CMPD Biology Section

Swabbing and Body Fluid Identification Training Manual

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Provisions for Modification and Updating of This Manual

Any updates, modifications, additions, or deletions to this manual will have the following information and an updated issue date located at the bottom of each page.

Summary of Revisions

Date Issued	Summary of Changes Made
11/21/2011	Created
6/4/2012	Combined Swabbing and BFI Manuals, added general statement about training
7/17/2012	Added information about legal training.
6/10/15	IR and Violet Light screening; and Hematrace were added to the Blood Identification Chapter

Training Program for Swabbing and Body Fluid Identification
Biology Section CMPD Crime Laboratory

Table of Contents:

Section 1: General Information

General: This training manual is designed to be used in conjunction with the General Lab Training Program, the Laboratory's Safety, Quality, Policy and Operations Manuals, along with the Biology Section Quality Manual and SOP's. Each analyst will be required to meet the general training requirements such as ethics, safety, legal aspects, and BloodBorne Pathogens before starting work in the laboratory. In addition, legal readings specific to Biology will be required.

Required Legal Readings:

- Giglio v. United States
- Frye v. United States
- Brady v. Maryland
- Daubert v. Merrell Dow Pharmaceuticals
- Melendez-Diaz v. Massachusetts
- Rule 702
- NC General Statute 15A-268 (Biology specific)

There are occasions where it will be necessary for non-Biology laboratory personnel to swab items such as guns for DNA. These individuals will undergo a modified training program on swabbing under the supervision of Biology personnel. A memo will be written by the trainer to show what each non-Biology analyst is trained to swab.

Purpose: Training cannot encompass all aspects of analyzing evidentiary items, but should provide a foundation from which the individual can draw upon and make appropriate decisions with case work. This manual is to be used as a guideline for the trainee and the trainer. Handouts may also be used to help the trainee visualize the information.

- To help the trainee develop good laboratory practices in the performance of analytical procedures.
- To help the trainee learn the proper procedures for evidence handling and storage. Emphasis will be placed on the importance of the chain of custody.
- To help the trainee achieve proficiency and understanding of the detection, identification, and comparison of biological specimens.
- To prepare the trainee for court testimony of casework results and conclusions.
- To provide the trainee with sources of reference material covering all aspects of duties and responsibilities of the Technician/Criminalist, i.e. analysis, court testimony, quality control, safety, etc.

Training Expectations: The following criteria ***must*** be met for advancement of a trainee. They are such that inadequate performance of any one of these components is unacceptable for the position as a whole.

- Demonstrate knowledge and understanding of analyses
- Demonstrate the ability to make reasonable decisions
- Demonstrate the ability to interpret results and troubleshoot problems
- Maintain accurate and concise notes and records
- Possess clear, concise verbal and written communication skills
- Demonstrate neat and proficient laboratory technique
- Pass the oral board
- Obtain correct results on competency tests
- Work supervised cases
- Perform acceptably in the mock trial

Required readings are included throughout the training. Notes should be taken on the readings as material read may be pertinent to future examinations and knowledge base. Some modules will have practical exercises and some modules will have quizzes. Discussions between trainer and trainee will include court testimony and question and answer sessions. The trainee will first observe the trainer perform the various tests using each of the accepted CMPD techniques. After observation, the trainee will perform the functions or tests under the trainer's supervision. Upon demonstration of proper technique the trainee will be given exercises to complete independently. Notes and results will be recorded according to CMPD laboratory protocol.

This training program is divided into two parts: the swabbing manual and the body fluid identification manual. This training is intended to provide the trainee with the knowledge, skills and abilities necessary to perform forensic body fluid identification. It is understood that while performing actual casework the trainee may choose to do only the initial, less specific test in order to preserve sample for DNA analysis, it does not relieve the trainee of the responsibility for knowing how to perform and interpret all tests.

Information on reagent preparation is found in Section 8 of the Biology QA Manual.

Section 2: Swabbing for Touch and Wear Area DNA

Introduction

Swabbing items for touch and wear area DNA is not an event, but a process. The process requires diligence on the part of the analyst to ensure the samples are kept free of contamination, that both the process and the results are well-documented and that

the resulting sample is collected and stored appropriately. There are 5 modules in this block of training. Each module is intended to give the trainee the background information and skills necessary to perform that part of the process.

The modules are as follows:

1) Contamination and decontamination
2) Information gathering
3) Documentation with notes and drawings
4) Photography
5) Swabbing

Required readings and exercises are included throughout. All modules have either a quiz or practical exercises. All modules will be signed by trainee, trainer and supervisor.

Required Reading:

- Generating forensic DNA profiles from 'contact' DNA on cartridge cases and gun grips. Lisa Branch MSc Thesis Spring 2010 California State University, Sacramento **http://csus-dspace.calstate.edu/xmlui/handle/10211.9/990**

- http://www.investigativegenetics.com/content/pdf/2041-2223-1-14.pdf Investigative Genetics 2010, 1:14. Forensic trace DNA: a review Roland AH van Oorschot, Kaye N Ballantyne, R John Mitchell.

- Journal of Forensic Sciences 1999; 44(6) 1270-1272. A systematic analysis of secondary DNA transfer. Ladd C, Adamowicz MS, Bourke MT, Scherczinger CA, Lee HC.

Module 1: Contamination and Decontamination

While the end products of evidence screening are usable DNA samples, understanding how contamination occurs and taking steps to prevent contamination are two of the crucial components of the analysis process. They must be taken into account every time evidence is handled to ensure that the results of any testing are of the highest quality. While it is not possible to prevent all contamination, it is a goal that all analysts should strive for.

Required Reading:

- Forensic Science International: Genetics Supplement Series Volume 1, Issue 1, August 2008, Pages 421-422. Progress in Forensic Genetics 12 - Proceedings of the 22nd International ISFG Congress, May a speaking individual contaminate the routine DNA laboratory? M. Finnebraaten[a], T. Granér[a] and P. Hoff-Olsen

- **Source: NFSTC DNA Analyst Training, Clean Technique** http://projects.nfstc.org/pdi/Subject02/pdi_s02_m01_02.htm

- DNA QA 15 "Contamination" R:\Department\Crimelab\Sensitive\Lab Documents\Biology SOPs\DNA SOPs and QA Manual\DNA QA in PDF

Lecture from the trainer should include:

- The various potential sources of contamination and ways contamination can be prevented.

Upon completion of this module, the trainee is expected to know:

- How contamination happens and how it can be prevented.

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What are ways that the trainee can transfer their own DNA to evidence?
- ✓ What are ways that the environment can contaminate evidence and how can it be prevented?
- ✓ How can cross contamination occur and be prevented?

QUIZ

Module 2: Information Gathering

Before swabbing an item for DNA testing it is necessary to know what information is being sought by the requesting officer. The information may appear in the laboratory request but there are times you will need to seek clarification from the officer.

There are four major questions that should be answered before you swab an item:

Will the item be sent to another section of the laboratory? This will help you determine how much of an item can be swabbed.

Does the item belong to the suspect or victim or someone else? In other words, whose DNA are we looking for on the object?

What did the suspect, victim or other person(s) involved do with the item? Would he/she have grabbed the item, used it to pry a door or dumped it from a purse etc.?

Where was the item found? Environmental conditions can affect results, and sometimes will preclude swabbing an item (e.g. fire debris containing solvents). Items

that are collected directly from a person will not be swabbed nor sent for DNA testing. Make it a habit to review the case scenario before you request the evidence. That way you won't end up with items that should not or cannot be swabbed.

Lecture from trainer should include:

- ❖ Issues and problems encountered with information gathering.
- ❖ How to obtain information, when it is necessary to reject the evidence due to lack of information.
- ❖ How too much information may bias your analysis.

Upon completion of this module, the trainee is expected to know:

- Issues and problems encountered with information gathering.
- How to obtain information.

Module 3: Documentation

Some type of documentation of an item should be done prior to swabbing. This must include a written description and may include drawings and/or photographs. If you choose to write only a description, provide enough detail so that several years from now you will be able to remember the item. You will likely never see the item again until you are in trial.

Lecture from trainer should include:

- ❖ What written descriptions should include.
- ❖ What addition information should be included and when.

Upon completion of this module, the trainee is expected to know:

- What should be included in case notes.

Exercise:

- Review 10 different case files that contain notes and drawings to become familiar with the ways analysts at CMPD take case notes.

Module 4: Photography

Photographs are an excellent way to document an item, as long as they accurately depict the item. Good photographs are in focus. This is achieved by focusing directly on the item or area of the item that is significant. Lighting is also a significant factor in obtaining good photographs. Attention should be paid to the lighting in the room and taken into account when determining what camera settings to use. Because any markings you make on items might be washed or worn off by additional processing, you may want to also photograph an item after it is tested and marked.

Lecture from trainer should include:

- ❖ What settings to use on the camera and when.
- ❖ Where electronic files for case photos are stored

Upon completion of this module, the trainee is expected to know:

- How best to use the camera to document evidence.

Exercise:

- Use the different cameras available to take photos of the different items. Be sure to include close up detailed photos.
- Print several photos on the section template and save the photos to a CD.

Module 5: Swabbing

Handouts

Required Reading:

- ➢ Journal of Forensic Sciences 2002:47(3):442-450. Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact, Wickenheiser, R. A.

- ➢ Journal of Forensic Sciences 1997; 42(2): 320-322. Sweet D, Lorente M, Lorente JA, Valenzuela A,Villanueva E.An improved method to recover saliva from human skin: The double swab technique.

Lecture from trainer should include:

- ❖ This is a hands on module.
- ❖ Information on handling, labeling, documenting, swabbing and packaging each type of item will be discussed.
- ❖ What to do if another section of the laboratory needs to analyze the item.

Upon completion of this module, the trainee is expected to know:

- How to best collect swabs for DNA from the various items.
- How to handle, label, document, and package those swabs.

Exercises:

- **Swab each of these items:**

1. Pistol
2. Rifle
3. Magazine
4. Cartridge case
5. Cartridges in magazine
6. Tools (screwdriver, pry tool, hammer)
7. Bag (athletic and plastic)
8. Cell phones
9. Bottles, cans, cups and straws
10. Lighter
11. Sunglasses or eyeglasses
12. Knives
13. Pants pockets
14. Head gear (ball cap, wave cap, mask)
15. Cords/rope
16. Gloves (latex and cloth)
17. Shirt (touch an wear)

Section 3 - Body Fluid Identification

Blood Identification

Introduction

Since blood is often found in cases of violent assault, the location and identification of blood samples can be significant information in a criminal investigation. The presence, or in some cases the absence of blood, can help answer such questions as whether a crime occurred, where it occurred, what weapon was used, who was involved and sometimes, the mechanism of the assault.

In forensic science, various chemical, microscopic and immune response tests can be used to characterize a stain as blood. These tests vary in sensitivity and specificity, a factor that must be taken into consideration when performing the tests and in reporting the results. Degradation and chemical contamination of the evidence may also have significant negative effects on the functioning of these tests. Careful handling of evidence will prevent contamination or cross-contamination of samples as well as further damage from existing contaminants as well as the loss of trace evidence.

There are four modules in this section of training:

1) Microscope basics
2) Blood basics
3) Alternate Light Source
4) Chemical catalytic tests
5) Hematrace
6) Case Approach

Module 1: Microscope Basics

There are two types of light microscopes used in the Biology section. The compound microscope is used for examination of cells and crystals while the stereomicroscope is available to examine larger items such as suspected hairs.

Slides are prepared for microscopic examination for the presence of spermatozoa and these require staining which the trainee will learn about in the Semen Identification Section. Since most of the blood cells encountered will be stained with Christmas tree stains, it is useful for the trainee to know how blood cells will appear.

The *compound microscope* employs a series of light and mirrors in what is known as Köhler illumination. This provides an image that is evenly lit and free of any shadows from the light filament; however, the resulting image is inverted. The trainee must take this into account when attempting to locate items on a slide.

The *stereomicroscope* requires the use of an external light source. The trainee has the ability to see and object in three dimensions and the image is upright. They can also manipulate the sample for better viewing; however, stereomicroscopes have limited magnification.

Lecture from trainer should include:

- ❖ The differences between a compound and stereo microscope
- ❖ How to stain and view biological slides
- ❖ How and when microscopes are used in the Biology section

Upon completion of this module, the trainee is expected to know:

- The parts of a compound and stereoscope microscope
- How to care for and handle the microscope
- Uses of the microscope in the Biology section
- How to handle microscopic specimens
- How to stain biological specimens

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What are the differences between a compound and a stereoscope microscope?
- ✓ Under what magnification do you use to first focus your sample?

Exercise:
Examine the various exhibits on the Handout. Scan each slide, first at 200X magnification then again at 400X magnification.

Module 2: Blood Basics

While forensic analysis emphasizes the testing of stains for the presence of blood, it is important to have some basic knowledge about blood in order to understand the basis of the various tests. The following information is considered relative to the forensic examination of items and to blood testing. The information supplied is based on characteristics of human blood.

Notes on crime scene blood samples

Due to the violent nature of many crimes a trainee will regularly receive samples from cases where the injured/deceased has lost a large volume of blood. In significant injury, particularly those involving the head, the serum and solid portions of the blood may separate. In these situations the crime scene investigator may collect apparent blood stains that don't appear to be red. The trainee should not automatically assume that these stains are not blood. They should be tested to resolve the question. There will generally be enough hemoglobin present to give a positive reaction. The separation of blood components may also be observed on items of clothing; there will be a red area surrounded by an area of off-white or yellow staining. To ensure there are sufficient leucocytes for DNA the trainee should consider testing samples from both areas if they are available.

Blood from crime scenes may come from veins, arteries or both. On clothing or other items arterial blood will typically appear bright red while venous blood will have a bluish cast. This does not affect the testing as much as it does the selection of samples for testing. The differences may indicate that more than one person was involved in an altercation and more than one sample should be tested.

While red-brown is the typical color, blood may appear in a range of colors. Putrefied blood may take on a greenish cast. Heated samples may be purple or resemble red hair dye or wood stain and they may have an orange or wine-red tint. In any case it is best to perform the test than to assume and have it brought to one's attention later.

Lecture from trainer should include:

- ❖ The characteristics and parts of blood
- ❖ History of blood typing
- ❖ Hazards of working with blood specimens.

Upon completion of this module, the trainee is expected to know:

- The functions and structures of blood
- General knowledge of the history of blood typing
- How to handle blood specimens

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What is the structure of blood?

- ✓ What is the function of blood?
- ✓ What are the components of blood serum?
- ✓ Where do you handle liquid blood and why?

Required Reading:

- Butler, J. M. *Advanced Topics in Forensic DNA Typing,* Chapter 1**:** Sample collection, storage and characterization. Elsevier/Academic Press, New York, 2011

- Kimball, John W. Kimball's Biology Pages: Blood. 2011. Accessed online on 9/1/2011 at http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/Blood.html

- Lee, H. C. The identification of bloodstains: microscopic methods. *Vol. I*, Ed. R. Saferstein, Prentice Hall, Englewood Cliffs, NJ, 1982. pp 271-272

QUIZ

Module 3: Near Infrared and Violet Light Sources for Detection of Stains

Before any tests for the presence of blood can be conducted, the stain(s) must be located. The initial examination of an item of evidence for the presence of stains is a visual one. This is straightforward on light and smooth fabrics. Blood screening of dark items has proven more difficult to perform because the dark color of both the blood and the substrate, and the lack of reflectivity by blood effectively mask the dark stains. In the past it has been necessary to blindly test some fabrics to determine whether blood is present. This is less than optimal because any blood patterns present may be distorted by blind swabbing.

Light sources such as the Foster + Freeman 82S Infrared and 82S Violet are may be effective on many dark or textured fabrics because they allow visualization of stains that are typically masked. Using strong lights in a narrow spectrum and blocking light from other parts of the spectrum using filters, the light systems reveal previously unseen stains. In situations where the bloodstain is too dilute to be visible, the examination of an item with the light source may block enough light to reveal stains that may have been previously missed altogether or discovered only after serological examination.

The Foster + Freeman Crime-Lite 82S near infrared (near IR) and violet light system consist of a power unit with LED bulbs that emit a specific wavelength of light, an IR

sensitive camera to allow visualization and photography, filter(s) that only allow selected wavelengths of light to be seen and protective goggles.

Lecture from trainer should include:

- ❖ Evaluating stains on evidentiary material
- ❖ Advantages and Disadvantages of using near infrared and violet light sources

Upon completion of this module, the trainee is expected to know:

- When to use the near infrared and violet light sources
- ❖ Advantages and Disadvantages of using near infrared and violet light sources

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ How does the near IR light source assist in locating stains?
- ✓ How does the violet light source assist in locating stains?
- ✓ Is an alternate light source a presumptive or confirmatory test? Explain your answer.

Demonstration: Observe the trainer use the different light sources to view blood samples and other samples shown to be difficult to detect with the naked eye.

Exercises:

- View multiple blood stained fabric swatches under each light source Record observations according to CMPD laboratory protocol.
- Photograph at least ten samples using the 82S camera. Print the photos and retain.

Required Reading

- Bailey , J.A., R.S. Casanova and K. Bufkin. A method for enhancing gunshot residue patterns on dark and multicolored fabrics compared with the modified Griess test. *J Forensic Sci* 2006, 51 (4) pp 812-814.

- De Forest, P, et al. Blood on Black-Enhanced visualization of bloodstains on dark surfaces. *NIJ Report 227840,* August 2009. Retrieved online on 6/12/2014 from https://www.ncjrs.gov/pdffiles1/nij/grants/227840.pdf

- Edelman, G. J, Hoveling, R.J. M., Roos, M., Van Leeuwen, T.G. and M C. G. Aalders. Infrared imaging of the crime scene: possibilities and pitfalls, *J Forensic Sci* 2013, 58 (5) pp. 1156-1162.

- Gorn, M James, S. H. Using infrared photography to document clothing evidence in a reconstruction of a homicide. *Journal of Bloodstain Pattern Analysis* 2012, 28:4

- Lin, A. et al. Forensic applications of infrared imaging for the detection and recording of latent evidence. *J Forensic Sci* 2007. 52 (5), pp 1148-1150.

- Mangold, K, JA Shaw and M. Vollmer. The physics of near-infrared photography. *European Journal of Physics*; 34 (2013) S51-S71. Retrieved online on 6/12/2014 at http://www.coe.montana.edu/ee/jshaw/publications/NIR%20Photography%20-%20Mangold%20et%20al%20-%20EJP2013.pdf

- Morgan, S.L., and M.L. Myrick .Rapid visualization of biological fluids at crime scenes using optical spectroscopy. NIJ report 283526, July 2011.

- Sankpill, J.P. Infrared radiation: Defending against the invisible workplace hazard. *Protection Update: News from the International Safety Equipment Association,* Oct 2009. Retrieved online on 6-12-2014 at .*https://www.safetyequipment.org/userfiles/File/ProUp_Oct09.pdf*

Module 4: Chemical Catalytic Tests

Chemical catalytic tests for blood function by detection of the peroxidase-like activity of hemoglobin. Hemoglobin and its derivatives, such as the heme found in degraded blood, are not enzymes; however, they function like one in these tests by catalyzing the oxidation, or break down, of hydrogen peroxide in the presence of organic compounds found in the reagent. In simple terms, positive results for the presence of hemoglobin (and thus blood) are indicated by either a color change or in the case of luminol reagent, the release of visible light (referred to as chemiluminescence). Before being used in testing, the component in the reagent that is being oxidized must be in the reduced form.

Three different chemical reagents are used by this laboratory:

1) Kastle-Meyer reagent which contains phenolphthalin, a reduced form of phenolphthalein. Abbreviated as PHT or KM.
2) Leucomalachite green, which contains a reduced form of malachite green. Abbreviated as LMG.
3) Luminol reagent or 3-aminopthalhydrazide

Notes on crime scene samples

Crime scene blood samples are not always diluted in water. Examples: It is common for samples of blood from the mouth to be highly diluted by saliva, and for vaginal blood to be diluted by both semen and the vaginal fluid itself.

Crime scene samples can be collected anywhere; therefore, they may be contaminated with many materials or residues that could affect testing. Arson case samples may have not only a soot covering but chemical accelerants may have been used. There are always limits to the testing, and it might not be possible to obtain clear results but testing should still be attempted.

Lecture from trainer should include:

- ❖ How to make each reagent**,** the chemistry behind each reaction, the specificity and sensitivity of each reaction, the limitations of these tests and how these test affect DNA testing.

Upon completion of this module, the trainee is expected to know:

- How to make reagents
- How to perform catalytic tests
- The chemistry, specificity and limitations for each test
- Be familiar with luminol and BlueStar®
- Use of controls

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ Describe the basis for how presumptive blood tests work.
- ✓ What reacts with the presumptive test other than blood?
- ✓ Why is the test done in two steps?
- ✓ How is PHT prepared?
- ✓ How is LMG prepared?
- ✓ What is the use for luminol? What are the components?
- ✓ What is special about zinc in these reactions, and how is it disposed?

Demonstrations: Test Neat blood and dilutions in TE of 1:10 and 1:1000 with Kastle-Meyer and leucomalachite green reagents. Mix the luminol solution and spray on weak and aged blood samples.

Exercises:

- Prepare working reagents of phenolphthalein and leucomalachite green using the appropriate SOP.

- Using both color tests, perform testing on as many of the listed objects or materials as possible.
- Using both color tests, perform testing on samples of various plants, including fresh horseradish, tomato, potato, onion, beans, lettuce, cabbage and beets. For any positive results, crush the samples and allow them to dry then repeat the tests.

- Using both color tests, perform testing on samples of various commercial sauces. List any ingredients that might be considered a source of peroxidases.

- Using each color reagent, perform testing on various body fluids/swabs.

- Using each color reagent, perform testing on blood from different animals.

- Perform testing using each of the color tests on neat samples and samples with the following dilutions: 1:10, 1:100, 1:1000, 1:10000, 1:100,000, 1:1,000,000.

  For the first series of tests, place three drops of each sample on the same piece of filter paper and test while the sample is still wet. Observe results and record any differences.

  For the second series of tests place three drops of each sample on the same piece of filter paper but allow it to dry before performing the test. Observe results and record any differences.

  For the third and final series of tests, place three drops of each sample on swabs. Heat the swabs gently (to mimic aging). Once totally dry, perform the test. Observe results and record any differences.

- Perform testing using each color reagent on older stains on various substrates.

- Perform testing using each of the color tests on dilutions of blood in neat saliva, neat urine and in neat semen.

- Mix luminol reagent and perform testing on fresh blood diluted to 1:10,000, 1:100,000 and 1:1,000,000. Repeat with dilutions of older blood, noting any differences.

Required Reading:

- Cox, M. A study of the sensitivity and specificity of four presumptive tests for Blood, *J Forensic Sci*, 1991, 36 (5), pp 1503-1511

- Gaensslen, R.E., Blood Identification, Section 6: Catalytic tests. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983. pp 101-116

- Laux, D. Effects of luminol on the subsequent analysis of bloodstains, *J Forensic Sci*, 1991, 36 (5), pp 1512-1520

- Lee, H.C., Identification and Grouping of Bloodstains: Catalytic tests, *Forensic Science Handbook, Vol. I*, Ed. R. Saferstein, Prentice Hall, Englewood Cliffs, NJ, 1982. pp 272-276

- Tobe, S., Watson, N. & N. Daeid. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular weight DNA, J *Forensic Sci*, 2007, 52 (1), pp. 102-109

QUIZ

Module 5: Hematrace

The ABAcard® HemaTrace test strips are manufactured by Abacus Diagnostics, Inc. The human hemoglobin present in the extract will combine with a monoclonal antihuman hemoglobin antibody that is labeled with a dye. Any antibody-antigen formed then migrates through an absorbent membrane to the test area of the strip. The test area has an immobilized polyclonal antihuman hemoglobin that will capture the Ag-Ab complex to form an Ab-Ag-Ab sandwich. The pink dye becomes visible as a band in the test region at concentrations of human hemoglobin above about 0.05 µg/ml. An internal control consisting of human hemoglobin antibody–dye conjugate cannot bind to the antibody in the test area but is captured by an antibody in the control area. A correctly functioning positive test will therefore show two pink bands, one in the test area and one in the control area. A correctly functioning negative test will show only one pink band, in the control area. If there is any problem with the test there will be no visible bands.

Lecture from trainer should include:

- What is hemoglobin and where is it found

- ❖ Principles behind the test.
- ❖ Sensitivity, specificity, and limitations of the *ABACard*® Hematrace test strip
- ❖ Use of controls
- ❖ "High Dose Hook Effect"

Upon completion of this module, the trainee is expected to know:

- What hemoglobin is and where it is found
- Sensitivity, specificity, and limitations of the *ABACard*® Hematrace test
- "High dose hook effect"
- Antigen/antibody interaction
- Use of controls

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What is hemoglobin?
- ✓ Describe the controls used in the Hematrace test.
- ✓ Based on your reading and exercise results, how sensitive is the *ABACard*® test in the detection of blood?

Demonstration: The trainee will observe the trainer perform the Hematrace test.

Exercises:

- To test the specificity of the *ABACard*® test, test the cards against human semen, vaginal fluid, saliva, feces, urine, a known positive blood control, and a negative control (distilled water).
- Using the blood samples prepared for the exercise in Module 3 conduct Hematrace testing using the *ABACard*®
- Complete presumptive and confirmatory testing on various provided samples (minimum 20), to include varying blood dilutions, and mixtures. The contents of each sample will be unknown to the trainee.

Required Reading

- ➢ Atkinson, C., Silenieks, E., Pearman, C.Validation of ABAcard® HemaTrace® Kits to Determine the Presence of Higher Primate Hemoglobin in Bloodstains

- Swander, C.J., Stites, J.G.Evaluation of the ABAcard® HemaTrace® for the forensic identification of human blood. MAFS Annual Meeting.

- Kristaly, A., Smith, D.A.S. Validation of the ABAcard® HemaTrace® for the rapid forensic identification of human blood.

QUIZ

Module 6: Case Approach

It is not possible to cover every scenario that may be encountered while performing case analyses during training. The following information is provided as a starting point for the trainee's training. The trainee will be expected to ask questions of the trainer or other designated persons whenever a totally new situation is encountered.

Safe methods for handling of items and protection from contamination have been covered in the swabbing section of this manual and in the Laboratory Safety Manual and Quality Assurance Manual. Additional information will also be found in the Biology Quality Assurance Manual.

Lecture from trainer should include:

- What to do for preparation of person, area and utensils for examination.
- What to do in an examination
- How to choose what stains to test
- Sample collection
- Handouts with guideline information

Upon completion of this module, the trainee is expected to know:

- What to do for preparation of person, area and utensils for examination.
- What to do in an examination
- How to choose what stains to test
- Sample collection

Demonstration: The trainer will perform examinations for blood on (5) blood cases while the trainee observes and takes notes.

Exercise:

- The trainee will be given (5) case type samples in packages and will perform blood testing and documentation of these mock case samples.

Required Reading:

- Taupin, J. M. & C. Cwiklik. *Scientific Protocols for Forensic Examination of Clothing*, Chapter 4: Stains and deposits. CRC Press, New York, 2011.

Supplemental Reading:

- BLUESTAR® forensic website,http://www.bluestar-forensic.com/gb/bluestar-chemistry.php Accessed 9/12/2011

- Budowle, B., et al. The presumptive reagent fluorescein for detection of dilute bloodstains and subsequent STR typing of recovered DNA. *J Forensic Sci* 2000, 45 (5). pp 1090-1092

- Cox, M. Effect of fabric washing on the presumptive identification of bloodstains, *J Forensic Sci*, 1990, 35 (6), pp 1335-1341

- Nilsson, A. The forensic luminol test for blood: unwanted interference and the effect on subsequent analysis. The Swedish National Laboratory of Forensic Science (SKL), Linköping University, 2006. pp 1-9

- Tontarski, K.L., et al. Chemical enhancement techniques of bloodstain patterns and DNA recovery after fire exposure, *J Forensic Sci*, 2009, 54 (1), pp 37-48

SEMEN IDENTIFICATION

Introduction

Preliminary chemical tests are used to initially screen samples suspected of containing semen. These tests are based on the detection of a major component of semen, acid phosphatase. While a positive reaction to the presumptive test generally indicates the presence of semen, confirmation of semen must be established by the identification of spermatozoa, or in the absence of spermatozoa, the detection of p30, a human seminal plasma specific protein.

There are six modules in this block of training:

1) Biology of semen
2) Alternate Light Source (ALS) examination
3) Chemical Catalytic test

4) Microscopic examination
5) p30 Detection Test
6) Case Approach

Module 1: Biology of Semen

In human males, spermatogenesis does not begin until puberty. The initial stages of spermatogenesis take place within the testes and progress to the epididymis where the developing gametes mature and are stored until ejaculation. The seminiferous tubules of the testes are where spermatozoa manufacturing begins. At this point, spermatozoa are non-motile and are transported to the epididymis in testicular fluid with the aid of peristaltic contraction. While in the epididymis, the spermatozoa gain motility and become capable of fertilization. However, sperm motility is reserved for ejaculation and the transport of the mature sperm through the remainder of the male reproductive tract is achieved through muscle contraction rather than the spermatozoa's own motility. Semen is defined as the viscous secretion of the male reproductive organs. The major components consist of the seminal plasma and spermatozoa. Seminal plasma is produced by the accessory sex glands of the male (prostate, seminal vesicles, and bulbourethral glands) and functions primarily as a medium for transporting spermatozoa from the male to the female reproductive tract. The sole function of the sperm cell is fertilization of the ovum.

Spermatozoa are approximately 50 to 70 microns long and usually consist of a head (contains a condensed haploid nucleus) and a tail (propels the sperm to the egg and helps it to burrow through the egg coat; the tail and head are both enclosed within a single plasma membrane. Approximately 200 to 500 million spermatozoa are released per ejaculation; normal volume per ejaculate is 2 milliliters or greater.

Lecture from trainer should include:

- ❖ Morphology and characteristics of semen

Upon completion of this module, the trainee is expected to know:

- What are the characteristics and components of semen
- How to identify semen

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What is semen? Specify which components are semen specific and which are not.
- ✓ What glands contribute to seminal fluid?
- ✓ What methods are used to identify spermatozoa?
- ✓ Are spermatozoa haploid or diploid?
- ✓ On average, what is the total volume of seminal fluid per normal ejaculate? What is considered a normal sperm count per milliliter of seminal fluid?

Exercises:

- Briefly describe and draw a human spermatozoon labeling the major parts.

- Using reliable Internet resources and resources within the CMPD laboratory find images of abnormal human spermatozoa and draw and label them.

QUIZ

Module 2: Alternate Light Source (ALS) examination

Before any tests for the presence of semen can be conducted, the stain(s) must be located. The initial examination of an item of evidence for the presence of stains is a visual one. Evidence examination conducted using only visible light wavelengths emitted from conventional bulbs may not reveal all potential semen stains. Conventional light may mask the presence of semen stains depending on the composition of the evidence or the material that it is located on. The use of alternate light sources may help reveal evidence that was otherwise hidden.

The location of stains may be aided by the use of the Mini-Crimescope, Luma-Lite, or Omniprint 1000. In general, an ALS consists of the light itself (laser or incandescent bulb), filter(s) that only allow selected wavelengths of light to be seen, some type of device to deliver light to the item of evidence, and protective goggles. The principle behind the light sources is that semen contains components which react to light between 450 and 455 nm wavelengths. Performed in a darkened room while wearing colored goggles, stains will fluoresce when viewed at different wavelengths of visible light. Questioned stains can then be subjected to the appropriate presumptive and confirmatory tests.

The reaction must be interpreted with caution as substances other than semen may also react to an ALS. These include, but are not limited to, urine, saliva, yogurt, cleaners, and bleach alternatives. Samples showing a reaction to the ALS require additional examination to detect and/or confirm the presence of semen.

Lecture from trainer should include:

- ❖ Evaluating stains on evidentiary material
- ❖ Advantages and Disadvantages of using an ALS

Upon completion of this module, the trainee is expected to know:

- What is the use of the ALS
- Advantages and Disadvantages of using the ALS

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ How does an alternate light source assist in locating stains?
- ✓ Is an alternate light source a presumptive or confirmatory test? Explain your answer.

Demonstration: Observe the trainer use the different ALS sources to view semen samples and other samples shown to exhibit fluorescence.

Exercises:

- View identical semen stains (i.e. different dilutions) on different substrates using different ALS sources and various wavelengths. Record observations according to CMPD laboratory protocol.

- Prepare samples of different substances known to fluoresce with ALS (urine, saliva, yogurt, bleach, cleaners) on various substrates. View with different ALS sources and various wavelengths. Record observations according to CMPD laboratory protocol.

Required Reading:

- ➢ Baechtel, F.S. The Identification and Individualization of Semen Stains, *Forensic Science Handbook Vol. II* Ed. R. Saferstein, Prentice Hall, Englewood Cliffs, NJ, 1988

- ➢ http://www.enotes.com/forensic-science/alternate-light-source-analysis; accessed September 26, 2011

Module 3: Chemical Catalytic Test

The chemical test used for screening of semen is the acid phosphatase or AP test. Acid phosphatase is an enzyme secreted by the prostate gland into seminal fluid. Its concentration in seminal fluid can be up to 400 times greater than in any other body fluid. Since acid phosphatase is not unique to the prostate and can be found in other biological fluids (including vaginal fluid), it is considered a presumptive chemical test for the presence of semen.

The AP test is based on the detection of the enzyme, acid phosphatase. In the presence of acid phosphatase, the sodium alpha-naphthyl acid phosphate is hydrolyzed to alpha-naphthol, which then combines with the diazo blue B dye to yield a purple-colored change.

A negative AP test does not necessarily mean that no semen is present in a stain. Since the test relies upon the amount of acid phosphatase present, it is possible that semen is present in such low (or dilute) amounts that a positive reaction does not occur.

Lecture from trainer should include:

- ❖ Theory behind the AP test and the purpose of the chemicals used
- ❖ Sensitivity and specificity of the AP test
- ❖ Limitations of the AP test
- ❖ Acid phosphatase persistence under different conditions (i.e. vaginal tract vs. clothing items)
- ❖ False positives (substances other than AP that may react with the AP reagent)
- ❖ Use of controls
- ❖ Importance of the AP test in evidence examination
- ❖ Significance of observing spermatozoa heads vs. intact spermatozoa

Upon completion of this module, the trainee is expected to know:

- How to make the reagent
- How to perform the test
- The chemistry, specificity and limitations for the test
- Significance of observing spermatozoa heads vs. intact spermatozoa
- Use of controls

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ List sources of AP other than semen.

- ✓ List the major chemical components used to make the single step AP reagent. Identify the purpose of each.
- ✓ Semen contains more AP than any other body fluid. What is the 2nd most concentrated body fluid and how many times less concentrated is it?
- ✓ Describe the controls used in the AP test.
- ✓ Based on your reading and exercises, how sensitive is the AP test in the detection of a positive result?
- ✓ What would a positive AP test result look like and what would it tell you?
- ✓ Explain the difference between seminal acid phosphatase and vaginal acid phosphatase.
- ✓ If you do not detect a positive AP result on an evidence sample, is it possible to identify spermatozoa? Explain.

Exercises:

- From scientific literature, compare acid phosphatase and spermatozoa persistence

- Prepare swabs of non-vasectomized semen consisting of the following dilutions and conduct presumptive testing: Neat, 1:2, 1:5, 1:10, 1:20, 1:50, 1:75, 1:100, 1:1000

- Prepare swabs of vasectomized semen consisting of the following dilutions and conduct presumptive testing: Neat, 1:2, 1:5, 1:10, 1:20, 1:50, 1:75, 1:100, 1:1000

- Conduct presumptive testing on a minimum of 20 provided samples consisting of semen of varying ages, semen on varying substrates, mixtures with other body fluids, and semen stains subjected to contaminants and environmental conditions.

Required Reading:

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983

- Kind, S.S., The use of acid phosphatase in searching for seminal stains, *J. of Crim. Law Criminology and Police Science*, Vol No 5, Jan. – Feb 1957

- Sensabaugh, G.F. The quantitative acid phosphatase test. A statistical analysis of endogenous and postcoital acid phosphatase levels in the vagina. *J Forensic Sci*, 24(2), pp 346-365, 1979

- Collins, K.A. and A.T. Bennett. Persistence of spermatozoa and prostatic acid phosphatase in specimens from deceased individuals during varied post-mortem intervals. *The American Journal of Forensic Medicine and Pathology*, 23(3). pp 228-232, 2001

- Davies, A. and Wilson, E. The Persistence of Seminal Constituents in the Human Vagina. *Forensic Science*, 3 (1974) 45-55

QUIZ

Module 4: Microscopic Examination

After the presumptive test is performed, the presence of semen must be confirmed. A conclusive test for the presence of semen is the microscopic identification of spermatozoa.

Since acid phosphatase degrades over time and the acid phosphatase level can vary between individual males, a negative AP test does not necessarily mean that semen is not present. Based on experience and results of the visual and UV exams, further testing may be conducted even in the absence of a positive AP test result.

The laboratory uses the “Christmas Tree” (Kernechtrot-Picroindigocarmine) staining method to stain slides for examination. Nuclear fast red, a red stain, stains the cell nucleus and spermatozoa heads (i.e. nuclear material), while picroindigocarmine, a green stain, stains plasma and spermatozoa tails (i.e. extracellular material).

Lecture from trainer should include:

- Spermatozoa persistence under different conditions (i.e. vaginal tract vs. clothing items)
- Spermatozoa persistence vs. acid phosphatase persistence
- Factors affecting the number of spermatozoa in a human male ejaculate
- Human spermatozoa morphology
- Use of staining reagents
- Importance of the microscopic examination in evidence examination
- Importance of distinguishing human spermatozoa from other animal spermatozoa

Upon completion of this module, the trainee is expected to know:

- Spermatozoa persistence under different conditions
- Spermatozoa persistence vs. acid phosphatase persistence

- Factors affecting the number of spermatozoa in a human male ejaculate
- Human spermatozoa morphology
- Use of staining reagents
- Importance of the microscopic examination in evidence examination
- Importance of distinguishing human spermatozoa from other animal spermatozoa

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ Name the staining solution and identify the components of the solution that we use.
- ✓ Why do spermatozoa exhibit differential staining?
- ✓ Describe the extraction procedure for removing sperm from a stain.
- ✓ What chemical can assist you in minimizing the effect of debris on a slide when viewing for sperm? Why is this chemical effective?
- ✓ Are sperm different morphologically between species?
- ✓ List reasons why spermatozoa may not be found in a semen sample.
- ✓ What factors may affect the persistence of spermatozoa in a living rape victim?
- ✓ What, if any, differences would you expect to find in regards to spermatozoa persistence in a living rape victim vs. a victim of rape and murder?
- ✓ How long would you expect there to be sperm in the female reproductive tract? In a stain on bedding?

Demonstration: Observe the trainer prepare stained slides using the different preparation techniques.

Exercises:

- Using reliable Internet resources and resources within the CMPD laboratory, find images of animal spermatozoa. Draw them and compare to normal human spermatozoa. Record observations according to CMPD laboratory protocol.

- Using all of the non-vasectomized samples prepared, the neat vasectomized prepared sample, and the samples provided from the exercise in Module 3 make slides and examine them under the microscope for the presence of spermatozoa. Record observations according to CMPD laboratory protocol.

Required Reading:

- Allard, J.E. The collection of data from findings in cases of sexual assault and the significance of spermatozoa on vaginal, anal and oral swabs. *Science and Justice,* 37. pp 99-108, 1997

- Randall, B. Persistence of vaginal spermatozoa as assessed by routine cervicovaginal (Pap) smears. *J Forensic Sci,* 32 (3) pp 678-683, 1987

- http://animalsciences.missouri.edu/reprod/Notes/sperm/morph.htm, Accessed October 4, 2011

- http://bio3520.nicerweb.com/Locked/chap/ch07/sperm_morphology.html, Accessed October 4, 2011

QUIZ

Module 5: p30 Detection Test

If an AP test suggests the presence of semen; however, no spermatozoa can be found on the slide made from the evidence sample, semen may be confirmed by the identification of p30. p30 is a glycoprotein produced in the prostate gland and is found only in males.

The *ABACard*® p30 test qualitatively detects p30 and is an accepted marker for semen detection in both vasectomized and azoospermic individuals. The test is based on an immuno-diffusion reaction.

Lecture from trainer should include:

- What p30 is and where it is found
- Species other than human where p30 may be found
- p30 persistence under different conditions (i.e. vaginal tract vs. clothing items)
- p30 persistence vs. acid phosphatase persistence vs. spermatozoa persistence
- Sensitivity, specificity, and limitations of the p30 *ABACard*® test
- "High dose hook effect"
- Antigen/antibody interaction
- Use of controls
- Importance of p30 in evidence examination

Upon completion of this module, the trainee is expected to know:

- What p30 is and where it is found

- Species other than human where p30 may be found
- p30 persistence under different conditions (i.e. vaginal tract vs. clothing items)
- p30 persistence vs. acid phosphatase persistence vs. spermatozoa persistence
- Sensitivity, specificity, and limitations of the p30 *ABACard*® test
- "High dose hook effect"
- Antigen/antibody interaction
- Use of controls
- Importance of p30 in evidence examination

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What does azoospermic mean? How does a person become azoospermic.
- ✓ What is p30?
- ✓ In what part of the male reproductive system does p30 originate?
- ✓ What is the significance of p30 and when and/or why would you test for it?
- ✓ How do we detect p30?
- ✓ Describe the controls used in the p30 test.
- ✓ Based on your reading and exercise results, how sensitive is the *ABACard*® test in the detection of semen?

Demonstration: Observe the trainer perform the p30 test using the *ABACard*® OneStep test

Exercises:

- To test the specificity of the *ABACard*® test, test the cards against human blood, vaginal fluid, saliva, feces, urine, a known positive semen control, and a negative control (distilled water).
- Using the vasectomized semen samples prepared for the exercise in Module 3 conduct p30 testing using the *ABACard*®
- Complete presumptive and confirmatory testing on various provided samples (minimum 20), to include varying semen dilutions, mixtures, and aspermic semen samples. The contents of each sample will be unknown to the trainee.

Required Reading:

- Sensabaugh, G.F., Isolation and characterization of a semen-specific protein from human seminal plasma. A potential new marker for semen identification. *J. Forensic Sci*., Vol 23, pp. 106-115, 1978

- Abacus Diagnostics, *One Step ABAcard® p30 Test for the Forensic Identification of Semen*, Revision 8/98, Abacus Diagnostics, West Hills, CA, 1998

- Poyntz, F.M. and Martin, P.D., Comparison of p30 and Acid Phosphatase Levels in Post-Coital Vaginal Swabs from Donor and Casework Studies, *For. Sci. Int*., Vol 24, 17-25, 1984

- Kristaly, A et al., *Validation of the OneStep ABACard® PSA Test for the Rapid Forensic Identification of Semen*, presented at the Spring 1999 Meeting of the Southern Association of Forensic Scientists, Decatur, GA

- Hochmeister, MN et al., Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid, *J. For. Sci*., Vol 44, No.5, Sept 1999

QUIZ

Module 6: Case Approach

Physical evidence analysis must consist of its physical detection and its recognition as evidence relevant to the case. The premise of an alleged sexual assault is that body fluids will be transferred between individuals; therefore, the finding of seminal components on a rape victim may be important evidence that sexual intercourse has occurred. The importance of seminal fluid identification includes corroborating a victim's story, aids in the establishment of sex crimes because often there is no witness, and it helps to identify involved individuals.

Before any tests can be conducted, a stain must be located. Sometimes potential seminal fluid stains are readily visible on items of evidence. However, sometimes the condition of the evidence (i.e. how soiled is the fabric, has the fabric been washed, is only a small amount of semen thought to be present) can make the visible detection of potential semen stains difficult.

Lecture from trainer should include:

- Components of the sexual assault evidence collection kit and their use
- Ways to prevent cross-contamination of seminal fluid/spermatozoa between samples
- Body fluid screening techniques on clothing and other items
- How to report semen screening test results

Upon completion of this module, the trainee is expected to know:

- Components of the sexual assault evidence collection kit and their use
- Ways to prevent cross-contamination of seminal fluid/spermatozoa between samples
- Body fluid screening techniques on clothing and other items
- How to report semen screening test results

Demonstration: The trainer will perform examinations for semen on (5) cases while the trainee observes and takes notes.

Exercise:

- The trainee will be given (5) case type samples in packages and will perform semen testing and documentation of these mock case samples.

Required Reading:

- Taupin, J. M. and Cwiklik, C. *Scientific Protocols for Forensic Examination of Clothing*. 2011. pp 127-131

- Kafarowski, E. et al. The Retention and Transfer of Spermatozoa in Clothing by Machine Washing. *Can. Soc. Forens. Sci. J.,* Vol. 29, No.1 (1996), pp. 7-11

Saliva

Introduction

Saliva is difficult to conclusively identify because of the lack of sufficient amounts of detectable substances specifically unique to saliva. While it is not unique to saliva, the enzyme α-amylase can be used as a tool for saliva stain identification. Salivary amylase is an important digestive enzyme and is responsible for the hydrolysis of starches of both the straight and branched chained types. As indicated, α-amylase can be found in sources other than saliva. High α-amylase concentration is found in saliva, pancreatic juice, and fecal material. Limited α-amylase activity is present in other body fluids (urine, blood, semen), wheat malt, sweet potatoes, and various bacteria and fungi. α-amylase activity in human secretions could be ranked relatively as (units): vaginal secretions (less than 1); seminal plasma (1); serum (2); urine (20); perspiration (40); milk (45); feces (very high); and saliva (12,000). Saliva can be the source of evidence in sexual offenses where oral contact is alleged, bite marks, or on cigarette butts discarded at a scene. Methods for testing α-amylase depend on the enzyme's ability to hydrolyze starch.

Phadebas® Test

This method of detecting amylase utilizes a water -insoluble cross-linked starch polymer carrying a blue dye as a substrate. It is hydrolyzed by α-amylase to form water soluble blue fragments. The amount of amylase present in the sample is proportional to the concentration of blue dye liberated during analysis. The concentration of blue dye in the supernatant can be analyzed for absorbance with a spectrophotometer or it can be used only qualitatively. For forensic purposes, it is generally sufficient to use the Phadebas® tablets to test for positive or negative results.

Screening for saliva is based on detection of high levels of amylase in the sample. It is not a confirmatory test as amylase is found in other body fluids.

Saliva contains ABH substances, especially in secretors. Saliva samples (spit or buccal swabs) are often taken as reference materials for determination of secretor status. Stains can be typed using absorption-elution or absorption-inhibition.

Saliva is also a rich source of DNA, and buccal swabs are routinely collected for reference DNA typing.

Lecture from trainer should include:

- ❖ The various substances that may contain α-amylase and where it is produced
- ❖ The mechanism and theory of the Phadebas® test
- ❖ Sensitivity and limitations of the Phadebas® test
- ❖ Interpretation of test results
- ❖ The utility of controls

Upon completion of this module, the trainee is expected to know:

- The various substances that may contain α-amylase and where it is produced
- The mechanism and theory of the Phadebas® test
- Sensitivity and limitations of the Phadebas® test
- Interpretation of test results
- The utility of controls

Training questions: Answers to training questions should be written out and used as reference material. The trainer will be responsible for ensuring answers to these questions are complete and accurate.

- ✓ What is the component in saliva that is tested for forensically and what is its function?

- ✓ Where else can the substance mentioned above be found?
- ✓ What is the principle of the Phadebas® testing procedure?
- ✓ Describe the procedure for testing saliva with Phadebas® tablets.
- ✓ Is the Phadebas test conclusive for saliva? Explain.

Demonstration: Observe the Phadebas® test being performed using the appropriate controls and take notes regarding the procedure.

Exercises:

- Run the Phadebas® procedure on a dilution series of saliva up to 1:10,000.

- Test various body fluids, which may include but are not limited to semen, blood, urine, vaginal fluid, fecal stain, and breast milk (as available). Use a known saliva source (not your own) for the standard.

- Test saliva from various substrates (e.g., chewed gum, cigarette butt, drinking straw, envelope flap, bottle or can, etc.). Use a known saliva source (not your own) for the standard.

- Test saliva from various animals (as available). Use a known saliva source (not your own) for the standard.

Required Reading:

- Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice, 1989.pp. 184-189

- Keating SM, et al. The detection of amylase on swabs from sexual assault cases. J of For Sci Soc, 34; 1994: 89-93

- Phadebas® Amylase Test, Pharmacia, Uppsala Sweden, 1994

- Whitehead, P. H., and A. E. Kipps. 1975. The significance of amylase in forensic investigations of body fluids. Forensic Sci 6 (3): 137–44

- Validation of Phadebas® tablet at CMPD

QUIZ

Training Log: Swabbing

Training Area	Date Completed/Initials	Trainer's Initials	Supervisor's Initials
General:			
Laboratory			
Legal			
Module 1:			
Reading			
Lecture			
Quiz			
Module 2:			
Lecture			
Module 3:			
Lecture			
Exercise			
Module 4:			
Lecture			
Exercise			
Module 5:			
Reading			
Lecture			
Exercises			
Supplemental Reading			
PowerPoint talk			
20 supervised cases			
3 months casework			

Training Log: Blood

Training Area	Date Completed/Initials	Trainer's Initials	Supervisor's Initials
Module 1:			
Lecture			
Exercise			
Module 2:			
Lecture			
Reading			
Quiz			
Module 3:			
Lecture			
Demonstration			
Exercises			
Reading			
Module 4:			
Lecture			
Demonstration			
Exercises			
Reading			
Quiz			
Module 5:			
Lecture			
Demonstration			
Exercises			
Reading			
Quiz			
Module 6:			
Lecture			
Demonstration			
Exercise			
Reading			
Sup. Reading			
PowerPoint			
Oral Board			
Competency			

Supervised cases			
Mock Trial			

Training Log: Semen

Training Area	Date Completed/Initials	Trainer's Initials	Supervisor's Initials
Module 1:			
Lecture			
Exercise			
Quiz			
Module 2:			
Lecture			
Demonstration			
Exercise			
Reading			
Module 3:			
Lecture			
Exercise			
Reading			
Quiz			
Module 4:			
Lecture			
Demonstration			
Exercise			
Reading			
Quiz			
Module 5:			
Lecture			
Demonstration			
Exercise			
Reading			
Quiz			
Module 6:			
Demonstration			
Exercise			
Reading			
PowerPoint			

Training Log: Saliva

Training Area	Date Completed/Initials	Trainer's Initials	Supervisor's Initials
Saliva			
Module 1:			
Lecture			
Demonstration			
Exercise			
Reading			
Quiz			
PowerPoint			
Oral Board			
Competency			
Supervised cases			
Mock Trial			