

**Department of Forensic Science**

**FORENSIC BIOLOGY  
TRAINING MANUAL**

**CASE APPROACH AND IDENTIFICATION  
OF BIOLOGICAL SUBSTANCES**

## TABLE OF CONTENTS

- 1 [Overview of Training Program](#)
  - 1.1 Purpose and Scope
  - 1.2 Coordination of the Program
  - 1.3 Training Period
  - 1.4 Location of Training
  - 1.5 Guidelines for Comprehensive Oral Competency Examination
  - 1.6 Guidelines for Final Comprehensive Mock Trial
  - 1.7 Transition from Trainee to Examiner
  - 1.8 Instructions for the Training Coordinator
  - 1.9 Instructions for the Trainee
  
- 2 [Safety](#)
  - 2.1 Bloodborne Pathogens
  - 2.2 Hazards
  - 2.3 Safety Procedures
  
- 3 [Receiving and Handling Physical Evidence](#)
  - 3.1 *Taq* DNA Polymerase
  - 3.2 PCR Primers
  
- 4 [Introduction to the Microscope](#)
  - 4.1 Purpose and Scope
  - 4.2 Technical Notes
  - 4.3 Tasks
  - 4.4 Evaluation
  
- 5 [Indication of Blood](#)
  - 5.1 Purpose and Scope
  - 5.2 Technical Notes
  - 5.3 Tasks
  - 5.4 Evaluation
  
- 6 [Identification of Semen](#)
  - 6.1 Purpose and Scope
  - 6.2 Technical Notes
  - 6.3 Tasks
  - 6.4 Evaluation
  
- 7 [Related Procedures](#)
  - 7.1 Purpose and Scope
  - 7.2 Technical Notes
  - 7.3 Tasks
  - 7.4 Evaluation
  
- 8 [Deoxyribonucleic Acid \(DNA\)](#)
  - 8.1 Purpose and Scope

- 8.2 Tasks
- 8.3 Evaluation

9 [General Report Writing](#)

- 9.1 Purpose and Scope
- 9.2 Tasks
- 9.3 Evaluation

10 [Testimony and Expert Witness Qualification](#)

- 10.1 Purpose and Scope
- 10.2 Tasks
- 10.3 Evaluation

Appendix A [References and Required Readings](#)

Appendix B [Study Questions](#)

## 1 OVERVIEW OF TRAINING PROGRAM

Trainees with prior applicable experience may follow an abbreviated training plan as deemed appropriate by the Biology Program Manager.

Modifications for Forensic Scientist I (Forensic Scientist – Biologist) trainees will be made on an individual basis as deemed appropriate by the Biology Program Manager.

### 1.1 Purpose and Scope

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

The program will provide exposure to methods, techniques and procedures presently used and accepted by the courts and forensic biologists. Additionally, it will provide for an exposure to the pertinent literature available in the field and to the laws governing the handling of evidential materials. Most of the training will be concentrated on the methods currently used in the Virginia Department of Forensic Science Forensic Biology Section, thus allowing the trainee to become proficient in these as applied to both known and case materials. Methods previously employed by the Department should be reviewed in the event the trainee eventually becomes involved in a cold case analysis.

Because each case a forensic examiner analyzes has the potential of involving him/her as an expert witness in courtroom testimony, testimony training is equally important as the analytical training. Therefore, the training will also provide exposure to court procedures and assistance in developing the skills necessary for effective expert witness testimony. It is the training coordinator's responsibility to ensure that the trainee is thoroughly prepared for legal questioning. This can be done by a combination of mock trials, prearranged as well as impromptu question and answer sessions, pertinent literature review, and observation of courtroom testimony given by experienced examiners.

The sequence in which the tasks are presented in the outline should not necessarily be considered as a mandatory order of instruction. If a particular sequence is considered to be mandatory, that sequence will be specified in the task lists below. If a Procedures Manual has been read and the information retained, the trainee is not required to re-read it in its entirety simply because it is listed as a task in a particular area of study. Exposure to legal aspects and testimony will be continuous throughout the training.

Oral and practical examinations and/or mock trials encompassing several topics will be staged periodically.

The case approach and identification of biological substances training will be followed by DNA Analysis training (refer to the FB TM DNA Analysis). This case approach and ID of biological substances training program will culminate with a satisfactory performance on a formal oral competency examination and satisfactory performance in a minimum of two mini-mock/mock trials prior to proceeding with the DNA training.

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

### 1.2 Coordination of the Program

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

### 1.3 Training Period

It is estimated that this training program can be completed in two to three months, which is to include successful completion of the formal oral competency examination and a minimum of two informal mini-mock trials.

Some individuals may require less time than others, depending on such factors as experience and education. The qualifications of the trainee will be evaluated and modifications will be made to this training program as appropriate. The length of the training period is a matter which will be left to the discretion of the Biology Program Manager, the trainee's supervisor, section supervisor (if different), and the training coordinator.

#### 1.4 Location of Training

Whenever practical, the bulk of an individual's training will occur in the laboratory to which he/she will be assigned. If this is not possible, the training will be conducted at the most convenient laboratory. Certain phases of instruction may be scheduled at any of the four laboratories. Such arrangements will be made through the Biology Program Manager and Regional Director(s). Oversight and direction of the training will be provided by the Biology Program Manager.

#### 1.5 Guidelines for Oral Competency Examination

1.5.1 An oral competency examination of the trainee will be conducted by the training coordinator, the section supervisor, the group supervisor (if different), the Biology Program Manager, and the Laboratory Director or designee to ascertain the technical knowledge of the individual. Questions will be used to ascertain whether the goals, as set forth in each technical portion of the training program, have been achieved. The questions that are asked and the outcome of the oral competency will be documented.

1.5.2 Immediately following the oral competency examination, the trainee may be released while the supervisor(s), the Biology Program Manager, and the Laboratory Director or designee evaluate the trainee's performance.

1.5.3 The outcome of the oral competency examination evaluation will be one of the following:

- Satisfactory
- Unsatisfactory

1.5.3.1 If the panel deems the trainee's performance to be unsatisfactory, steps will be taken to effect the appropriate action, as determined by the panel.

#### 1.6 Guidelines for the Mini-Mock/Mock Trials

1.6.1 The level of formality and required attendees will be determined by the section supervisor.

1.6.2 It is preferred that at least one of the mini-mock/mock trials be conducted using a case for which the trainee made case approach decisions and conducted the identification of biological substances testing.

1.6.2.1 A real case that was examined by the trainee under the direct supervision of a qualified examiner may be used.

1.6.2.2 A mock case created by the laboratory and provided to the trainee for this purpose may be used.

1.6.3 The mini-mock/mock trials will not exceed two (2) hours.

1.6.4 Prior to the trial, the "prosecutor" and the "defense attorney" may reach an agreement as to selected items to be introduced at trial in order to remain within the set time constraints.

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

- 1.6.6 There may be two “defense lawyers” at the trials, one of whom must be a qualified examiner in the Forensic Biology Section.
- 1.6.7 The trials may be stopped at any time upon the request of any of the involved parties.
- 1.6.8 Immediately following each trial, the trainee may be released while the attendees evaluate the trainee's performance.
- 1.6.9 The outcome of the mini-mock/mock trial evaluations will be:
  - Satisfactory
  - Unsatisfactory
- 1.6.9.1 If the attendees deem the trainee's performance to be unsatisfactory, steps will be taken to effect the appropriate action, as determined by the panel.
- 1.6.10 The evaluation may be followed by a short performance critique.

## 1.7 Instructions for the Training Coordinator

**NOTE:** Refer to the Department Quality Manual for the requirements for training documentation and for the retention requirements for those records.

The intent of the training program is to ensure that each and every trainee is provided with certain basic principles and fundamentals necessary for the complete education of an examiner in the Forensic Biology Section. All of the listed topics must be incorporated into the program. However, education and prior experience of the trainee will be used as a guide to determine the amount of time devoted to each topic. Some of the topics will suggest an order of events and this ranking should be followed. ANY DEVIATION FROM THE CONTENTS OF THIS PROTOCOL MUST BE CLEARED WITH THE BIOLOGY PROGRAM MANAGER PRIOR TO IMPLEMENTATION.

At the culmination of the training, the trainee should be able to demonstrate through the Oral Competency Examination and the minimum of two mini-mock trials:

- Knowledge of the principles and practices of forensic body fluid identification as these relate to the analysis of case material.
- Knowledge of the theory and application of instrumentation and specialized techniques used to examine biological evidence.
- The ability to independently assess and implement sound case approach and accurately test for and/or identify biological substances.
- The ability to accurately document the findings of all analyses in accordance with Department and Section policies and procedures, and to accurately report those findings in a Certificate of Analysis.
- The ability to skillfully present and defend case approach decisions and the results of testing associated with the identification of biological substances in a court of law.

- 1.7.1 The training coordinator, or designated examiner, will document the completion of each required training task by the trainee on the Training Documentation Form.
  - 1.7.1.1 The completed Training Documentation Form will be retained by the trainee in his/her training notebook.
  - 1.7.1.2 One copy of all completed Training Documentation Forms will accompany the training coordinator's final report (after completion of both this manual's requirements as well as those detailed in the FB TM DNA Analysis) to the Biology Program Manager stating that all aspects of the training program have been completed satisfactorily.

1.7.2 The training coordinator will continually evaluate the trainee's performance and submit a monthly training report of progress to the Biology Program Manager and Laboratory Director using the Qualtrax workflow in accordance with the requirements set forth in the Department Quality Manual.

1.7.2.1 A monthly training report will be submitted individually for each trainee.

1.7.2.2 Each monthly training report will be maintained and used as documentation of the trainee's progress toward qualification as an examiner.

## 1.8 Instructions for the Trainee

1.8.1 The trainee will keep a notebook of all work completed, including the Training Documentation Form and the training coordinator's monthly training reports.

1.8.2 The notebook will be organized by subject.

1.8.2.1 Within each subject category the following will be included:

- The types of tests or examinations observed and performed
- Notes and comments on each type of test
- Review of pertinent literature
- Answers to study questions (see 1.8.4)

1.8.2.2 For each procedure performed, comments/notes will include the following, as applicable:

- Principle behind the procedure
- A procedural outline including the purpose of critical reagents
- Sensitivity of the procedure
- Specificity of the procedure
- Contemporaneous results of testing
- Interpretation of results
- Possible interferences/problems
- Other comments including comparisons to other methods or procedures

1.8.3 The casework and quality control procedures can be found in the Forensic Biology Procedures Manuals.

1.8.4 A list of study questions is located in Appendix D. Each trainee is expected to write out the answers to the questions after completing the required tasks and readings for each subject area.

1.8.5 The training program provides the trainee with exposure to various types of samples. Similar samples have been grouped together. Each group of samples can be worked simultaneously, although they may be at different stages of the procedure.

1.8.6 The trainee will assist with casework only after successful completion of an applicable documented competency test and under the direct supervision of a qualified examiner. All FS Lab numbers and/or names must be redacted from the training notes/copies maintained in the training notebook.

1.8.6.1 The requirements for each applicable competency test will be defined in each chapter/module, as necessary. The requirement(s) of the defined competency test(s) may be combined such that successful completion of multiple requirements at any given time may qualify the trainee to participate in casework/handle evidence under supervision throughout the training modules without further competency testing.

## 2 SAFETY

### 2.1 Bloodborne pathogen

All trainees will attend a bloodborne pathogen training course and a chemical hygiene course organized by the Department's Safety Coordinator.

### 2.2 Hazards

Each individual working in the laboratory of the Forensic Biology Section will be made aware of the hazards inherent in his/her work. These hazards include, but are not limited to:

- Infectious agents, such as those associated with:
  - Hepatitis
  - HIV/AIDS
  - Sexually transmitted diseases
  - Parasitic infections
  - Bacterial infections
- Hazardous materials, such as:
  - Acids and bases
  - Organic chemicals

### 2.3 Safety Procedures

2.3.1 All trainees will read and become familiar with the Department of Forensic Science Safety Manual.

2.3.2 All trainees will follow personal protective measures.

2.3.2.1 Gloves, safety glasses and other protective clothing and equipment will be worn.

2.3.2.2 The production of aerosols will be avoided.

2.3.2.3 No mouth pipetting is allowed.

2.3.2.4 Trainees will read and become familiar with the prescribed precautions for the handling of all chemicals used in a particular procedure before performing the procedure.

2.3.2.4.1 This will include a review of any applicable Safety Data Sheets (SDS).

2.3.3 All trainees will follow biosafety practices.

2.3.3.1 Prescribed personal, work space and equipment cleaning procedures will be followed.

2.3.3.2 All biological materials and containers/supplies that have come in contact with biological materials and/or hazardous chemicals will be placed in biohazard bags, which will be disposed of according to the procedures outlined in the Department Safety Manual.

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

2.3.3.4 Organic and other hazardous chemicals (e.g., phenol, tetramethylbenzidine) will be retained in appropriately labeled containers in a designated, marked area in the section or building until disposed of following the procedures outlined in the Department Safety Manual (i.e., picked up by a disposal company).



### 3 RECEIVING AND HANDLING PHYSICAL EVIDENCE

#### 3.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Gain a working knowledge of factors influencing the deterioration of evidence as these relate to proper vs. improper packaging, handling and storage.
- Develop a thorough understanding of evidence handling procedures, including preservation of chain of custody, use of laboratory information management system (LIMS) and intra/inter-laboratory transfer of evidence.
- Gain knowledge of court procedures involving identification and introduction of evidence.
- Develop a thorough understanding of the necessity for:
  - Detailed, comprehensive notes.
  - Adequate labeling of evidentiary material.
  - Drawings/photographs

#### 3.2 Tasks

- 3.2.1 Read the FB PM QA.
- 3.2.2 Read the FB PM Documentation and Evidence Handling Requirements.
- 3.2.3 Read the FB PM Screening and Collection for DNA Analysis.
- 3.2.4 Observe operations in the Evidence Receiving Section.
- 3.2.5 Observe, when possible, and understand the purpose of pre-submission consultations.
- 3.2.6 Observe and obtain instruction from qualified examiners performing routine examinations on case material.
- 3.2.7 Participate in an oral question and answer session covering the receipt, transfer, routine examination, and note-taking/documentation of evidence.
- 3.2.7.1 This oral question and answer session serves as a competency. Successful completion qualifies the trainee to perform the tasks described in 3.2.8-3.2.10 as well as general receipt, transfer, opening/inventorying and documentation of evidence.
- 3.2.8 Receive, transfer and return evidence, including reconciliation of items of evidence and containers with the associated RFLE(s).
- 3.2.9 Assist in the preservation and storage of evidence.
- 3.2.10 Examine, describe and take notes on case material under the direct supervision of a qualified examiner.

**NOTES:** This examination of the evidence by the trainee does not include chemical testing unless the trainee has successfully completed an applicable competency specific to the type of chemical testing required as detailed in the appropriate chapter(s) of this training manual. The trainee may, however, handle, examine, describe and take notes on the evidence and observe the chemical testing by the supervising examiner.

This task will continue throughout the training process.

### 3.3 Evaluation

3.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes in the training notebook by the training coordinator.
- Mini-mock trial(s)/oral and/or question and answer session(s).

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

## 4 INTRODUCTION TO THE MICROSCOPE

### 4.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the theory behind the use of various types of microscopes.
- Develop an understanding of the theory behind and techniques for using bright field vs. phase contrast microscopy.
- Develop an understanding of the construction of various types of stereo and compound microscopes and the function of each component.
- Gain a working knowledge of factors determining the resolution of the microscope, including, but not limited to, total magnification and numerical aperture.
- Learn to use various types of microscopes (e.g., stereo, compound and phase contrast microscopes).
- Learn proper care and maintenance of the equipment.
- Learn to properly achieve Köehler illumination.

### 4.2 Technical Notes

#### 4.2.1 Illumination

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

#### 4.2.2 Contrast and Resolution

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

#### 4.2.3 Köehler illumination

**NOTE:** Refer to Appendix B of the FB PM Screening and Collection for DNA Analysis for the procedure for Köehler illumination.

- The best illumination for most purposes is a special type of critical illumination known as Köehler illumination (named after August Köehler, 1866-1948). Here, a specific secondary source is imaged in the specimen plane. The particular secondary source in this case is the uniformly illuminated lamp lens framed by the field diaphragm.
- With Köehler illumination the imaging of the lamp lens and field diaphragm in the specimen plane yields three distinct advantages: 1) the ray paths are predictable and controllable; 2) the illumination is uniform; 3) the source size - that is, the area illuminated - can be adjusted.

### 4.3 Tasks

**NOTE:** The tasks in this module may be performed on fabricated training smears or on evidence smears. Prior to performing any of these tasks on an evidence smear, the trainee must have examined a minimum of one (1) fabricated smear under the direct supervision of the training coordinator or designee. Satisfactory performance while examining a fabricated smear will be documented in a MFR to be maintained with the training records and will serve as the competency to perform any of these tasks on evidence. A fabricated smear created for this purpose or any of the fabricated training smears listed below may serve as the competency smear.

4.3.1 Apply proper alignment techniques necessary for phase contrast illumination when examining smears for spermatozoa.

4.3.1.1 Refer to the centering instructions for the microscope in use.

4.3.2 Apply proper techniques for obtaining Köhler illumination while examining spermatozoa on smears at various magnifications.

4.3.3 Perform bright field illumination techniques while examining spermatozoa on stained smears at various magnifications.

4.3.4 Perform phase contrast illumination techniques while examining spermatozoa on stained and unstained smears at various magnifications.

4.3.5 Take photographs of items while under magnification.

4.3.6 Perform routine maintenance on the equipment.

4.3.7 Read applicable literature and complete the applicable study questions.

### 4.4 Evaluation

4.4.1 Knowledge of the trainee will be evaluated through:

- Review of notes in the training notebook by the training coordinator.
- Mini-mock trials(s), practical exams and/or question and answer sessions.

4.4.2 The trainee should demonstrate the ability to obtain Köhler illumination and to properly use and maintain the microscopes used in the Section. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

## 5 INDICATION OF BLOOD

### 5.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the use of presumptive and confirmatory tests.
- Gain a thorough knowledge of the procedures used by the Department.
- Develop an understanding of the sensitivity and stability of reagents.
- Determine the specificity and limitations of the various methods used to indicate or identify blood.
- Develop an understanding of the use of controls.

### 5.2 Technical Notes

#### 5.2.1 Indication of Blood Using Catalytic Tests

- Most of the preliminary chemical tests for blood are based on the detection of hemoglobin by detecting its peroxidase-like activity. Ionic iron forms chelate (ring) structures with many organic compounds and very often such iron-chelates possess catalytic activity in oxidation reactions. An example of a biological catalyst is peroxidase which decomposes hydrogen peroxide or organic peroxides to form free hydroxyl radicals. The heme group of hemoglobin possesses peroxidase-like activity which may catalyze this breakdown of hydrogen peroxide. If no other organic oxidizable compound is present, these radicals decompose to form water and oxygen. If a benzidine derivative or phenolphthalin is present, it will oxidize the colorless reagent to form a colored product.
- The peroxidase-like activity of hemoglobin operates in both acidic and basic media, while some of the bacterial and plant enzymes (catalases and peroxidases) are more pH dependent. Therefore, the phenolphthalein test, which takes place in basic medium, and the tests using benzidine derivatives, which take place in acid medium, are not redundant. Fast positive reactions obtained with both tests on a red-brown or other appropriately colored substance can be considered very strong evidence (essentially proof for practical purposes) that the substance being tested is blood. The Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test has been routinely used by the Department for many years to indicate the presence of blood; however, the Department does NOT consider this an identification test.

#### 5.2.2 Luminol

- Luminol can be oxidized by heme to a product which luminesces under darkened conditions. This test is very useful in locating “latent” bloodstains, but should only be performed after a visual search has failed to reveal suspected blood. The reagent is applied as a mist from a spray bottle over the item being analyzed.
- The degree of luminescence is dependent on the substrate and will fade with time, but can be restored with an additional application of reagent mist. This may be particularly useful for weak stains that require prolonged exposure times to photograph, but care must be taken to avoid diluting the stains with unnecessary repeat spraying. If latent blood is suspected on a vertical surface, be prepared to photograph immediately, as the spraying may cause the blood to run down the surface.
- When appropriate, the necessary photographic equipment should be available to document any luminescence produced. If photographing, use a ruler with luminescent tape as a scale and 400 ASA film or higher.
- Once possible blood is located with luminol, the PTMB test must be performed. Since other substances are known to react with luminol, blood is not indicated unless the PTMB Test is positive. Luminol will not interfere with this subsequent test

## 5.2.3 BLUESTAR® Forensic Test

Information is available at <http://www.bluestar-forensic.com>

**NOTE:** The use of BLUESTAR® Magnum is not recommended by the Department.

## 5.3 Tasks

**NOTE:** The trainee may perform chemical testing for blood on evidence under the direct supervision of a qualified examiner during this training program. Prior to performing these tests on evidence, the trainee must perform luminol and/or BLUESTAR® AND the PTMB color test on at least one fabricated training sample under the direct supervision of the training coordinator or designee. Satisfactory performance of these tests on the fabricated training sample will be documented in a MFR to be maintained with the training records and will serve as the competency to perform these tests on evidence. A fabricated sample created for this purpose or any of the fabricated training samples listed below may serve as the competency sample.

- Competency must be tested for each type of test prior to performing that test on casework. This may be performed and documented in any order. Competency in each type of test may be demonstrated together on any one sample, or independently over time.

5.3.1 Refer to the FB PM Screening and Collection for DNA Analysis.

5.3.2 Read the applicable chapter(s) in the FB PM Report Writing.

5.3.3 Prepare the reagents used for the combined Phenolphthalein-Tetramethylbenzidine (PTMB) and Luminol tests.

5.3.4 Perform the PTMB chemical color test, as well as luminol and/or BLUESTAR® Forensic Test, on the following:

**NOTE:** Perform the procedure as outlined in the FB PM and record the results for each sample for each test performed.

- Bloodstains of varying dilutions prepared in normal saline (1:10, 1:100, 1:250, 1:500, 1:750, 1:1000, 1:2000)
  - Recipe for normal saline:
    - 9 g Sodium chloride
    - 1000 mL distilled water
    - Mix thoroughly until dissolved
- A minimum of five (5) bloodstains of varying ages
- A minimum of twenty (20) bloodstains subjected to various contaminants and environmental conditions
  - Including, at a minimum:
    - Super glue
    - Fingerprint powder
    - Ninhydrin
    - Redwop powder – rhodamine base
    - Bleach
    - Soap
    - Motor oil
    - Luminol

- Mold
  - Heat
  - Moisture
  - Heat and moisture, combined
  - Decomposition
- A minimum of forty (40) substances reported in the literature to give false positive reactions
- 5.3.5 Observe specificity and sensitivity of all tests performed and compare observations to the information found in the literature.
- 5.3.6 Observe and obtain instruction from qualified examiners performing routine examinations of case material.
- 5.3.7 Successfully test a competency set of a minimum of ten (10) unknown stains provided by the training coordinator or designee.
- 5.3.8 Read applicable literature and complete the applicable study questions.

#### 5.4 Evaluation

- 5.4.1 Knowledge of the trainee will be evaluated through:
- Review of notes in the training notebook by the training coordinator.
  - Mini-mock trials/oral and practical exams.
- 5.4.2 The trainee should develop and exhibit an unquestionably sound technique for testing stains for the presence of blood. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator, as well as an evaluation of the results obtained for the set of unknown samples.

## 6 IDENTIFICATION OF SEMEN

### 6.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the physical and chemical characteristics of semen (animal and human).
- Gain a thorough knowledge of the procedures used by the Department.
- Gain proficiency in the use of alternate light sources for locating semen stains.
- Gain proficiency in locating and evaluating stains on evidentiary material.
- Develop an understanding of the theory behind the use of chemical (color) tests and immunological tests for semen.
- Develop an understanding of the sensitivity, specificity and limitations of the Acid Phosphatase (AP) Test (qualitative and quantitative) and p30 test using the OneStep ABACard®.
- Gain proficiency in extraction techniques, staining techniques, and microscopic examination for spermatozoa.
- Gain proficiency in the use of the AP test and p30 test (ABACard®), including the use of controls and possible sources of error.
- Gain proficiency in techniques used to prevent cross-contamination of seminal fluid/spermatozoa between samples.

### 6.2 Technical Notes

- Screening items such as clothing or bedding for the presence of semen stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a “Wood’s Lamp” by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If proper eye protection is not worn, permanent damage to the eye may occur. The principle behind the light sources is that semen contains a component(s) which reacts to light between 450 and 455 nm wavelengths. While some sources cite flavins, other sources cite acid phosphatase as being the reactive component in semen. The reaction may either appear as a light stain against a dark background, or in some circumstances, the stain appears darker against a light background. The reaction must be interpreted with caution since other substances (such as, but not limited to, urine, saliva, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Samples exhibiting a reaction to an ALS require further examination to detect and/or confirm the presence of semen.
- When the presence of semen is suspected in a stain, the Acid Phosphatase Test, a preliminary chemical test used to screen stains for the presence of semen, is conducted initially. This test is based on the detection of acid phosphatase, a major component of semen. In the presence of acid phosphatase, the sodium  $\alpha$ -naphthyl acid phosphate is hydrolyzed to  $\alpha$ -naphthol, which diazotizes with the dye to yield a colored azo-dye. Samples giving a positive reaction to the screening test require further examination to confirm the presence of semen.
- Although a positive result (defined in the procedure manual) with the Acid Phosphatase Test is strongly indicative of semen, confirmation of its presence must be established by the identification of spermatozoa. In the absence of spermatozoa, the detection of p30, a human seminal plasma protein, is further indication of the presence of seminal fluid. The presence of semen on swabs from a Physical Evidence Recovery Kit is confirmed by the finding of spermatozoa on the correspondingly labeled smears or in an extract of the swabs themselves. Acid Phosphatase testing is optional when the correspondingly labeled smears are positive for spermatozoa. The presence of semen in stains is confirmed by the finding of spermatozoa in an extract of the stain. If the acid phosphatase test suggests the presence of semen, but no spermatozoa are identified on the correspondingly labeled smears or in an extract of the stain, semen may be indicated with a positive result using the ABACard® for p30.



### 6.3 Tasks

**NOTE:** The trainee may perform presumptive and/or confirmatory tests for seminal fluid on evidence under the direct supervision of a qualified examiner during this training program. Prior to performing these tests on evidence, the trainee must perform these tests on at least one fabricated training sample under the direct supervision of the training coordinator or designee. Satisfactory performance of these tests on the fabricated training sample will be documented in a MFR to be maintained with the training records and will serve as the competency to perform these tests on evidence. A fabricated sample created for this purpose or any of the fabricated training samples listed below may serve as the competency sample.

- Competency must be tested for each type of test prior to performing that test on casework. This may be performed and documented in any order. Competency in each type of test may be demonstrated together on any one sample, or independently over time.
- Qualification to examine evidence smears, as detailed in Chapter 4, need not be repeated.

6.3.1 Read the applicable chapter(s) in the FB PM Report Writing.

6.3.2 Refer to the FB PM Screening and Collection for DNA Analysis.

6.3.3 Examine and compare a minimum of twenty (20) samples on different substrates with the aid of all alternate light sources available in the section.

6.3.3.1 Samples must include known physiological fluids (including, but not limited to):

- Various semen dilutions prepared in distilled water
- Blood
- Saliva
- Perspiration
- Mixtures of physiological fluids

6.3.3.2 Samples must include substances known to react to an alternate light source (including, but not limited to):

- Milk
- Yogurt
- Lotion
- “Bleach alternative” detergent

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

6.3.5 Perform presumptive and confirmatory tests, as appropriate, on a minimum of fifty (50) known semen stains:

**NOTE:** Prepare the necessary reagents and perform the procedures as outlined in the FB PMs. Record the results for each sample for each test performed.

6.3.5.1 Stains must include, at a minimum:

- Semen stains of varying ages
- Semen stains on various substrates
- Semen dilutions (neat to 1:100)
- Semen stains subjected to various contaminants
- Semen stains subjected various environmental conditions

6.3.6 Examine and compare twenty (20) different prepared slides of animal spermatozoa in the reference collection.

6.3.7 Perform presumptive and confirmatory tests, as appropriate, on at least twenty (20) samples of various known physiological fluids.

6.3.7.1 Stains must include, at a minimum:

- Aspermic seminal fluid samples
- Various known physiological fluids not mixed with semen or seminal fluid
- Mixtures of semen with various known physiological fluids
- Mixtures of aspermic seminal fluid with various known physiological fluids

6.3.8 Test a minimum of twelve (12) semen or seminal fluid samples of varying dilutions using the OneStep ABACard® p30 Test to determine the sensitivity of that test. Compare the results.

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

6.3.10 Successfully test, using presumptive and confirmatory tests, as appropriate, a competency set of a minimum of twenty-five (25) unknown stains provided by the training coordinator or designee.

**NOTE:** These will consist of, at a minimum, varying dilutions of semen as well as samples with no spermatozoa.

6.3.10.1 Use appropriate cleaning techniques between samples to ensure that no cross-contamination occurs.

6.3.11 Read applicable literature and complete the applicable study questions.

## 6.4 Evaluation

6.4.1 Knowledge of the trainee will be evaluated through:

- Review of notes in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

6.4.2 The trainee should develop and exhibit an unquestionably sound technique for testing stains for semen/seminal fluid, using both presumptive and confirmatory tests. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator, as well as an evaluation of the results obtained for the set of unknown samples.

## 7 RELATED PROCEDURES

### 7.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding that there are ways in which to test for other biological substances (e.g., urine, feces, saliva), and why the Department no longer employs those methods.
- Develop a basic understanding of bloodstain patterns and surface deposition of stains.
- Develop an understanding as to how and when to group stains together for testing.
- Gain a thorough knowledge of the design and use of OCME, Victim, and Suspect Physical Evidence Recovery Kits and Buccal Swab Kits.
- Gain proficiency in the recovery and packaging of hairs and fibers and an understanding of when hair/fiber examinations are conducted.
- Gain proficiency in the recovery of body fluids from porous and nonporous surfaces.
- Develop an understanding of other forensic disciplines in order to recognize and preserve potential evidence related to these areas.

### 7.2 Technical Notes

- Screening items such as clothing or bedding for the presence of urine stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a “Wood’s Lamp” by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If proper eye protection is not worn, permanent damage to the eye may occur. The principle behind the light sources is that urine contains a component (urea) which reacts to light between 450 and 455 nm wavelengths. The reaction appears as a light stain against a dark background. The reaction must be interpreted with caution since other substances (such as, but not limited to, semen, saliva, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Samples exhibiting a reaction to an ALS require further examination to detect the presence of urine.
  - The urease test is a presumptive test for the presence of urine no longer used by the Department. It is based on the fact that urea is found in high concentration in urine. Although there are many different presumptive tests for the presence of urine, there are no confirmatory tests available for the identification of urine in a dried stain. The urease reagent reacts with urea, releasing ammonia from the stain, which turns red litmus paper to a blue color.
- Edelman’s test is a presumptive test for the presence of fecal material no longer used by the Department. It is based on the detection of urobilinogen found in high concentration in feces. Urobilinogen is formed in the intestine by the reduction of bilirubin. Urobilinogen is oxidized to urobilin, which is soluble in alcohol. In the presence of neutral alcoholic salts, a green fluorescent complex is formed between urobilin from human or Carnivore feces and zinc. Due to the presence of chlorophyll in Herbivore (ruminants, such as cattle, sheep, and deer) feces, fluorescence will be orange-pink. Although there are other presumptive tests to indicate the presence of fecal material, there are no confirmatory tests available for the identification of fecal material.
- Screening items such as masks or clothing for the presence of saliva stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a “Wood’s Lamp” by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If

proper eye protection is not worn, permanent damage to the eye may occur. The principle behind the light sources is that biological fluids may react to light between 450 and 455 nm wavelengths. The reaction may either appear as a faint light stain against a dark background, or in some circumstances, the stain appears darker against a light background. The reaction must be interpreted with caution since other substances (such as, but not limited to, urine, semen, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Since the Department does not conduct presumptive testing for the presence of saliva, samples exhibiting a reaction to an ALS may require DNA analysis. Common sense sampling from areas believed to have come in contact with saliva (e.g. the mouth area of a mask) may also require DNA analysis.

### 7.3 Tasks

- 7.3.1 Read the Section Specific Modules under Educational Resources in Qualtrax.
- 7.3.2 If possible, observe and receive instruction from qualified examiners in other forensic disciplines, particularly the Trace Evidence, Firearms, and Latent Print Sections.
- 7.3.2.1 Arrangements will be made by the training coordinator and Laboratory Director.
- 7.3.2.2 The period of observation/instruction should be brief and focus on items of evidence involved with examinations in the Forensic Biology Section.
- 7.3.3 Observe and receive instruction from qualified examiners noting possible bloodstain patterns during examinations, including surface deposition and how and when to group the stains for testing.
- NOTE:** Although there is the potential for some investigative information to be gleaned from the nature of bloodstain deposits (drop, smear, splatter), it is well advised that any examiner in the Forensic Biology Section exercise extreme caution in offering opinions regarding the ballistics of such patterns. Opinions should be reserved for those individuals with considerable expertise in this area
- 7.3.4 Observe and receive instruction from qualified examiners performing examinations of OCME, Victim, and Suspect Physical Evidence Recovery Kits, as well as Buccal Swab Kits.
- 7.3.5 Observe and receive instruction from qualified examiners recovering/packaging hairs and fibers using various recovery techniques such as scraping, using forceps, and using post-it type notes.
- 7.3.6 Observe and receive instruction from qualified examiners recovering various types of body fluids/potential body fluids from various types of porous and nonporous surfaces.
- 7.3.7 Read applicable literature and complete the applicable study questions.

### 7.4 Evaluation

- 7.4.1 Knowledge of the trainee will be evaluated through:
- Review of notes and worksheets in the training notebook by the training coordinator.
  - Mini-mock trials/oral and practical exams.
- 7.4.2 The trainee should examine a sufficient number of cases/items of evidence to develop and exhibit an unquestionably sound technique for grouping stains, determining the surface of stain deposition, recovering a packaging hairs and fibers, recovering body fluids from porous and nonporous surfaces, and examining OCME, Victim, and Suspect Physical Evidence Recovery Kit as well as Buccal Swab Kits. The trainee should also demonstrate the ability to easily recognize potentially valuable evidence involving other forensic disciplines and how to handle such items to prevent any loss of evidence. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

## 8 DEOXYRIBONUCLEIC ACID (DNA)

### 8.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the application of DNA analysis to forensic samples, including:
  - Type/size of specimens required
  - Methods of specimen preservation and storage
  - Documentation required in case file
- Become familiar with the DNA testing procedures currently and previously conducted on casework and Data Bank samples by the Department, including:
  - DNA RFLP testing
  - DNA PCR-based typing systems
- Become familiar with the purpose of the Combined DNA Index System.

### 8.2 Tasks

- 8.2.1 Observe and receive instruction from qualified examiners assessing the suitability of forensic specimens for DNA analysis, including appropriate documentation.
- 8.2.2 Read applicable literature.

### 8.3 Evaluation

- 8.3.1 Knowledge of the trainee will be evaluated through:
- Review of notes in the training notebook by the training coordinator.
  - Mini-mock trials/oral and practical exams.
- 8.3.2 The trainee should examine a sufficient number of cases/items of evidence to develop and exhibit an understanding of the types/sizes of samples required to conduct DNA analysis, the methods by which they are to be preserved and stored and the case file documentation required by the Department. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

## 9 GENERAL REPORT WRITING

### 9.1 Purpose and Scope

During the completion of this area of study the trainee will become familiar with the format and report wording presently used by DFS Forensic Biology examiners in regard to identification of biological substances.

### 9.2 Tasks

- 9.2.1 Refer to the FB PM Screening and Collection for DNA Analysis.
- 9.2.2 Read the Forensic Biology Procedures Manual Report Writing.
- 9.2.3 Using the results of body fluid examinations from a minimum of ten (10) actual cases, prepare a Certificate of Analysis for each and compare to the original Certificate released by the examiner.
- 9.2.4 Conduct technical and administrative peer reviews of examiners' Certificates for cases with body fluid examinations before the official peer reviews are conducted by a second qualified examiner and compare the results with those of the official peer reviewer.

### 9.3 Evaluation

- 9.3.1 Knowledge of the trainee will be evaluated through:
  - Review of notes Certificates of Analysis prepared by the trainee in the training notebook by the training coordinator.
  - Mini-mock trials/oral and practical exams.
- 9.3.2 The trainee should develop and demonstrate the ability to clearly, accurately, and concisely set forth body fluid examination results in a Certificate of Analysis.

This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

## 10 TESTIMONY AND EXPERT WITNESS QUALIFICATION

### 10.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become skilled in expressing results and conclusions orally in a simple, clear, concise, and technically correct manner.
- Become familiar with legal terminology and criminal law procedures, including:
  - Rules of evidence admissibility
  - Hearsay evidence admissibility
  - Public document admissibility
  - Examination of an expert witness
  - Opinion evidence
  - Proof of chain of custody
  - Frye
  - Daubert
  - Spencer hearing
  - Courtroom procedures
- Become skilled in properly communicating with attorneys, judges, and juries.
- Become familiar with the use of testimony aids.

### 10.2 Tasks

- 10.2.1 Observe examiners testify, when possible.
- 10.2.2 Observe at least one pretrial conference with a qualified examiner, when possible.
- 10.2.3 Participate in mini-mock trials/oral question and answer session(s).
- 10.2.4 Read applicable literature and complete the applicable study questions.

### 10.3 Evaluation

- 10.3.1 Knowledge of the trainee will be evaluated through mini-mock trials and/or question and answer sessions.
- 10.3.2 The trainee should demonstrate the ability to clearly and accurately testify to body fluid examination results and conclusions. This will be monitored by the evaluation of question and answer sessions and mini-mock trials conducted throughout the training program.
- 10.3.3 Refer to Chapter 1 of this manual for guidelines on the evaluation of the completion of the training overall, including testimony and expert witness qualification.

**APPENDIX A – REFERENCES AND REQUIRED READINGS**

McCrone, Walter C. And Delly, John Gustav, The Particle Atlas, Volume I, 2nd ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan (1973), page 23.

Forensic Science Handbook, edited by Richard Saferstein, Prentice-Hall, Inc., Englewood Cliffs, NJ (1982).

Kirk, Paul L., Crime Investigation, John Wiley and Sons, New York, NY (1974), pp. 650-651.

Gaensslen, R. E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, U. S. Government Printing Office, Washington, DC (1983).

Metropolitan Police Forensic Science Laboratory, Biological Methods Manual, London, England (1978).

MICROSCOPY

Optical Systems for the Microscope, Carl Zeiss Oberkochen/Wuertt., Edition December 1967. (No further information available.)

Photomicrography: Instant Photography Through the Microscope, Polaroid Corporation, Cambridge, Massachusetts, 4-26. (1995).

BLOOD

Baxter, S. J. and Rees, B., “The Use of Anti-human Hemoglobin in Forensic Serology,” *Medicine, Science, and the Law*, Vol. 14: 159-162 (1974).

Boorman, K. E., Dodd, B. E., and Lincoln, P. J., Blood Group Serology, Churchill Livingstone, Inc., London, England (1977).

Culliford, B. J. and Nickolls, L. C., “The Benzidine Test,” *JFS*, Vol. 9: 175-191 (1964).

Culliford, Bryan, The Examination and Typing of Bloodstains in the Crime Laboratory, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1971).

Garner, D. D., Cano, K. M., Peimer, R. S., and Yeshion, T. E., “An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood,” *JFS*, Vol. 21: 816-821 (1976).

Gaensslen, R. E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).

Garner, D. D., Cano, K. M., Peimer, R. S., and Yeshion, T. E., “An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood,” *JFS*, Vol. 21: 816-821 (1976).

Grispino, R. R., “The Effect of Luminol on the Serological Analysis of Dried Human Bloodstains,” *Crime Laboratory Digest*, Vol.17: 13-23 (1990).

Kirk, Paul L., Crime Investigation, John Wiley and sons, New York, NY (1974).

Lee, H. C., et al., “The Effect of Presumptive Tests, Latent Fingerprint and Some Other Reagents and Materials on Subsequent Serological Identification, Genetic Marker, and DNA Testing in Bloodstains,” *J. Forensic Ident.*, Vol. 39: 339-358 (1989).

MacDonell, H. L., Flight Characteristics and Stain Patterns of Human Blood, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1971).



Masters, R. W. and Schlein, F. C., “Factors Affecting the Deterioration of Dried Bloodstains,” JFS, Vol. 3: 288-302 (1958).

Saferstein, R., Ed., Forensic Science Handbook, Prentice-Hall, Inc., Englewood Cliffs, NJ (1982).

Thornton, J. I. and Maloney, R. S., “The Chemistry of the Luminol Reaction—Where to From Here?,” Forensic Science Group, University of California, Berkeley, CA (1981). (No further information available.)

Wraxall, Brian G. D., Bloodstain Analysis System Procedures Manual (October 1978), prepared for Forensic Serology Workshops at the Serological Research Institute 1977-1978.

### SECRETIONS

Abacus Diagnostics, OneStep ABACard® p30 Test For The Forensic Identification of Semen, Technical Information Sheet, Revised 10/98.

Adams, Elizabeth G. and Wraxall, Brian G., “Phosphatases in Body Fluids: The Differentiation of Semen and Vaginal Secretions,” Forensic Science, Vol. 3: 57-62 (1974).

Alderman, Philip M. “The Lurking Sperm: A Review of Failure in 8879 Vasectomies Performed by One Physician” JAMA Vol 259, No. 21: 3142-3144 (1988).

“Anti-Sperm Antibodies,” CAC Newsletter (Sept. 1986).

Auvdel, Michael J., “Amylase Levels in Semen and Saliva Stains,” JFS, Vol. 31: 426-431 (1986).

Auvdel, Michael J., “Comparison of Laser and Ultraviolet Techniques Used in the Detection of Body Secretions,” JFS, Vol. 32: 326-345 (1987).

Baechtel, F. Samuel, “Immunological Methods for Seminal Fluid ID,” Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp. 83-89, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).

Baxter, S. J. and Rees, B., “The Identification of Saliva in Stains in Forensic Case Work,” *Medicine, Science, and the Law*, Vol. 15: 37-41 (1975).

Baxter, S. J., “Immunological Identification of Human Semen,” *Medicine, Science, and the Law*, Vol. 13: 155-165 (1973).

Bitner, Sarah E., “False Positives Observed on the Seratec® PSA SemiQuant Cassette Test with Condom Lubricants,” JFS, Vol. 57: 1545-1548 (2012).

Boward, Emily S. et al., “A comparison of ABACard® p30 and RSID™-Semen test kits for forensic semen identification,” *Journal of Forensic and Legal Medicine*, Vol. 20: 1126-1130 (2013).

Brown, K. M. and Brow, C. G., “Specificity of Two Commercial Acid Phosphatase Determination Kits with Respect to Feminine Hygiene Products and Vaginal Contraceptives,” JFS, Vol. 19: 384-389 (1974).

Brown, Barry L., “Anatomy, Physiology, and Biochemistry of the Female Reproductive System,” Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp. 3-19, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).

Calloway, A. R., et. al., “Location of Seminal Stains by Their Phosphorescence and Its Use in Determining the Order of Deposition of Overlapping Seminal and Bloodstains,” *J. For. Sci. Soc.*, Vol. 13: 223-229 (1973).

Chang, Thomas S. K., "Seminal Cytology," Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp. 45-56, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).

Davies, Anne and Wilson, Elizabeth, "The Persistence of Seminal Constituents in the Human Vagina," *Forensic Science*, Vol. 3: 45-55 (1974).

Davies, Anne, "Evaluation of Results from Tests Performed on Vaginal, Anal, and Oral Swabs Received in Casework," *J. For. Sci. Soc.*, Vol. 17: 127-133 (1977).

Divall, Graham B., "Identification and Persistence of Seminal Constituents in the Postcoital Vaginal Tract," Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp. 57-63, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).

Duenholter, J. H., et al., "Detection of Seminal Fluid Constituents After Alleged Sexual Assault," *JFS*, Vol. 23: 824-829 (1978).

Enos, William F., Mann, Geoffrey T., and Dolan, William D., "A Laboratory Procedure for the Identification of Semen," *American Journal of Clinical Pathology*, Vol. 39: 316-320 (1963).

Enos, W. F. and Beyer, J. C., "Spermatozoa in the Anal Canal and Rectum and in the Oral Cavity of Female Rape Victims," *JFS*, Vol. 23: 231-233 (1978).

Enos, W. F. and Beyer, J. C., "Prostatic Acid Phosphatase, Aspermia, and Alcoholism in Rape Cases," *JFS*, Vol. 25: 353-356 (1980).

Gaensslen, R. E., Lee, Henry C., Mertens, John E., and Stolorow, Mark D., "Staining and Extracting Techniques," Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp. 135-144, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).

Gomez, Rolando R., et al., "Qualitative and Quantitative Determinations of Acid Phosphatase Activity in Vaginal Washings," *American Journal of Clinical Pathology*, Vol. 64: 423-432 (1975).

Graves, Howard C. B., Sensabaugh, George F., and Blake, Edward T., "Postcoital Detection of a Male-Specific Semen Protein," *New England Journal of Medicine*, Vol. 312: 338-343 (1985).

Groth, A. Nicholas and Burgess, Ann Wilbert, "Sexual Dysfunction During Rape," *New England Journal of Medicine*, Vol. 297: 764-765 (1977).

Hochmeister, M. N., Budowle, B., Rudin, O., Gehrig, C., Borer, U., Thali, M., and Dirnhofer, R., "Evaluation of Prostate-Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid", *JFS*, Vol. 44: 1057-1060.

Hueske, E. E., "Techniques for Extraction of Spermatozoa from Stained Clothing: A Critical Review," *JFS*, Vol. 22: 596-598 (1976).

"Identification of Seminal Fluids," *American Jurisprudence Proof of Facts*, Vol. 12: 319-333 (1962).

Joshi, U. N., Subhedar, S. K., and Saraf, D. K., "Effect of Water Immersion on Seminal Stains on Cotton Cloth," *Forens. Sci. Intern.*, Vol. 17: 9-11 (1981).

Kafarowski, E., Lyon, A. M. and Sloan, M. M., "The Retention and Transfer of Spermatozoa in Clothing by Machine Washing" *Can. Soc. Forens. Sci. J.*, Vol. 29: 7-11 (1996).

Kaye, Sidney, "Acid Phosphatase Test for Identification of Seminal Stains," *Journal of Laboratory and Clinical Medicine*, Vol. 34: 267-288 (1964),

- Kind, S. S., “The Acid Phosphahtase Test,” Methods of Forensic Science, Vol. 3, Interscience Publishers, New York, NY (1964).
- Kipps, A. E. and Whitehead, P. H., “The Significance of Amylase in Forensic Investigations of Body Fluids,” JFS Vol. 6: 1376-144 (1975).
- Laux, Dale L. Forensic Detection of Semen I: The Acid Phosphatase Test, *Midwestern Association of Forensic Scientists Newsletter*, Vol. 32, Fall 2003, pp. 6-10.
- Laux, Dale L., Tambasco, Anthony and Benzinger, Elizabeth A. Forensic Detection of Semen II : Comparison of the Abacus Diagnostics *OneStep ABACard p30 Test* and the Seratec *PSA Semiquant Kit* for the Determination of the Presence of Semen in Forensic Cases, *Midwestern Association of Forensic Scientists Newsletter*, Vol. 32, Fall 2003, pp. 11-18.
- Laux, Dale L. and Custis, Sarah E. Forensic Detection of Semen III: Detection of PSA Using Membrane Based Tests: Sensitivity Issues with Regards to the Presence of PSA in Other Body Fluids, *Midwestern Association of Forensic Scientists Newsletter*, Vol. 33, Winter 2004, pp. 33-39.
- McCloskey, K. L., et al., “Prostatic Acid Phosphatase Activity in the Postcoital Vagina,” JFS, Vol. 20: 630-636 (1975).
- Moyer, Dean L., et al., “Sperm Distribution and Degradation in the Human Female Reproductive Tract,” *Journal of Obstetrics and Gynecology*, Vol. 35: 831-839 (1970).
- Nelson, Donald F. and Kirk, Paul L., “The Identification of Saliva,” *Journal of Forensic Medicine*, Vol. 10: 14-21 (1963).
- Peabody, A. J., Burgess, R. M., and Stockdale, R. E., “Re-examination of the Lugol’s Iodine Test,” *Home Office Central Research Establishment Report No. 412* (Oct. 1981).
- Peonim, Vichan, et al., “Comparison between prostate specific antigen and acid phosphatase for detection of semen in vaginal swabs from raped women,” *Journal of Forensic and Legal Medicine*, Vol. 20: 578-581 (2013).
- Philp, T., Guillebaud, J. and Budd, D., “Complications of Vasectomy: Review of 16,000 Patients,” *British Journal of Urology*, Vol 56: 745-748 (1984).
- Pollack, O. J., “Semen and Seminal Stains – A Review of Methods Used in Medicolegal Investigation,” *Archives of Pathology*, Vol. 35: 140-196 (1943).
- Poyntz, F. M. and Martin, P. D., “Comparison of P30 and Acid Phosphatase Levels in Post-Coital Vaginal Swabs from Donor and Casework Studies,” *Forens. Sci. Intern.*, Vol. 24: 17-52 (1984).
- Prabhakran, D., et al., “A Rapid Test for Seminal Stain Acid Phosphatase,” *Journal of Police Science and Administration*, Vol. 9: 76-79 (1981).
- Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, Laboratory Division of the Federal Bureau of Investigation, U. S. Department of Justice, Washington, DC (July 6-8, 1983).
- Randell, B., “Glycogenated Squamous Epithelial Cells as a Marker of Foreign Body Penetration in Sexual Assault,” JFS, Vol. 33: 511-514 (1988).
- Rothwell, T. J. and Harvey, K. J., “The Limitations of the Lugol’s Iodine Staining Technique for the Identification of Vaginal Epithelial Cells,” *J. For. Sci. Soc.*, Vol. 18: 181-184 (1978).
- Rupp, Joseph C., “Sperm Survival and Prostatic Acid Phosphatase Activity in Victims of Sexual Assault,” JFS, Vol. 14: 177-183 (1969).

- Rushton, Claire, et al., “The Distribution and Significance of Amylase-Containing Stains on Clothing,” *J. For. Sci. Soc.*, Vol. 19: 53-58 (1979).
- Rutter, E. R., Kind, S. S., and Smalldon, K. W., “Estimation of Time Since Intercourse Acid Phosphatase/UV270 Absorbance Ratios,” *J. For. Sci. Soc.*, Vol. 20: 271-282 (1980).
- Schiff, A. F., “Reliability of the Acid Phosphatase Test for the Identification of Seminal Fluid,” *JFS*, Vol. 23: 833-844 (1978).
- Schiff, Arthur Frederick, “Modification of the Berg Acid Phosphatase Test,” *JFS*, Vol. 14: 538-544 (1969).
- Schumann, G. Berry, et al., “Prostatic Acid Phosphatase – Current Assessment in Vaginal Fluid of Alleged Rape Victims,” *American Journal of Clinical Pathology*, Vol. 66: 944-952 (1976).
- Sensabaugh, George F., “Isolation and Characterization of a Semen Specific Protein from the Human Seminal Plasma: A Potential New Marker for Semen ID,” *JFS*, Vol. 23: 106-115 (1978).
- Sensabaugh, George F., “The Acid Phosphatase Test,” Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp 65-81, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).
- Sensabaugh, G. F., “The Quantitative Acid Phosphatase Test: A Statistical Analysis of Endogenous and Postcoital Acid Phosphatase Levels in the Vagina,” *JFS*, Vol. 24: 346-355 (1979).
- Sharpe, Noble, “The Significance of Spermatozoa in Victims of Sexual Offences,” *Canad. Med. Assoc. Journal*, Vol. 89: 513-514 (1963).
- Sherins, Robert J. and Brown, Barry L., “Anatomy, Physiology, and Disorders of the Male Reproductive System,” Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, pp. 21-43, U. S. Department of Justice, U. S. Government Printing Office, Washington, DC (1983).
- Silverman, Eugene M. and Silverman, Alida G., “Persistence of Spermatozoa in the Lower Genital Tracts of Women,” *JAMA*, Vol. 240 No. 17: 1875-1877 (1975).
- Spear, T. F. and Khoskebari, N., “The Evaluation of ABACard® p30 Test for the Identification of Semen”, The CAC News, 1st Quarter 2001, pages 22-24.
- Speck, Ballantyne; Post Coital DNA Recovery, December 2014: NIJ Grant No. 2009-DN-BX-0023.  
<http://www.ncjrs.gov/pdffiles1/nij/grants/248682.pdf>
- Standefer, J. C. and Street, E. W., “Postmortem Stability of Prostatic Acid Phosphatase,” *JFS*, Vol. 21: 165-172 (1976).
- Stolorow, Mark D., et al., “Identification of Human Seminal Acid Phosphatase by Electrophoresis,” *Journal of the Association of Official Analytical Chemists*, Vol. 59: 1352-1356 (1976).
- “To P or Not to P (30, that is...) Saga of a Seminal Protein,” *Seri Newsletter*, No. 7 (December 1981).
- Wallace-Haagens, Mary Jean, et al., “Recovery of Spermatozoa from Human Vaginal Washings,” *Fert. And Ster.*, Vol. 26: 175-179 (1975).
- Weitzig, Laurence, et al., “Diagnostic Value of PSA and AP Tests for the Detection of Spermatozoa in Postmortem Swabs from the Genital and Anal Region in Males,” *JFS*, Vol. 60: 41-44 (2015).
- Willott, G. M., “An Improved Test for the Detection of Salivary Amylase in Stains,” *J. For. Sci. Soc.*, Vol. 14: 341-344 (1974).

Willott, G. M. and Allard, J. E., "Spermatozoa-Their Persistence After Sexual Intercourse," *Forens. Sci. Intern.*, Vol. 19: 135-154 (1982).

Willott, G. M. and Crosse, M. A., "Detection of Spermatozoa in the Mouth," *J. For. Sci. Soc.*, Vol. 26: 125-128 (1986).

Willott, G. M., "L-Tartrate Inhibitable Acid Phosphatase in Semen and Vaginal Secretions," *J. For. Sci. Soc.*, Vol. 12: 363-366 (1972).

Wilson, E. F., "Sperm's Morphologic Survival After 16 Days in the Vagina of a Dead Body," *JFS*, Vol. 19: 7-74 (1974).

Yu, He and Diamandis Eleftherios P., "Prostate-Specific Antigen Immunoreactivity in Amniotic Fluid," *Clin. Chem.*, Vol. 41: 204-210 (1995).

Yu, He and Diamandis Eleftherios P., "Prostate-Specific Antigen in Milk of Lactating Women," *Clin. Chem.*, Vol. 41: 54-58 (1995).

Editorial, "Nonprostatic Sources of Prostate-Specific Antigen: A Steroid Hormone-Dependent Phenomenon?" *Clin. Chem.*, Vol. 41: 7-9 (1995).

#### EXPERT WITNESS TESTIMONY

Brownlie, Alistair R., "A Lawyer Looks at Forensic Science: The Expert in Court," *J. For. Sci. Soc.*, Vol. 18: 5-12 (1978).

Burke, John J., "Testifying in Court," *FBI Law Enforcement Bulletin* (September, 1975).

Cato, B. H., "The Presentation of Scientific Evidence in the Courts – Improving its Effectiveness," *J. For. Sci. Soc.*, Vol. 14: 93-98 (1974).

McCarthy, J. F., "On Playing the Game of Expert Witness in a Two-Value Logic System," *JFS*, Vol. 19: 131-135 (1974).

Hon. B. Marc Mogil, J. D., "Maximizing Your Courtroom Testimony," *FBI Law Enforcement Bulletin*, pp. 7-9 (May 1989).

Phillips, K. A., "The 'Nuts and Bolts' of Testifying as a Forensic Scientist," *JFS*, Vol. 22: 457-463 (1977).

Starrs, J. E., "The Ethical Obligations of the Forensic Scientist in the Criminal Justice System," *Journal of AOAC*, Vol. 54: 906-914 (1971).

Steinmetz, Charles W., "The Expert Witness and Communication Competency," *Identification News*, pp. 3-6 (August 1982).

#### Symposium: Ethical Conflicts in the Forensic Sciences

- a. Peterson, Joseph L., "Introduction," *JFS*, Vol. 34: 717-718 (1989).
- b. Lucas, Douglas M., "The Ethical Responsibilities of the Forensic Scientist: Exploring the Limits," *JFS*, Vol. 34: 719-729 (1989).
- c. Giannelli, Paul C. "Evidentiary and Procedural Rules Governing Expert Testimony," *JFS*, Vol. 34: 730-748 (1989).

- d. Peterson, Joseph L. and Murdock, John E., “Forensic Science Ethics: Developing an Integrated System of Support and Enforcement,” JFS, Vol. 34: 749-762 (1989).
- e. Saks, Michael J., “Prevalence and Impact of Ethical Problems in Forensic Science,” JFS, Vol. 34: 772-793 (1989).

Tanton, R. L., “Jury Preconceptions and Their Effect on Expert Scientific Testimony,” JFS, Vol. 24: 681-691 (1979).

**APPENDIX B – STUDY QUESTIONS****Receiving and Handling Physical Evidence:**

1. What is a container?
2. Where is the Department’s LIMS?
3. What is a lock box?
4. How is evidence transferred from one laboratory to another?
5. What is the pathway that an item of evidence goes through from the time it enters DFS to the time it is returned to the agency?
6. Describe the duties of the “primary examiner”. How is the “primary examiner” determined?
7. What is chain of custody?
8. How is chain of custody maintained in your laboratory?
9. How is evidence stored in your laboratory?
10. How is evidence stored in your personal custody when you are not examining it?
11. Who has access to the various storage areas including your personal evidence locker?
12. What is a proper seal?
13. What is a temporary seal and when can it be used?
14. What is the difference between short and long term storage?
15. You receive a known blood sample in a lavender top blood tube. How do you preserve this sample to ensure that no degradation occurs?
16. You receive a call from an investigator saying he’s arrested the suspect in the case he submitted two weeks ago, but isn’t sure what to do. What do you tell him?
17. What key pieces of information should be included on every page of your notes?
18. Introducing Physical Evidence In Court (taken from Trial Technique Predicate Questions, Second Edition, National District Attorneys Association, Alexandria, Virginia):
  - a. Do you recognize this item of evidence?
  - b. How do you recognize it?
  - c. What is it?
  - d. How did it first come into your possession?
  - e. Where did you obtain it?
  - f. When did you obtain it?
  - g. Is this item in substantially the same condition now as when you first saw it?
  - h. What did you do with it?

**Introduction to the Microscope:**

1. Describe Köehler Illumination and how this is achieved on the microscope.

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

**Indication of Blood:**

1. What is blood and what is it composed of?
2. What is the purpose of blood in the body?
3. What is the PTMB test?
4. When is the PTMB test performed?
5. What is the mechanism behind the PTMB test?
6. What is the purpose of each chemical used in the PTMB testing?
7. If an oxidizer, such as potassium permanganate, was tested with the chemicals used for PTMB testing, what reaction would be expected and why?
8. What is the benefit of using the combined PTMB chemical test?
9. Which PTMB reagent works best in the acidic environment and which works best in the basic environment?
10. What does a positive PTMB result tell you?
11. What would you do if your P test was positive but the TMB test was negative?
12. What action would you take if your negative control was positive?



13. What is believed to be the mechanism behind a false negative result sometimes encountered with suede or leather material?
14. What is the mechanism for luminol?
15. What is the mechanism for the BLUESTAR® Forensic Test?
16. What is luminol/BLUESTAR® used?
17. What does a positive luminol or BLUESTAR® result look like and what does it mean?
18. You get a call from an investigator requesting luminol, which the Department no longer provides. How do you handle it?
19. You get a call from an investigator asking if luminol can be used to examine the back yard where it is believed that a husband shot his wife two weeks ago. What do you tell him?
20. You get a call from a patrol officer saying that he is processing the scene of a B&E where he sees blood on a broken window. He's never done this before. How do you advise him to proceed?
21. You get a "supercan" trashcan for a case in which the victim's body was found in the trashcan itself. What do you do with it?
22. What is the purpose of a positive control?
23. Name two presumptive tests for blood and two confirmatory tests for blood not used by the Department.

**Identification of Semen:**

1. What is semen?
2. What glands contribute to seminal fluid?
3. What is p30 and where is it found?
4. What is the significance of p30 and under what circumstances would you test for it?
5. Why is the ABACard® p30 Test no longer considered a confirmatory test for semen?
6. What factors can lead to a diminished sperm count in the male ejaculate?
7. Describe the mechanism and the purpose of the chemicals for the AP test. What would a positive result look like and what would a positive result tell you?
8. Describe the mechanism and the purpose of the chemicals for the p30 test.
9. Compare and contrast the different methods for detecting semen stains.
10. How does an alternate light source assist in locating stains? What alternate light sources are used by DFS (include filters used and wavelengths)?
11. What is the name of the stain used to stain smears for spermatozoa examination? What is the purpose of each chemical?
12. Describe the appearance of stained spermatozoa using phase contrast and bright field.
13. Describe the morphology of a spermatozoon.

14. What factors may affect the persistence of sperm in a living rape victim? What, if any, differences would one expect to find with regard to the persistence of sperm in a victim of rape and murder?
15. On average what is the total volume of seminal fluid per normal ejaculate? What is considered a normal sperm count per ml of seminal fluid?
16. What, if any, is the significance of observing only sperm heads vs. intact sperm on a vaginal/cervical smear?
17. What, if any, is the significance of observing only sperm heads vs. intact sperm in an extract from a bedsheet stain?
18. Explain the difference between seminal acid phosphatase and vaginal acid phosphatase.
19. If you do not detect a positive AP result on a swab or in a stain, is it possible to identify sperm? Explain your answer. Is it possible to detect male DNA?
20. You get a call from an investigator saying he has a girl who is pregnant due to a rape that occurred about 6 weeks ago. She wants to get an abortion now. What do you advise the investigator?
21. How do you preserve a used condom?
22. If you have some bedding with stains and the stains test AP NEG what would be your next step?
23. What does the literature report about the finding of spermatozoa on articles of clothing/bedding after laundering?
24. Is it possible for spermatozoa to be transferred to other articles of clothing/bedding during laundering?
25. If you have some swabs that test AP POS and an extract of the swabs is NEG for the sperm search, what is your next step?
26. How long would you expect there to be sperm in the female reproductive tract? How about in a stain on bedding?
27. You get a call from an investigator who says he's working a case in which a victim was raped by her husband. Her previous intercourse with him was 3 days ago. What do you advise the investigator?

**Other Biological Substances:**

1. Describe the mechanism for the Urease test, including chemicals used and why.
2. Describe the mechanism for Edelman's test including chemicals used and why.
3. Are there any false positives for the Urease test? Edelman's test?
4. Why doesn't DFS test for urine or feces anymore?
5. What are some other methods used for the detection of urine and feces?
6. Name at least one method that can be used to indicate the presence of saliva. Why doesn't DFS test for saliva anymore?
7. Is there a test to indicate the presence of vaginal fluid? If there is such a test, why doesn't DFS use the test?
8. You receive a piece of bone and a piece of tissue from a decomposed body. How do you preserve these samples for possible future testing?

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

**Related Procedures:**

1. What is a PERK?
2. What is a FNE? SANE?
3. What is the difference between a Victim PERK, Suspect PERK, OCME PERK and Buccal Swab Kit?
4. List the components of each of the Kits listed above.
5. Why might PERK vaginal/cervical swabs be slightly blue? Slightly green?
6. Some laboratories around the country may still conduct conventional serological typing before DNA analysis is conducted. Why doesn't DFS do that? What are the pros/cons for using this approach?
7. Is it important to document the surface of a stain's origin on an item of clothing? Explain how you would determine if a stain is deposited on the inside or outside of a piece of clothing.
8. An investigator calls and says he has a case that was analyzed by a DFS employee who has since left the laboratory. ABO and enzyme typing were previously done. Now he has a suspect for the case and wants ABO and enzyme typing conducted on the suspect's sample so that it can be compared to the previous results. What do you tell him?
9. I checked the lot number on the urease reagent and noticed that it had expired. You used this expired chemical in testing a high profile case and testing cannot be redone. Can you rely on the results you obtained? Why or why not?
10. You are examining a bed sheet and notice a possible footwear impression on it. What do you do?
11. You open a bag containing a knife that may be the weapon used in a murder. You see a possible fingerprint in red stains on the handle. What do you do?
12. You receive a call from an officer at the scene of an assault. He observes what he believes to be blood on the sidewalk, but doesn't know how to collect it. What do you tell him?
13. You receive a call from an officer at the scene of a breaking and entering. Apparently the unknown perpetrator cut himself when he broke the window to gain entry. There is blood on glass on the floor and blood on glass still in the window. He needs to know how to collect these samples. What do you tell him about collecting, packaging, and submitting the blood to the lab?

**Testimony and Expert Witness Qualification**

1. What is the difference between quality assurance and quality control?
2. How is quality control maintained in your laboratory?
3. Name 3 quality control measures you take in the laboratory?
4. Explain the following:
  - Rules of evidence admissibility
  - Hearsay evidence admissibility
  - Public document evidence admissibility

- Competency of an expert witness
  - Examination of an expert witness
  - Opinion evidence
  - Proof of chain of custody
  - Frye Rule
  - Daubert Hearing
  - Spencer Hearing
  - Courtroom procedures
5. Qualifying a Body Fluids Expert as a Witness (taken from Trial Technique Predicate Questions, Second Edition, National District Attorneys Association, Alexandria, Virginia):
- Please state your name and address.
  - What is your occupation?
  - Where are you employed?
  - How long have you been employed with (name of agency/company)?
  - What are your specific responsibilities in your work?
  - What is the extent of your training as a body fluids expert?
  - How much of your time is now devoted to the study and examination of body fluids?
  - Are you a member of any organizations related to your work?
  - Which organizations?
  - Have you authored any papers for either private circulation or publication relating to your field?
  - What subjects did you write about?
  - Were the papers you authored published?
  - If privately circulated, among whom?
  - Is your entire work devoted to your field, or do you have other duties?
  - Have you ever testified as an expert on body fluids?
  - In court or before administrative agencies?
  - In what jurisdiction have you testified as an expert before?
  - Approximately how many times?
  - Would you explain to the jury the nature of the work you do?
  - What body fluids do you analyze?
  - What is the significance of the information you obtain as a result of this type of analysis?
  - Explain tests used for analyzing blood, sperm, seminal fluid and p30.