What is a PERK?
2. What is a FNE?
3. What is the difference between a victim PERK, suspect PERK, Blood or Buccal Swabs and Hair Samples Kit, and an OCME PERK? List the components of each.
4. Why might PERK vaginal/cervical swabs be slightly blue? Slightly green?
5. Some laboratories around the country may still conduct conventional serological typing before DNA analysis is conducted. Why doesn't DFS do that? What are the pros/cons for using this approach?
6. Is it important to document the surface of a stain's origin on an item of clothing? Explain how you would determine if a stain is deposited on the inside or outside of a piece of clothing.
7. An investigator calls and says he has a case that was analyzed by a DFS 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?
8. I checked the lot number on the urease 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?
9. You are examining a bed sheet and notice a possible footwear impression on it. What do you do?
10. You open a bag containing a knife that may be the weapon used in a murder. You see a possible fingerprint in red stains on the handle. What do you do?
11. 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?

12. 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?

TRAINEE CHECKLIST FOR RELATED PROCEDURES

Name of Trainee:_____

1. Trainee has observed and obtained instruction from qualified examiners performing the following:

   Bloodstain pattern examinations, including surface of stain deposition, and how and when to group stains together for testing.

   Date:_____ Training Coordinator/Examiner:_____

   Comments:_____

   Examination of Victim and Suspect Physical Evidence Recovery Kits, and Blood or Buccal Swabs and Hair Samples Kits.

   Date:_____ Training Coordinator/Examiner:_____

   Comments:_____

   Recovery and packaging of hairs and fibers.

   Date:_____ Training Coordinator/Examiner:_____

   Comments:_____

   Recovery of body fluids from porous and nonporous surfaces.

   Date:_____ Training Coordinator/Examiner:_____

   Comments:_____

2. Trainee has observed and obtained instruction from qualified examiners in other forensic disciplines.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee understands the capabilities and limitations of other forensic disciplines and recognizes when to preserve potentially valuable evidence that may involve these other areas.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee has a basic knowledge of bloodstain patterns and surface deposition of stains and understands how and when to group stains together for testing.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Trainee displays an unquestionably sound technique for recovering body fluids from porous and nonporous surfaces.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee thoroughly understands the design and use of Victim and Suspect Physical Evidence Recovery Kits and the Blood or Buccal Swabs and Hair Samples Kit.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee understands when hairs and fibers collection is necessary, and when hair/fiber examinations are conducted. Trainee exhibits an unquestionably sound technique for their recovery and packaging.

   Date:_____ Training Coordinator:_____

   Comments:_____

8. Trainee's notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

9. Trainee has participated in a mock trial and/or a practical or oral examination. Performance was satisfactory.

   Date:_____ Training Coordinator:_____

   Comments:_____

10 DEOXYRIBONUCLEIC ACID (DNA)

10.1 Goals

10.1.1 To become familiar with the application of DNA analysis to forensic samples, including:

10.1.1.1 Type/size of specimens required.

10.1.1.2 Methods of specimen preservation and storage.

10.1.1.3 Documentation required for case file.

10.1.2 To become familiar with DNA testing procedures that are or have been conducted on casework and Data Bank samples by the Virginia Department of Forensic Science.

10.1.2.1 DNA RFLP testing.

10.1.2.2 DNA PCR-based typing systems.

10.1.3 To become familiar with the purpose of the Combined DNA Index System (CODIS).

10.2 Tasks

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

10.2.2 Read applicable literature. Refer to Appendix A.

10.3 Training Evaluation

10.3.1 Knowledge

10.3.1.1 Review of notes in training notebook by training coordinator

10.3.1.2 Mini-mock trials/oral and practical examinations.

10.3.1.3 Completion of checklist by training coordinator.

10.3.2 Skills

10.3.2.1 Observation by training coordinator or designee.

10.3.2.2 Completion of checklist by training coordinator.

CHECKLIST FOR DEOXYRIBONUCLEIC ACID (DNA)

Name of Trainee:_____

1 Trainee is familiar with the type and size of specimens required for DNA analysis, the methods of specimen preservation and storage, and the required documentation for the case file.

Date:_____ Training Coordinator:_____

Comments:_____

2 Trainee is familiar with DNA RFLP testing and DNA PCR-based typing systems previously or currently used for casework and Data Bank sample analysis by the Virginia Department of Forensic Science.

Date:_____ Training Coordinator:_____

Comments:_____

3 Trainee is familiar with the purpose of the Combined DNA Index System (CODIS).

Date:_____ Training Coordinator:_____

Comments:_____

4 Trainee has worked with qualified examiners in assessing the suitability of forensic specimens for DNA analysis and is familiar with the related documentation.

Date:_____ Training Coordinator:_____

Comments:_____

5 Trainee's notebook is organized and complete.

Date:_____ Training Coordinator:_____

Comments:_____

6 Trainee has participated in a mock trial and/or a practical or oral examination. Performance was satisfactory.

Date:_____ Training Coordinator:_____

Comments:_____

11 GENERAL REPORT WRITING

11.1 Goals

11.1.1 To become familiar with the format and wording presently used by examiners in the Forensic Biology Section of the Virginia Department of Forensic Science.

11.2 Tasks

11.2.1 Utilize the results of examinations on a minimum of 10 actual cases to write reports and compare them to the original reports.

11.2.2 Conduct technical and administrative peer reviews of examiners' reports after the official peer reviews have been completed. Compare results with the peer reviewers.

11.3 Training Evaluation

11.3.1 Knowledge

11.3.1.1 Review of notes in training notebook by training coordinator.

11.3.1.2 Mini-mock trials/oral examinations.

11.3.1.3 Completion of checklist by training coordinator.

11.3.2 Skills

11.3.2.1 Satisfactory performance on report writing.

11.3.2.2 Completion of checklist by training coordinator.

11.4 Basic Report Terminology

11.4.1 Visual examination (with or without aids such as an alternate light source) is considered a screening test. Negative screening test results will be reported as "No [body fluid] was detected." Positive screening test results will be tested further and reported according to those test results.

11.4.2 For presumptive testing, the terms "indicated" and "no [body fluid] was detected" will be used.

11.4.3 For confirmatory testing, the terms "identified" and "not identified" will be used.

11.5 Reporting Results of Analysis

11.5.1 When body fluid presumptive/identification tests (including visual examination for blood) are conducted, the conclusions (based on the results) will be reported as "Results".

11.5.1.1 The observation of the absence of blood on trauma-related evidence (evidence in homicides, assaults, rapes, etc.) will be reported as this may provide investigative information.

11.5.2 In the report, it is not necessary to distinguish whether an item was swabbed or cut by the examiner during analysis. This information is maintained in the case file notes.

11.5.2.1 Example: Where the blade was swabbed and the swab tested - "Blood was indicated on the blade of the knife."