NORTH CAROLINA STATE BUREAU OF INVESTIGATION

TRAINING PROGRAM FOR BODY FLUID IDENTIFICATION TECHNICIANS

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Appendices:

1. PURPOSE AND SCOPE

The purpose of this manual is to provide a consistent training program for the analysis of forensic evidence by Technicians at the Molecular Genetics Section at the North Carolina State Bureau of Investigation. This program provides individuals with the theoretical background and the working knowledge to assist Qualified Analysts conducting scientific tests on forensic evidence using validated procedures under the direction/supervision of a Qualified Body Fluid Analyst. Heavy emphasis shall be placed on quality assurance of all tests performed, data integrity via thorough documentation, and excellence in obtaining consistent and congruous results.

The training program detailed in this document provides the following:

- Theoretical knowledge of the principles of body fluid identification testing.

- Working knowledge of the principles and practices of serological theories such as antigen antibody reactions, immunodiffusion, etc. as they relate to the forensic analysis of body fluids.

- The ability to perform accurate and consistent forensic analysis on forensic case material.

2. REQUIREMENTS FOR QUALIFICATION

2.1 Prerequisites

Individuals must possess a strong scientific background and have completed a Bachelor's of Science in a Science and possess course work in biology, chemistry, biochemistry and genetics.

2.2 Competency Tests

A Qualified Technician must pass a competency test in the specific area that he/she will be assisting the Qualified Analyst. These tests are to determine the trainee's ability to consistently analyze body fluids from a variety of sources. Competency tests may include blood, mixed fluids, and simulated cases when appropriate.

2.3 Written Examination

A written examination shall examine the trainee's understanding of the theoretical and working knowledge of body fluid identification tests and how they are applied in the laboratory.

2.4 Proficiency Testing

The Qualified Technician will participate in a Proficiency Testing Program in the specific area in which they were trained as soon as possible after training has been completed.

3. INSTRUCTIONS FOR THE TRAINING OFFICER

3.1 This program is designed to provide each trainee with the theoretical background and working knowledge to reliably assist in the analysis of forensic evidence utilizing body fluid identification tests. The training officer must pay very close attention to detail and ensure that all quality assurance guidelines are being followed for every sample processed in the training program. By ensuring each trainee maintains a high degree of concentration and awareness during the performance of his/her training, the proper techniques will be learned and later successfully applied to actual casework.

3.2 The order of topics listed in this manual are not necessarily in the chronological order that the tests will be performed. It may be necessary to learn and perform some techniques out of order. In this case, it is the responsibility of the training officer to provide the trainee with a clear explanation of any missing points or steps and later logically tie everything together.

3.3 It is the responsibility of the Training Officer to point out pertinent scientific literature and technical manuals included in the bibliography to the trainee so that they may become familiar with these readings.

3.4 It is the responsibility of the Training Officer to explain potential safety hazards to each Trainee BEFORE performing a task that may involve said potential safety hazard.

4. INSTRUCTIONS FOR THE TRAINEE

4.1 The trainee is required to keep files on all work completed. These files should include but are not limited to the Training Manual Log Sheet, worksheets, and copies of reports issued in cases they participate in. These files will be checked periodically by the Training Officer and/or SAC.

4.2 The readings assigned are very important. While it is not necessary to memorize reagent recipes, it is necessary to become familiar with the principles of each test and the protocol and be able to perform all duties independently. The trainee is expected to become familiar with the literature that pertains to the forensic analysis of body fluid identification testing that is included in the bibliography.

5. SAFETY ISSUES, INTRODUCTION TO THE LAB, AND ORGANIZATION

5.1 There are many potential hazards that exist in the laboratory. While the exposure to all hazards can be minimized or avoided, it is the responsibility of the Training Officer to ensure the Trainee is aware of all potential hazards. These potential hazards include but are not limited to the following:

<u>Infectious Agents</u>

A. Viral agents, including HIV and Hepatitis
B. Bacteria, including sexually transmitted diseases
C. Fungi
D. Parasites

<u>Hazardous Materials</u>

A. Caustic Agents (Acids and Bases)
B. Carcinogens/Mutagens
C. Teratogens
D. Organic Chemicals

<u>Electrical Hazards</u>

Shock from any piece of electrical equipment

Burn Hazards

Autoclaves Bunsen burners
Sterilizers Hot Plates

Eye Damage

Alternate light sources

5.2 Laboratory Safety Procedures

5.2.1 Individuals must be trained in laboratory safety by the Section Safety Officer prior to the commencement of training. Various manuals are provided that must be followed to ensure safety of all laboratory personnel. The following manuals are to be used for reference and guidance for laboratory safety: MSDS Notebook, Molecular Genetics Section Manuals, Chemical Hygiene Program, Bloodborne Pathogen Program, and the DOJ Safety Manual. The trainee will also be briefed on the fire evacuation plan for the laboratory.

5.2.2 It is the responsibility of the training officer to alert the trainee to safety hazards specific to this laboratory, including all items mentioned in 5.1.

5.3 Laboratory Orientation

The trainee may be taken throughout the laboratory and shown areas of interest to their work. They will also be provided with a written job description, an organizational chart, and various manuals including the Crime Laboratory Procedures Manual, SBI Policy Manual and the Section Quality Assurance Manual.

6. ASEPTIC TECHNIQUE AND CONTAMINATION CONTROL

6.1 This Section uses the Polymerase Chain Reaction (PCR) technology which allows very small amounts of DNA to be amplified over a billion times. Because of the sensitivity of this technique, contamination control is a very serious issue that must be emphasized and practiced with every sample, starting with the identification of the body fluids present on the evidence. The Evidence Handling Procedures must be strictly followed.

6.1.1 All items used in the identification, transfer and isolation of forensic samples must be sterile and/or free of contaminate DNA.

6.1.2 Gloves must be worn at all times while handling samples. This is to protect both the analyst and sample.

6.1.3 A fresh, sterile pipette tip must be used for each transfer of fluid or chemical to be used for DNA analysis.

6.1.4 Special precautions must be taken to ensure that the bench and surrounding areas have been properly decontaminated.

6.1.5 Scissors, tweezers, and other instruments used for cuttings or extractions shall be sterilized in between each sample.

6.1.6 The preparation of the known and unknown samples shall be separated by time.

7. DOCUMENTATION

7.1 Goals

7.1 To provide protocols for the preparation of reagents and performance of tests to ensure consistent, reliable results.

7.2 To provide a thorough record of events for each case analysis.

7.2 Protocol Notebook

The Body Fluid Identification Procedures Manual shall be made available to each Technician. The analyst shall not deviate from any procedure without permission from the SAC. Any deviation (purposely or by mistake) from the protocol shall be thoroughly documented on the worksheet at the time of occurrence.

7.3 Buffers and Solutions Recipe Book

The working copy of the appropriate QC forms, which includes procedures for preparing solutions, will be maintained in the QC notebook.

7.4 Worksheets

The purpose of the worksheets are to provide a means to thoroughly document each step of the analytical process. Each worksheet is to be completed either during or as soon as possible following the step.

8. RECEIVING AND HANDLING OF EVIDENCE

8.1 Goals

8.1.1 To obtain a working knowledge of factors and conditions that influence the deterioration of evidence as it relates to packaging, handling, and storage conditions and time.

8.1.2 To develop a thorough understanding of evidence handling procedures.

8.1.3 To develop a thorough understanding of the necessity for detailed comprehensive notes and adequate labeling of evidential materials.

8.2 Tasks

8.2.1 Read and become thoroughly familiar with the SBI Evidence Handling Procedures regarding receiving, identifying, and handling of evidence; as well as specific guidelines for handling biological evidence within the Section.

9. ANALYSIS OF BODY FLUID SAMPLES

9.1 Goals

9.1.1 To develop a basic understanding of the methodology and theory of chemical, microscopic, and immunological testing procedures used to identify blood and the species from which the blood may have originated.

9.1.2 To develop a basic understanding of the methodology and theory of chemical, microscopic, and immunological testing procedures used to identify semen and sperm.

9.1.3 To develop a basic understanding of the methodology and theory of chemical testing procedures used to identify amylase.

9.1.4 To develop skills that will allow the trainee to independently and successfully analyze forensic samples.

9.1.5 To become familiar with the sensitivity and limitations of the procedures used.

9.1.6 To develop a cognizant understanding of contamination issues and the steps necessary to avoid contamination.

9.1.7 To understand the use of controls during each procedure.

9.1.8 To become familiar with and understand the function of any buffers, solutions, or reagents used.

9.1.9 To become familiar with all documentation required.

9.2 Tasks

9.2.1 Prepare any buffers, solutions, or reagents necessary for testing.

9.2.1 Understand and perform quality control checks necessary on buffers, solutions, reagents and test kits used.

9.2.3 Perform testing on various sample types which allow for ample testing for each analytical procedure used **(see Appendix III for specific training tasks for each procedure)**. The training samples will represent materials commonly encountered in forensic casework. The Training Officer will initially present the trainee with a short lecture on the analytical procedure in question. The trainee

is then given samples on which to practice. Once a block of instruction is completed, the trainee will receive a written test and a set of competency samples to analyze. The exact number of samples will be determined by the training officer in accordance with experience and ability of the trainee. Upon successful completion of the block of instruction, the trainee will start on the next block of instruction.

9.2.4 Blocks of instruction the trainee will complete to identify blood include:

9.2.4.1 Phenolphthalein testing (presumptive chemical tests)

9.2.4.2 Takayama testing (microcrystalline identification)

9.2.4.3 Species origin testing (immunological methods)

9.2.4.3 ABAcard HemaTrace testing

Note: See Appendix III for specific training tasks for each procedure.

9.2.5 Blocks of instruction the trainee will complete to identify semen include:

9.2.5.1 Acid Phosphatase testing (presumptive chemical tests)

9.2.5.2 Spermatozoa identification (microscopic identification)

9.2.5.3 ABAcard P30 testing

Note: See Appendix III for specific training tasks for each procedure.

9.2.6 Blocks of instruction the trainee will complete to identify amylase include Phaedebas testing (presumptive chemical tests).

Note: See Appendix III for specific training tasks for this procedure.

9.2.7 Complete documentation for all procedures.

10. REPORT WRITING

10.1 Goals

10.1.1 To develop the skills necessary to effectively report body fluid identification results.

10.1.2 To develop a working knowledge of the terminology for analysis and results.

10.1.3 To become familiar with pertinent scientific literature.

10.2 Tasks

10.2.1 Read and understand pertinent scientific literature provided in the Bibliography (Appendix III).

10.2.2 Thoroughly understand and be able to accurately and concisely answer the questions in Appendix II.

10.2.3 Draft Body Fluid Identification Reports. May include but not be limited to proficiency tests, draft reports from cases previously analyzed by the section, mock cases, draft reports from current cases (NOTE: These MUST be reviewed and approved by a Trained Analyst before Technical Review and Administrative Review.)

11. COMPETENCY TESTING

11.1. Goals

Upon successful completion of all blocks of instruction, the trainee will be given a series of competency tests that closely mimic forensic evidence. The trainee must score 100% accuracy in these tests. This test is the final one that the trainee must complete before being allowed to work on forensic cases.

11.2 Tasks

Successful completion of the competency tests.

12. Qualified Technician

12.1 Goals

Once the trainee completes their competency tests, they are placed with a qualified Body Fluid Analyst. Qualified technicians will work cases under the direct supervision of a Qualified Body Fluid Examiner.

12.2 Tasks

12.2.1 The qualified technician will assist the Analyst in conducting the analysis of cases, the preparation of the notes, and may write the report.

12.2.2 The Analyst will observe all results, initial the notes, and evidence, and review all work conducted. The case will be assigned to the experienced examiner, and the experienced examiner will sign the laboratory report.

Revision History		
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APPENDIX I. NORTH CAROLINA STATE BUREAU OF INVESTIGATION: Body Fluid Identification Training Manual Log Sheet

Training Area	Date Completed/Initials	Trainers Initials
lucation		
Safety		
1. Chemical Hazards/MSDS Sheets		
2. Electrical Safety		
3. Blood borne Pathogen Training		
4. Readings		
Laboratory Orientation and Organization		
1. Lecture		
2. Readings		
Blood Identification		
1. Lecture		
2. Readings		
Semen Identification		
1. Lecture		
2. Readings		
Saliva Identification		
1. Lecture		
2. Readings		
Quality Control and Documentation		

1. Use of QC Reagent Book		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
2. Use of QC forms		
3. Documentation using Worksheets		
eceiving and Handling Evidence		
Read SBI Evidence Procedures		
boratory Training		
Aseptic Technique and Contamination Control		
leaning of Equipment		
se of Bio-safety Hood		
andling of Samples		
Blood Identification		
henolphthalein Testing		
1.1 Read and Understand Procedure		
1.2 Demonstration of Test		
1.3 Testing washed and heated samples		
1.4 Sensitivity Testing		
1.5 Specificity Testing		
1.6 Supervised Testing of Known Samples		
1.7 Competency testing		
akayama Testing		
2.1 Read and Understand Procedure		
2.2 Demonstration of Test		
2.3 Supervised Testing of Known		

Samples		
Training Area (continued)	**ate Completed/Initials**	**rainers Initials**
2.4 Testing of materials prepared for Phenolphthalein Testing		
2.5 Testing of catalase, peroxidase, and old bloodstains		
2.6 Absorbing and testing blood placed on various matrices		
2.7 Competency Testing		
pecies Origin Tests		
3.1. Read and Understand Procedures		
3.2. Demonstration of Tests		
3.3 Supervised Testing of Known Samples		
3.4 Testing of human and animal blood samples		
3.5 Competency Testing		
men Identification		
cid Phosphatase Testing		
1.1 Read and Understand Procedures		
1.2 Demonstration of Tests		
1.3 Supervised Testing of Known Samples		
1.4 Testing of a variety of stains		
1.4 Competency Testing		
onfirmatory Testing		
2.1 Read and Understand Procedures		
Training Area (continued)	**ate Completed/Initials**	**rainers**

		Initials
2.2 Demonstration of Tests		
2.3 Supervised Testing of Known Samples		
2.4 Testing of a variety of stains		
2.5 Competency Testing		
hristmas Tree Stain		
3.1 Read and Understand Procedures		
3.2 Demonstration of Tests		
3.3 Supervised Testing of Known Samples		
3.4 Testing of a variety of stains		
3.5 Competency Testing		
30 Analysis		
4.1 Read and Understand Procedures		
4.2 Demonstration of Tests		
4.3 Supervised Testing of Known Samples		
4.4 Testing of a variety of stains		
4.5 Competency Testing		
bacard Analysis		
5.1 Read and Understand Procedures		
5.2 Demonstration of Tests		
5.3 Supervised Testing of Known Samples		
5.4 Testing of a variety of stains		
5.5 Competency Testing		
Training Area (continued)	**ate Completed/Initials**	**rainers**

		Initials
liva Identification		
hadebas test Testing		
1.1 Read and Understand Procedure		
1.2 Demonstration of Test		
1.3 Supervised Testing of Known Samples		
1.4 Testing of a variety of stains		
1.5 Competency Testing		
Training Area (continued)	**ate Completed/Initials**	**rainers Initials**
port Writing		
to Draft a Satisfactory Report		
mpetency Testing		
uccessful Completion of Practical Competency Tests		
uccessful Completion of Final Written Test		

APPENDIX II. NORTH CAROLINA STATE BUREAU OF INVESTIGATION: General Questions For The Trainee

1. Describe the basis for presumptive tests for blood in general.
2. Name two classes of substances that react with the presumptive tests other than blood.
3. Why is a three step procedure better than a one step blood testing procedure?
4. List the advantages and disadvantages to ABA Card testing.
5. List and explain the appropriate controls used in Ouchterlony species identification.
6. What environmental factors affect the stability of a bloodstain?
7. How does heat, such as autoclaving, affect a bloodstain?
8. Which is more sensitive, a blood presumptive test or a human origin test?
9. What is semen and list as many components that you can think of that are found in semen? Specify which are semen specific and which are not.
10. List three sources of Acid Phosphatase.
11. What components identify semen conclusively?
12. Briefly describe and draw a spermatozoa labeling the major parts.
13. Name the staining procedure used for sperm identification and identify the names of the stains that we use.
14. Are sperm haploid or diploid?
15. What does azoospermic mean? How does a person become azoospermic (list three)?
16. What is p30?
17. How do we detect p30? Describe the controls used in each test.
18. Describe the principle of the Phadebas testing procedure.
19. Describe the procedure for testing saliva with Phadebas tablets.
20. Is Phadebas testing a confirmatory or presumptive test for saliva?

APPENDIX III. NORTH CAROLINA STATE BUREAU OF INVESTIGATION: Background and Reference Information

1. Bloodstain Identification

1.1 Phenolphthalein (presumptive) test

Background

Presumptive tests, or catalytic tests, for blood center on the erythrocyte portion of the formed elements. Each of these red blood cells carries approximately 280 million molecules of hemoglobin, each possessing four heme units. A heme compound is represented by an iron center coordinated with four nitrogen containing compounds, each of which bind to one oxygen molecule to transport it in the circulatory system. This heme group acts like a peroxidase, an enzyme which can catalyze the oxidation of an organic compound by utilizing peroxide. While heme is actually a conjugated protein, and not an enzyme, it acts like a peroxidase to dissociate hydrogen peroxide into two hydroxyl free radicals, which are highly reactive and tend to oxidize organic substances. In catalytic testing, these organic compounds are color reagents which change in color when transforming from the reduced to the oxidized state. This technique allows for a quick visual screening of blood but should not be judged as a confirmation of the presence of blood. Presumptive tests are designed to be used in conjunction with confirmatory tests for blood if enough sample is available.

The phenolphthalein test is a presumptive catalytic test for the presence of blood. The heme portion of hemoglobin possess a peroxidase-like activity which catalyzes the breakdown of hydrogen peroxide into free hydroxyl radicals. These hydrozyl radicals then oxidize the reduced phenolphthalin, producing a pink color.

To maintain this pink color, the reaction is carried out in alkaline conditions. If the pH is lowered to acidic conditions, the solution will again become colorless, but is in the form of phenolphthalein. The working solution is maintained in zinc to keep phenolphthalin in the reduced state. Use care in the preparation of the phenolphthalin, since flammable gases may be generated. For this reason an electric heating mantle is used. Also be cautious since zinc dust, in the presence of water, may act as a fire catalyst.

This test is particularly useful because there are less known false positives than other presumptive tests. The literature reports that certain plants including horseradish, tomato, turnip, and Jersulem artichoke possess

elevated levels of peroxidase which may give a positive reaction with phenolphtlain. This false reaction may be eliminated by heating the filter paper to 100^{o}C for 30 minutes, which destroys the peroxidase activity. The literature also reports that bacteria which possess a high catalase activity may give a false positive reaction. If a pink color appears after the addition of phenolphthalin to the filter paper, but before the addition of the hydrogen peroxide, then the presence of an oxidant is indicated. Any reaction that occurs more than 5 seconds after the addition of the hydrogen peroxide is considered a false positive and is not recorded. Metals and rust do not interfere with this testing. However, it may be slightly less sensitive than some other catalytic tests.

References:

Gaensslen, RE (1989) Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice, pp 103-105

Lee, HC (1982) Identification and Grouping of Bloodstains, in R. Saferstein, ed., Forensic Science Handbook, Vol. 1, Prentice Hall, Englewood Cliffs, N.J., pp 283-297

Blake, ET, Dillon DJ (1973) Microorganisms and the presumptive tests for blood. *Journal of Police Science and Administration* 1: 395-400

Higaki RS, Philp WMS (1976) A study of the sensitivity, stability, and specificity of phenolphthalein as an indicator test for blood. *Canadian Society of Forensic Sciences* 9: 97-102

Training Tasks:

1. Testing the effects of washing and heating on blood detection

   Use fresh blood and make a stain on clean cotton sheeting. Subject a portion of the stain to each of the following conditions:

   a. Wash in cold water in a sink, air dry
   b. Wash in hot water in the sink, air dry
   c. Wash by hand with soap, air dry
   d. Soak in water overnight, air dry
   e. Char on a microscope slide
   f. Heat to 100^{o} C

2. Sensitivity Testing

Take a fresh liquid blood sample and make serial dilutions. Make stains from the dilutions on clean cotton sheeting and then air dry them. Test each stain with phenolphthalein.

3. Specificity Testing

Test a variety of samples with phenolphthalein. Samples may include but not be limited to:

Horseradish	Pineapple	Red Grapes	Red Cabbage	Cantaloupe
Radish	Celery	Onions	Spinach	Lettuce
Carrots	Broccoli	Tomatoes	Peas	Raisins
Mushrooms	Artichoke	Turnip	Ketchup	Cucumber

Also collect and test cola stains, chicken blood, beef blood (from meats).

1.2. Takayama test (Confirmatory Testing)

Background

The Takayama test will confirm the presence of blood and is designed to be used in conjunction with presumptive testing for blood. Hemoglobin is composed of a heme prostetic group and globin. When a blood stain dries, the iron in the heme group is in the ferric state (FE+3) due to the formation of methemoglobin. In order for the Takayama test to work, the dried stain must be hydrolyzed to metheme and globin via alkaline hydrolysis by sodium hydroxide. Next the iron in the ferric (Fe +3) state must be reduced to the ferrous state (Fe +2) via the use of glucose, a reducing sugar. Once in the ferrous state, the iron will combine with pyridine to form pyridineferroprotoporphyrin, which is an insoluable crystalline product. A positive result is visualized microscopically by the formation of salmon colored rhomboidal or stellate crystals

The only materials that will give a positive reaction other than blood are commercially produced preparations of catalase and peroxidase, items not occurring in nature.

References:

Gaensslen, RE, (1989) Sourcebook in Forensic Serology, Immunology,and Biochemistry, 2nd ed., National Institutes of Justice, pp. 85-87.

Lee, HC, (1982) Identification and Grouping of Bloodstains, in R. Saferstein, ed., Forensic Science Handbook, Vol. 1, Prentice Hall, Englewood Cliffs, N.J., pp. 283-297

Training Tasks:

1. Test the materials prepared for phenolphthalein testing and record the results.
2. Test purified catalase and peroxidase and old bloodstains provided by the training officer.
3. Place blood on various matrices, absorb it and test it.

1.3. Human (or Species) Origin test - via Ring Precipitin, Ouchterlony and Human Hemoglobin Test Methods

Background

The precipitin test is one method of distinguishing between human and animal blood. It utilizes the biological properties of antibody-antigen complex formation to allow a visual representation of a reaction. Antibodies are very large molecules and are represented by five classes of immunoglubulins, IgG, IgA, IgM, IgD, and IgE. An antigen is a substance which has the ability to produce an immunological response when introduced into a foreign animal. The antibodies are produced by a host animal when the animal is injected with a foreign protein containing antigens and the host becomes sensitized. The immune system of the host recognizes the foreign antigen and produces antibodies to react with it in a very specific manner.

In common forensic testing, the antibodies to human antigens are raised in rabbits which results in rabbit anti-human antiserum. Dr. Uhlenhuth in 1901, presented evidence of the specificity for human antigens to only agglutinate with complementary antibodies. Therefore, if the antibodies in the anti-human antiserum comes in contact with human antigens, the specificity of the reaction allows for the formation of the human antigen-antibody complex and prohibits the formation of other non-specific complexes. In addition to testing for human antigens, the same test can be performed on a variety of animals. For example, goat anti-swine antiserum can be used to determine if a blood sample originated from a pig.

It should be noted that there is some anti-sera cross reactivity between the antigens in a closely related species. For example, ant-ram sera may cross react with goat and cow antigens. In humans, some monkeys or

higher primates may produce a response. The Cappel rabbit antiserum to human serum will produce (I) a positive reaction in a 1:3 dilution of monkey blood in a 24 hour test (ii) a positive reaction up to a 1:100 dilution for human blood in a 24 hour test and (iii) a positive reaction up to 1:1000 for human blood in a 48 hour test.

The antigen-antibody reaction occurs in two steps. The first is called sensitization where the antigen and antibody form weak bonds, probably a combination of electrostatic, hydrophobic, and vander Waals interactions, and coordinate themselves for phase two. In this step, the complex is formed and the lattice structure begins to multiply which is representative of precipitation. This visualization step creates a white band which can be recognized for analysis.

Several methods have been developed to monitor the formation of the antigen-antibody complex including the Ring Test, Single Diffusion in One or Two Dimensions, or Double Diffusion in One or Two Dimensions. The latter is also referred to as the Ouchterlony, or immunodiffusion, method. While the different tests exhibit a range of sensitivity, some general characteristics are the same due to the antigen-antibody interaction mechanism.

A blood sample can fail to produce a precipitin band, also known as a false negative. This may occur if the sample is degraded due to age, heat, sunlight, chemical treatment with detergents, aluminum oxide, pulverized iron ore, or when mixed with some types of soil. Also note that the complex is best formed when the antigen and antibody are present in approximately equal concentrations. Either component present in excess can result in a weak reaction or a false negative.

With certain tests, several substances have been documented to exhibit a reaction which may mimic a true antigen-antibody reaction, also known as a false positive. These include aluminum and iron chlorates, aluminum chromate, salts of alkyl sulfonates and alkyl sulfates, peroxide, some dilute acids and bases, tannic acid, and spruce bark extract.

ABAcard HemaTrace

In the ABAcard HemaTrace procedure, human hemoglobin (hHb) will react with the mobile, monoclonal, dye particle conjugated, anti-hHb antibody forming a mobile antigen-antibody complex. This mobile complex migrates towards the test ('T') area where an immobilized polyclonal antihuman Hb antibody is located. An antibody-antigen-antibody sandwich is formed when the 'T' immobilized antibody captures the above complex. If hHb is present at above 0.05 ug/ml, the conjugated

pink dye particles will form a pink colored band in the test 'T' area. An internal positive control 'C' area indicated that the test has worked properly and proper procedure have been followed. The migrating hHb antibody-dye complex will not bind to the antibody in the test 'T' area but will bind to an immobilized anti-immunoglobulin antibody in the control 'C' area. The captured pink dye particles will form a pink band in this area. A single pink colored line in the control 'C' area indicates a negative result, while two colored lines, one in the test 'T' area and another in the control 'C' area indicate a positive result (provided no "high dose hook effect"). This test is positive for human, primate and ferret blood.

References

Gaensslen, RE, (1989) Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice,. pp. 43-56

Lee, HC, (1982) Identification and Grouping of Bloodstains, in R. Saferstein, ed., Forensic Science Handbook, Vol. 1, Prentice Hall, Englewood Cliffs, N.J., pp. 283-297

Saferstein, R, (1990) Criminalistics: An Introduction to Forensic Science, 4th ed., Prentice Hall, Englewood Cliffs, N.J., pp. 315-319

Fletcher, SM, Dolton, P, Harris-Smith PW (1983) Species identification in blood and saliva stains by enzyme-linked immunoassay (ELISA) using monoclonal antibody. *Journal of Forensic Science Society* 21: 301-305

Hochmeister, MD et al (1999) Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. *Journal of Forensic Sciences* 44:597-602

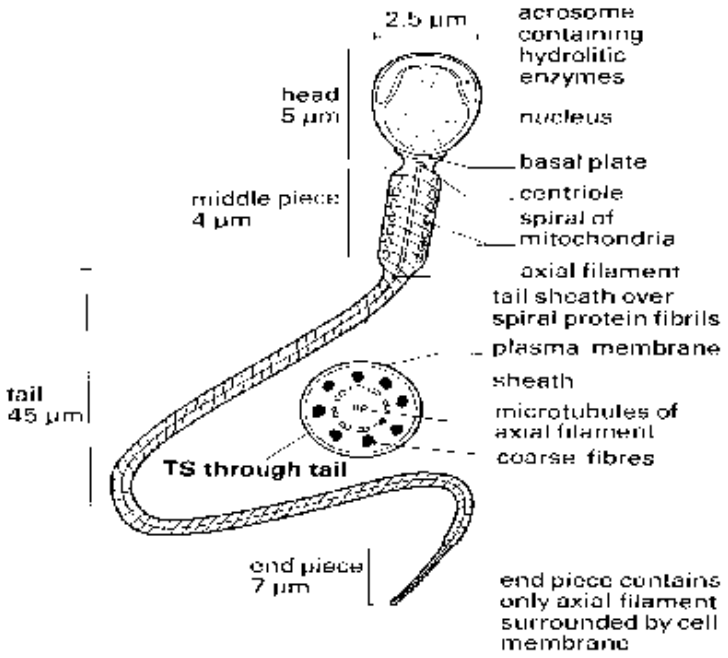
Training Task:

Test a wide variety of human and animal bloods that will be provided by the training officer.

2. Seminal Stain Identification

Semen testing is comprised of two parts. One is a presumptive test for the presence of acid phosphatase, which is used as a preliminary screen. And the second is confirmatory testing, which includes sperm searches and detection of p30. P30 is used when the preliminary screening is positive and no sperm are detected.

Semen is a white gelatinous liquid ejaculated by the male. It consists of water, flavins, citric acid, fructose, other sugars, spermine, free amino acids, phosphorycholine, acid phosphatase, prostoglandins, and sperm (except in vasectomized and aspermic males). There are four portions of the ejaculate. The first is the alkaline secretions of the Cowpers (Bulbourethral) gland, which clear the urethra and neutralize the acidic environment. The second portion originates from the prostate gland and contains alkaline secretions that are rich in proteins and proteolytic enzymes, and acid phosphatase and p30. The third portion is the sperm cell fraction and the final is the seminal plasma which is produced in the seminal vesicles.



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2.1 Acid Phosphatase Presumptive Chemical Testing

Background

The detection of acid phosphatase (AP) for use as a presumptive test for the presence of semen is based on the observation that levels of AP were 200 times higher in semen stains than in any other material tested. Seminal acid phosphatase (SAP) is formed in males and is a product of the prostate gland, whose arrangement in the reproductive system, allows for the possibility of the presence of SAP without spermatozoa. Conditions including vasectomy, and exposure to drugs and chemicals can reduce sperm counts. Production of SAP is tied to testosterone, which means that lower levels of SAP are probable in adolescent males. It has also been noted that increased levels are present in prostatic cancer patients.

In general, AP concentration is higher in the area surrounding a stain and spermatozoa are concentrated more in the central region. The theory and reaction used in AP testing is based on the biochemical aspect of AP as an enzyme. When presented with sodium alpha napthyl phosphate, AP will cleave the sodium phosphate group and produce the alcohol of the fused ring structure, naphthol. Naphthol will then react with Brentamine fast blue B and the azo group at the carbon adjacent to the hydroxyl to create a purple color. There are several other substrates which react via the same mechanism of phosphate removal and formation of an azo dye, including phenylphosphate, p-nitro phosphate, and thymolphthalein monophosphate.

False positives may occur on substances such as human milk, some vegetable extracts, and various other substances rarely encountered in forensic casework. Some vaginal treatments such as deodorants or spermicides could lead to false negatives.

References:

Gaensslen, R.E (1989) , Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice, pp. 155-169

Training Task:

Test a wide variety of stains that will be provided by the training officer.

2.2 Christmas Tree Stain (PICS and NFR)

Background

Sperm cells can be stained by several techniques including carmine, iodine-KI mixture, and methyl blue. However, the most common method is referred to as the Christmas tree technique and utilizes nuclear fast red (NFR) and picroindigocarmine (PIC) as stains. In this procedure, the sperm heads are stained red due to the intercalation of nuclear fast red into the nucleus of the sperm head. A clear acrosomal cap is also noted. The picroindigocarmine is used to stain the cytoplasm of the epithelial cells green to increase the contrast between the two most predominant cell types in forensic casework.

References:

Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice, 1989. pp. 150-152.

Gaensslen RE, et al, Staining and Extraction Techniques in Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, FBI, Wash. D.C. (1983), pp. 135-144

Training Task:

Test a wide variety of stains that will be provided by the training officer.

2.3 P30 Analysis

Sensabaugh identified, in 1973, a 30,000 MW antigenic protein in seminal fluid which he labeled p30. It was discovered when a reaction occurred between an antiserum prepared by Li and Beling. p30, also known as Prostate Specific Antigen (PSA), is present in the epithelial cells of the prostate ducts, so it can be present regardless of the presence of spermatozoa. Since it is a product of the prostate, there is none present in females. Levels of p30 concentration vary, but in a study by Brown, in one ml of semen, levels ranged from 300 to 4200 micrograms, with a mean of 1200 micrograms. In one ml of blood, the mean concentration of p30 is 0.0026 micrograms, unless the patient is inflicted with prostatic cancer where levels are in the 0.5 microgram range. This demonstrates that the concentration is significantly higher in semen than blood or other body fluids. Baechtel reported that prostatic tissue, prostatic carcinoma, and urine may also contain some level of p30 for obvious reasons. PSA

has also been detected in orangutan and macaque semen, but these samples also had much lower levels of SAP, and could be distinguished from human serum by AP testing.

References:

Baechtel SF (1988) Identification and Individualization of Semen Stains, in R. Saferstein, ed., Forensic Science Handbook, Vol. 2, Prentice Hall, Englewood Cliffs, N.J., pp. 347-392

Gaensslen, R.E.(1989) Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice, pp. 169-71

Johnson E, et al (1993) , Detection of Prostate Specific Antigen by ELISA, *Journal of Forensic Sciences* 38: 250-258

Proceedings of the International Symposium on Forensic Immunology, US Department of Justice, 1986

Training Task:

Test a wide variety of stains that will be provided by the training officer.

2.4 Abacard

Background

This test detects an antigen-antibody reaction. A small extract of a stain is placed into a well and the sample migrates across a strip to the test and control areas. If p30 is present in the semen specimen, it will react with the mobile monoclonal antihuman p30 antibody and a complex is formed. A polyclonal antihuman p30 antibody is immobilized on the membrane in the test area which captures the complex so than an antibody-antigen-antibody sandwich is formed. The dye particle-p30 complex concentrate in the test area. When the p30 concentration in the sample exceeds 4 ng/ml the pink dye particles will form a pink colored band. The p30 antibody-dye conjugates cannot bind to the antibody in the test area, but are captured by an immobilized anti immunoglobulin antibody present in the control area. A pink band in this area indicates that the test performed properly. Therefore, a pink line must be formed in both the test and control areas for a positive result.

References:

Baechtel SF,(1988) Identification and Individualization of Semen Stains, in R. Saferstein, ed., Forensic Science Handbook, Vol. 2, Prentice Hall, Englewood Cliffs, N.J., pp. 347-392

Gaensslen, R.E.(1989) Sourcebook in Forensic Serology, Immunology, and Biochemistry, 2nd ed., National Institutes of Justice, pp. 169-171

Johnson E, et al. (1993) Detection of Prostate Specific Antigen by ELISA, *Journal* of Forensic *Sciences* 38 : 250-258

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Rapid Immunoassay for the qualitative detection of p30 for the forensic identification of semen, Abacus Diagnostics instruction manual, August 1998.

Kristaly A, Smith DAS. 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.

Training Task:

Test a wide variety of stains that will be provided by the training officer.

2.4 Chemical Indications of Saliva

Amylase, namely alpha amylase for forensic purposes, is an enzyme found in plants and animals which hydrolyzes glucose polymers at the C^1-O^4 bond of the alpha 1,4 linkage. The amylase found in human saliva is of this alpha form, and this hydrolyzing characteristic is used to detect for the presence of saliva stains. Forensic methods generally use starch as the substrate. Starch is made up from amylose and amylopectin, both of which contain the alpha 1,4 linkage which can be hydrolyzed by salivary amylase. However, amylopectin contains the glucose side chain bound by an alpha 1,6 linkage which cannot be broken. Therefore, the amylopectin is not degraded completely.

A test for amylase is only presumptive for the presence of saliva because it is found in other body fluids. However, the concentration of amylase is more than 600 times more concentrated on average in

saliva than sweat, the next highest amylase containing component of the bodily fluids.

Phadebas test

Background

This method of detecting amylase utilizes a water –insoluble cross-linked starch polymer carrying a blue dye as a substrate. It is hydrolyzed by alpha 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.

References:

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

Keating SM, et al. (1994) The detection of amylase on swabs from sexual assault cases. *Journal of Forensic Science Society,* 34: 89-93

Phadebas Amylase Test, Pharmacia, Uppsalla Sweden, 1994.

Training Task:

Test a wide variety of stains that will be provided by the training officer.