

Serology Quality Manual

ARKANSAS STATE CRIME LABORATORY

Serology Quality Manual

2010

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FORENSIC SEROLOGY

1 INTRODUCTION (GOALS AND OBJECTIVES)

The Forensic Serology unit is dedicated to providing forensic analysis of physical evidence to the criminal justice system. To this end, we analyze evidence, such as swabs, clothing, weapons and bedding, that may contain body fluids. Other physical evidence such as hairs, fibers and debris may be collected for future analyses. These analyses are performed in a chain-of-custody environment using proper and appropriate procedures in order to ensure the most accurate and relevant analytical results.

1.1 Organization and Management Structure

1.1.1 Organization

The Serology Unit is part of the Physical Evidence Section. The Physical Evidence Section has a supervisor and is under the supervision of the Scientific Operations Director.

1.1.2 Management

This manual has been approved by the Section Supervisor, Scientific Operations Director, and Executive Director and as such is accepted as the routine operating policy of the Forensic Serology Unit within the Arkansas State Crime Laboratory. To discuss possible revisions, meetings between the Section Supervisor and the analysts will be held as needed. Any changes to this manual must be approved through formal chain of command processes, with affected manual pages and files updated. Previous versions of revised documents are maintained in a separate Historical Archive Manual. All analysts must be notified of the changes and must be given necessary training.

2 PERSONNEL QUALIFICATIONS AND JOB DESCRIPTIONS

2.1 Job Descriptions

2.1.1 Chief Criminalist/Serologist

EXAMPLES OF WORK

- A. Supervises a professional staff of Trace Evidence Analysts and Serologists by interviewing and recommending for hire; training or providing training opportunities; assigning and reviewing work and evaluating the performance of incumbents.
- B. Coordinates section activities by reviewing, prioritizing and assigning new cases; providing assistance to staff in regard to appropriate testing methods and findings; and reviewing selected final reports.
- C. Reviews investigator's summary sheet to become familiar with the details of the crime and examines items such as gunshot residue, fire debris, hair, fiber, body fluids, paint, glass and soils submitted as potential evidence to determine appropriate testing methods.
- D. Designs and conducts a series of analytical tests (including chemistry, chromatography, mass spectography; and transmitted light, stereo and electron microscopy and body fluid identification tests) to try to determine physical and chemical properties of evidence items and identity of evidence items.
- E. Prepares reports of findings and conclusions for submission to legal authorities and courts of law.
- F. Testifies in court as an expert witness on the analysis of evidence and conclusions reached.
- G. Writes articles, presents training and provides consultation to law enforcement officers, prosecutors, defense attorneys and other public officials on crime scene investigation; methods of collecting, transporting and preserving evidence to ensure its integrity and maintenance of the chain of custody.
- H. Researches scientific literature and exchanges information with peers in other states in order to stay abreast of the latest scientific advances in the analysis of criminal evidence and/or determine the best method of testing a particular piece of evidence.
- I. Performs administrative duties by preparing activity reports, inventory reports; maintaining employee history information and equipment maintenance logs; requisitioning supplies and equipment; and researching and recommending policies/procedures.

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J. Conducts on-site crime scene investigations at the request of law enforcement agencies after gaining approval from the Executive Director or the Scientific Operations Director.

K. Performs related responsibilities as required or assigned.

L. May delegate duties as required.

WORKING RELATIONSHIPS

The Crime Laboratory Chief Criminalist/Serologist has regular contact with other laboratory sections, law enforcement officials, attorneys, criminal/civil court personnel, and peers in other states.

SPECIAL JOB DIMENSIONS

The employee will experience frequent exposure to hazardous, toxic, repulsive and/or infectious materials. Occasional in or out-of-state travel and on-call duty are required.

KNOWLEDGE, ABILITIES AND SKILLS

- A. Knowledge of the principles and practices of chemistry, chemical analysis, and forensic analytical methods and techniques.
- B. Knowledge of laws, regulations and agency policies governing trace evidence analysis.
- C. Knowledge of laboratory equipment.
- D. Ability to plan, organize and oversee the work of subordinates.
- E. Ability to conduct and direct the activities of the physical evidence section.
- F. Ability to write descriptive results of analysis and appear as expert witness in court.
- G. Ability to conduct research, prepare and present training on methods of collecting and preserving evidence.

2.1.2 *Forensic Serologists*

EXAMPLES OF WORK

- A. Reviews investigator's summary information to become familiar with the details of the crime and examines items of evidence to determine appropriate testing methods.

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- B. Conducts a series of analytical tests to identify biological fluids and locate areas which may be suitable for DNA testing. Hairs and fibers (tape lifts) may be collected for future analyses.
- C. Prepares reports of findings and conclusions for submission to legal authorities and courts of law.
- D. Testifies in court as an expert witness on the analysis of evidence and conclusions reached.
- E. Writes articles, presents training and provides consultation to law enforcement officers, prosecutors, defense attorneys and other public officials on crime scene investigation; methods of collecting, transporting and preserving evidence to ensure its integrity and maintenance of the chain of custody.
- F. Researches scientific literature and exchanges information with peers in other states in order to stay abreast of the latest scientific advances in the analysis of criminal evidence and/or determine the best method of testing a particular piece of evidence.
- G. Conducts on-site crime scene investigations at the request of law enforcement agencies after gaining approval from the Executive Director or the Scientific Operations Director.
- H. Performs related responsibilities as required or assigned.

WORKING RELATIONSHIPS

The Forensic Serologist has regular contact with other laboratory sections, law enforcement officials, attorneys and peers in other states.

SPECIAL JOB DIMENSIONS

The employee will experience frequent exposure to hazardous, toxic, repulsive, and/or infectious materials. Occasional in or out-of-state travel and on-call duty are required.

KNOWLEDGE, ABILITIES AND SKILLS

- A. Knowledge of the principles and practices of biology and forensic analytical methods and techniques.
- B. Knowledge of laws, regulations and agency policies governing evidence analysis.
- C. Knowledge of laboratory equipment.
- D. Ability to assign and coordinate work activities and monitor the performance of co-workers and/or subordinates.
- E. Ability to conduct forensic analysis of criminal evidence.

- F. Ability to write descriptive results of analysis and appear as expert witness in court.
- G. Ability to conduct research, prepare and present training on methods of collecting and preserving evidence.

2.2 Educational Requirements

2.2.1 Crime Laboratory Chief Criminalist

The position requires the formal education equivalent of a bachelor's degree in chemistry or closely related field; plus two years experience in a forensic laboratory. Other job related education and/or experience may be substituted for all or part of these basic requirements upon approval of the S.O.M.

2.2.2 Forensic Serologist

The position requires the formal education equivalent of a bachelor's degree in biology, chemistry or closely-related field. Other job related education and/or experience may be substituted for all or part of these basic requirements upon approval of the S.O.M.

2.3 Special Training Requirements

Knowledge of principles and practices of biology, chemistry, chemical analysis, forensic analytical methods and techniques are required.

2.3.1 Training Prior to Casework

The analyst must demonstrate the ability to perform specific tests properly before performing independent casework. This is ensured by requiring the analyst to undergo in-house training that must include:

- A. Working with a qualified analyst in the specific area of analysis to be tested. The hours of training will be logged by the trainee and reported to the supervisor. At the completion of this course, the training analyst or supervisor will determine if additional training is needed.
- B. Reading and signing off on assigned literature pertaining to the subject matter. This material is assigned by the training analyst or the supervisor.
- C. Passing a written proficiency examination.
- D. Passing an analytical proficiency test given in the area of analysis to be tested.
- E. Participating in at least one moot court.

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2.3.2 *Continuing Education*

2.3.2.1 Current Literature

Analysts must read peer-reviewed scientific journals as needed. Analysts must be prepared to share pertinent information at staff meetings. Articles may be assigned by the supervisor and analysts may be asked to locate articles.

2.3.2.2 Training Sessions

Each analyst will attend at least one training session every year. This may include structured in-house training in a scientific discipline to which the analyst is assigned or is being trained. In addition, each employee may participate in the Arkansas Inter-Agency Training Program.

2.3.2.3 Documentation of Training

Each analyst will be supplied with a binder. All training certificates, college transcripts, proficiency test results, and reviews of testimony will be kept in the binder. It is the responsibility of the individual analyst to keep the binder up to date.

2.3.2.4 Meetings

Section meetings will be held once a month or as often as deemed necessary by the supervisor.

3 FACILITIES/SECURITY

3.1 Arkansas State Crime Laboratory

The Laboratory facilities and security are described in the laboratory Quality Manual (Section 3).

3.2 Forensic Serology Unit

The Forensic Serology Unit is secured by lockable doors. Keys are only issued to analysts within the section and to administration. Each analyst has a set of lockable drawers and cabinets. Keys to these are issued only to the analyst, to the section supervisor and to administration. The scrape down rooms are secured by lockable doors. Keys are kept in a secure area and obtained by an analyst when needed.

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4 EVIDENCE CONTROL

General guidelines regarding evidence may be found in the laboratory Quality Manual (Section 4).

4.1 Secure Storage

4.1.1 *Temporary storage within the section*

While in the possession of an analyst, evidence must be controlled at all times. This requires that the evidence be observed or secured. If the analyst is to leave the evidence for an extended period of time, it must be stored in a secure area. A secure area must have access limited to those specifically designated by the administration. Access to the Trace Evidence Unit is only through locked doors. In addition, analysts are provided with individual locked cabinets for storage.

4.1.2 *Long-term storage within the section*

Evidence removed from larger items (i.e. tape lifts, cuttings, debris, etc.) is stored in evidence cabinets labeled "PE Secure Storage".

4.2 Evidence Handling

All evidence must be handled in a manner that preserves the integrity and usefulness of the evidence as much as possible. This is discussed more specifically in section 6 of this manual.

In general, the original condition of the evidence should be maintained as much as possible while performing all necessary analyses. Nondestructive techniques are preferred over destructive techniques, given that the results obtained through each method of analysis are equally valid and useful. Where modification of the evidence is necessary, all modifications performed by the analyst should be noted in the case file. If such modifications are an integral part of a standard method of analysis, a notation of the method of analysis used will suffice.

Any substance (swabs, cuttings, hairs, fibers, tape lifts, glass, particles, paint layers, etc.) removed from any item of evidence and retained by the Physical Evidence Unit shall be treated as evidence and must be given a barcode. Photographs are generally considered documentation and will not be treated as evidence.

4.3 Documentation of Evidence and Packaging

Case notes should include:

- The case number assigned by the evidence receiving section.
- The evidence number for each piece of evidence submitted for analysis.

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- A detailed description of packaging, noting seals and identifying marks.
- A description of the contents of each item of evidence.
- Any comments by the analyst concerning the packaging or condition of the evidence or of any variation from routine procedure.

4.4 Release of Evidence

The release of evidence is set forth by the laboratory Quality Manual (Section 4.9).

Upon completion of the analysis, items of evidence may be transferred to the Evidence Receiving Section, to another analyst or may be retained.

Occasionally evidence needs to be sent to another laboratory for analysis. The analyst will transport the item(s) to Evidence Receiving and complete an Inter-Laboratory Evidence Transfer Form (ASCL-FORM-07).

4.5 Disposition

Swabs, cuttings, hair slides, tape lifts and other items necessary to retain will be stored in the cabinets designated for storage.

4.6 Destruction

The Physical Evidence Section does not destroy any evidence. All bulk evidence is returned to the respective law enforcement agency.

Case information from Justice Trax may be printed for court purposes. Upon returning from court the printed records may be destroyed.

4.7 Evidence Assessment

4.7.1 Evidence Assessment by Supervisor

The supervisor or his/her designee will evaluate each case to determine:

- what the law enforcement officer wants/needs with regards to each item of evidence.
- if the ASCL is equipped to perform the requested analysis. If not, the supervisor will assist the officer in location of a laboratory that performs said analysis.
- which analyst(s) will be assigned to the case.

The above may require a documented conversation with the officer for clarification of the analysis needed. The supervisor may require the assigned analyst to make the assessment and plan a course of action.

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4.7.2 *Evidence Assessment by Analyst*

The case information should be reviewed by the analyst and conversations with investigators may be necessary. The Physical Evidence analyst may make a determination as to the probative value of the submitted items.

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5 VALIDATION

The Physical Evidence Section uses procedures that are well established and generally accepted in the field of chemical and/or forensic analysis. In Section 6 of this manual, references are provided to document that the techniques used are based on widely established scientific principles.

Validation is conducted in accordance with the laboratory Quality Manual (Section 5).

After a new procedure has been validated, it should be incorporated into the Trace Evidence Quality Manual.

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6 ANALYTICAL PROCEDURES (SOP)

These methods cover the most commonly encountered evidence types and are meant to serve as guidelines. The analyst has the discretion to choose a procedure(s) that is appropriate for a particular piece of evidence.

GUIDELINES FOR SCREENING FOR BIOLOGICAL STAINS

1. Review the summary provided on the submission sheet. Review officer's report (if submitted) and talk with the detective or attorney if necessary.
2. Document and label all packaging.
3. Determine whether trace evidence materials need to be collected.
4. Lay out a clean piece of paper to examine evidence.
5. Clean scissors and tweezers/forceps with 10% bleach then rinse with water.
6. Open package without destroying other seals and initials if possible.
7. Describe item in case notes.
8. Diagram or photograph item if helpful in creating a record of evidence.
9. Collect trace evidence prior to conducting biological stain examinations, if deemed necessary.
10. Visually examine the item for possible biological material. An alternate light source may be used when no visible seminal stains are noted. Chemically test (phenolphthalein and/or AP) any stains of interest. Document the results in the case notes.
11. Evaluate each possible biological stain to determine the appropriate amount to be consumed. Conserving material for future testing is a priority.

6.1 Collection of Hairs and Fibers

6.1.1 Evidence Assessment by Analyst

- Hairs and fibers should not be collected on items where the victim and suspect are co-habiting. It may be necessary to examine some items (i.e. murder weapon) for a transfer of hairs and/or fibers.
- Determine which items are from the victim, suspect, scene, etc.

6.1.2 Testing Techniques

6.1.2.1 Collection from Sexual Assault Kits

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- Examine the contents of the sexual assault kit to locate the “Pubic Hair Combings” envelope and “Underwear” bag. Also note any extra items that may have been included for hair and fiber examination.
- If samples were not collected according to the information supplied on the package, no further analysis is needed for that item. Record in notes.
- Open the “Pubic Hair Combings” envelope and remove all hairs from the comb, cotton, and/or napkin. Place the hairs in a folded piece of paper or tissue and package in a labeled coin envelope. Return the “Pubic Hair Combings” envelope to the kit.
- Place envelopes and/or tape lift transparency sheets in a manila envelope.

6.1.2.2 Collection from Clothing or Other Items

- Visually examine item and note description of item and fabric content, if listed.
- Take care to preserve evidence that other sections may need to examine (i.e. blood stains, latent prints, etc.). It may be necessary to collect fibers and/or hairs with forceps and place in an envelope or on tape rather than taping the item directly.
- A section of clear adhesive tape is pressed on the item and pulled away. Fibers and/or hairs adhere to the tape which is then placed on a clear transparency sheet. Continue collecting with sections of tape until the entire item has been covered.
- Label the tape lifts on the transparency sheet.
- Known samples of all the fiber types and colors are cut from the item and placed on the transparency sheet with clear tape or in an envelope. White cotton, denim, light-colored fabrics and smooth fabrics (such as nylon windbreakers) are not suitable target fibers.
- Place transparency sheets and/or envelopes in a manila envelope.

6.1.3 Notes/Documentation

- Evidence and packaging should be noted.
- Photographs or photocopies of the items and/or packaging may be taken
- Describe items and include fabric content, if labeled.

6.1.4 Assessment of Results

6.1.4.1 Review of Evidence Collected

- Review evidence which was collected.
- If known samples are present and/or additional work needs to be completed, continue to the appropriate analysis section or turn the case over to the section supervisor for reassignment.
- If known samples were not submitted, write report requesting known samples (if needed).

6.1.4.2 Report Writing Suggestions

- Tape lifts were collected from the items listed. They are being retained.
- Hairs and/or fibers were collected from the items listed. They are being retained.
- If further analysis is desired, the following items are needed: (may request one or more of the following)
 - Head hair sample (40 – 60 pulled hairs) from the victim and any suspects.
 - Pubic hair sample (30 – 40 pulled hairs) from the victim and any suspects.
 - Fiber samples (clothing, carpet, etc.) from any suspects.

6.2 Analytical Procedures - Seminal Fluid Screening

6.2.1 BCIP

bromo-chloro-indolyl phosphate (bcip) test for the presumptive screening of suspected seminal stains

6.2.1.1 Principle

Seminal acid phosphatase is detected in stains of seminal origin through its hydrolysis of the BCIP substrate to an insoluble, stable blue product

6.2.1.2 Materials

1. 5-Bromo-4-chloro-3-indolyl Phosphate (BCIP), toluidine salt
2. Dimethylsulfoxide (DMSO)
3. Sodium Acetate
4. Cotton-tipped applicator swabs
5. Acetic Acid

6.2.1.3 Reagent Preparation

1. Acetate Buffer, 0.01M, pH 5.5. Dissolve 1.36gm sodium acetate in 900ml distilled water (for each liter of buffer to be made) and titrate pH to 5.5 with acetic acid.
2. BCIP Substrate Solution. Dissolve 0.25gm BCIP in a few drops of DMSO (solution will have a slight yellow color). Add 500ml acetate buffer slowly with thorough mixing. Initially the mixture will have a cloudy or milky appearance but will clear when the substrate is dissolved in the full

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500ml of buffer. Store under refrigeration (or frozen if desired - with no loss in potency). Final BCIP concentration: 0.5mg/ml.

If only a 100ml volume of BCIP stock solution is desired, adjust ratios of reagent and solvent components accordingly.

6.2.1.4 Procedure

1. Moisten (do not saturate) swabs with deionized water
2. Stroke a small area of the questioned stain firmly several times with the moistened swab (avoid swabbing the entire stain) and place the swab in a test tube.
3. After all the swabs have been collected, add approximately 200µl BCIP substrate to each tube and incubate in a 37⁰C water bath for 15 minutes.
4. Required Controls (with each run):

Control A: A reagent blank - a moistened swab applied to an unstained cloth and placed directly in a tube.

Control B: A positive control - a moistened swab applied to a known semen stain and placed directly in a tube.
5. After incubation, acid phosphatase activity will be indicated by the development of a blue to aqua-blue color on the swabs.

6.2.1.5 Interpretation of Results

The blue/aqua-blue BCIP hydrolysis product is indicative of acid phosphatase activity.

No color change indicates the absence of acid phosphatase.

6.2.1.6 Trouble Shooting

- a. Saturated swabs will soak the questioned stain and not take an adequate sample for testing.
- b. Inadequate pressure will result in insufficient sample being transferred to the swab.
- c. Allowing the test swabs to stand for too long after stain sampling and prior to reagent addition will allow degradation. A maximum of two hours is allowable

6.3 Extraction of Suspected Semen Stains For Analysis of Soluble and Particulate Seminal Components

6.3.1 Principle

Sperm cells and/or P30 are accepted markers for detecting semen. This is accomplished by microscopic and chemical examination of the extract.

6.3.2 Materials

1. HEPES
2. Pipette
3. 1.5ml microcentrifuge tubes

6.3.3 Materials for HEPES Preparation

1. N-2-hydroxyethylpiperazine-N¹-2-ethanesulfonic acid (HEPES)
2. Sodium Chloride
3. Sodium Hydroxide

6.3.4 Reagent Preparation (HEPES)

HEPES-Buffered Saline (HBS): Dissolve 8.42gm NaCl (0.144M) and 2.38gl HEPES (0.01M) in 1000 ml distilled water and titrate to pH 7.2.

6.3.5 Procedure

The following procedure will provide an extract of the soluble substances and a pellet of the particulate material for analysis.

1. Specimen cuttings of approximately 3-7 mm² (cloth) and approximately ¼ to 1/3 of a cotton tipped applicator are placed in 1.5ml polypropylene tubes. Adjust cutting size to the thickness of the material.
2. Add approximately 250-300 µL buffer (HEPES) to each sample and allow at two hours for extraction at room temperature with occasional agitation. **Do not** vortex (foaming denatures protein material). Extraction may also be accomplished overnight under refrigeration.
3. Centrifuge to maximize the recovery of extract and pellet. Punch small holes in the top of the centrifuge tube. This can be accomplished using a dissecting probe. Place the cutting in the top of the tube and centrifuge. This will allow as much fluid as possible to be extracted from the sample. Centrifuge for approximately three minutes.
4. The 1.5ml tube now contains the supernatant that is ready for Seratec PSA Semiquant or ABACard testing and a pellet or particulate material which may contain sperm cells. See Christmas Tree, Spermatozoa search, P-30 assays, etc.

Note: If test cannot be run immediately, the tube may be refrigerated or frozen overnight.

6.3.6 *Preparation Of Slide From Pellet*

1. Separate pellet from supernatant.
2. Lightly break up pellet with dropper and take up approximately 20 microliters of pellet material. Use dropper to deposit material on microscope slide.
3. Dry slide in oven and proceed with Christmas tree staining procedure.

6.4 Christmas Tree Stain For Spermatozoa

6.4.1 *Reagent Preparation*

Nuclear Fast Red (Kernechtrot Solution)

Aluminum Sulfate	5.0grams
Hot distilled water	100ml
Nuclear Fast Red	0.1grams

Stir, allow to cool and filter.

Picroindigocarmine (PICS)

Saturated, aqueous Picric Acid	300ml
Indigocarmine (concentrations may be reduced if needed)	1.0gram

Dissolve indigocarmine into the picric acid solution

6.4.2 *Staining Procedure*

1. Dried slides are soaked in 100% Ethanol for several minutes
3. Add Nuclear Fast Red stain. Allow to sit approximately 15-20 minutes
- 4 GENTLY wash stain off with distilled water
5. Oven dry slide
6. Add PICS stain and rinse with Ethanol after approximately 15 seconds
7. Oven dry slide
8. Examine slides under the microscope
9. Use Permunt as needed.

6.4.3 *Results*

- (a) Sperm - Anterior Head = light red
- (b) Epithelial cells – Nucleus = light red
- (c) Posterior Head = dark red
- (d) Cytoplasm = light green
- (e) Mid-piece = green

- (f) Tail = green

All positive identification of sperm cells must be confirmed by another qualified analyst.

- All slides prepared by the analyst or provided in the sexual assault kit must contain the laboratory case and item number. If slides do not contain a “frosted” area, a diamond tip applicator may be used.
- When verifying sperm cells, the confirming analyst must make sure that the laboratory case number and corresponding item number on the slide match the laboratory number of the analyst worksheet.

All positive slides made from evidence material will be returned with the evidence.

All negative slides made from evidence will be discarded appropriately.

All medical examiner slides are returned after examination.

6.5 Identification of Semen Using The Seratec PSA Semiquant

6.5.1 Principle

The Seratec PSA Semiquant test is designed to qualitatively detect P-30 for the forensic identification of semen. P-30 is an acceptable marker for detecting semen in criminal cases including vasectomized or azoospermic individuals.

6.5.2 Materials

1. Test Device (Seratec card)
2. Pipette
3. Clock or timer
4. Centrifuge

6.5.3 Procedure

1. Allow the sample to warm to room temperature if it has been refrigerated.
2. Centrifuge sample for approximately 3 minutes.
3. Remove the Seratec card and the dropper from the sealed pouch.
4. Label the Seratec card with the appropriate item number and case number.
5. Add approximately 200 μ l (or 5 drops with the dropper) of the supernatant from the extract to the sample well marked “S”.
6. Read results at 10 minutes.
7. No results are valid past 10 minutes.

6.5.4 Controls

1. Positive Control: a positive control is run when new lot numbers are obtained. All results are recorded in the Quality Control of Critical Reagents Logbook and indicated on the Semen Exam Worksheets.
2. Negative Control: a negative (reagent) control is required daily. The results are recorded on the Semen Exam Worksheet. The positive reaction of a negative control renders the test inconclusive.

6.5.5 Interpretation

1. *Positive Results:* Three pink lines, one in the test area “T”, one in the control area “C”, and one internal standard line, indicate the test result is positive.
2. *Negative Results:* Two pink lines, one in the control area “C”, and one internal standard line, indicate the test result is negative. This may indicate that (a) No detectable p30 is present or (b) Presence of “High Dose Hook Effect” which may give false negative results due to the presence of high concentration of p30 in the sample, as for example in undiluted seminal fluid.
3. *Invalid:* No pink line visible in the control area “C”, or no internal standard line visible, the test is inconclusive.

6.6 Identification of Semen Using The ABACard

6.6.1 Principle

The ABACard P-30 test is designed to qualitatively detect P-30 for the forensic identification of semen. P-30 is an acceptable marker for detecting semen in criminal cases including vasectomized or azoospermic individuals.

6.6.2 Materials

1. Test Device (ABACard)
2. Pipette
3. Clock or timer
4. Centrifuge

6.6.3 Procedure

1. Allow the sample to warm to room temperature if it has been refrigerated.
2. Centrifuge sample for approximately 3 minutes.
3. Remove the ABACard and the dropper from the sealed pouch.
4. Label the ABACard with the appropriate item number and case number.
5. Add approximately 200 µl (or 8 drops with the dropper) of the extract to the sample well marked “S”.
6. Read results at 10 minutes.

7. No results are valid past 10 minutes.

6.6.4 Controls

1. **Positive Control:** a positive control is run when new lot numbers are obtained. All results are recorded in the Quality Control of Critical Reagents Logbook and indicated on the Semen Exam Worksheets.
2. **Negative Control:** a negative (reagent) control is required daily. The results are recorded on the Semen Exam Worksheet. The positive reaction of a negative control renders the test inconclusive.

6.6.5 Interpretation

1. **Positive Results:** Two pink lines, one in the test area "T" and one in the control area "C", indicate the test result is positive.
2. **Negative Results:** Only one pink line in the control area "C", indicates the test result is negative. This may indicate that (a) No detectable p30 is present or (b) Presence of "High Dose Hook Effect" which may give false negative results due to the presence of high concentration of p30 in the sample, as for example in undiluted seminal fluid. In such cases the sample may be retested using a 10 to 10,000 fold dilution.
3. **Invalid:** No pink line visible in the control area "C", indicates the test is inconclusive.

6.7 Identification of Semen Using the Rapid Stain Identification of Human Semen (RSID-Semen) Test

6.7.1 Principle

The RSID-Semen test is designed to qualitatively detect human semenogelin for the forensic identification of semen. Semenogelin is a unique protein specific to seminal fluid, produced by the seminal vesicles, and is responsible for the coagulum associated with ejaculation.

6.7.2 Materials

1. Test Device (RSID-Semen card)
2. RSID-Universal Buffer
3. Pipette
4. Clock or Timer
5. 1.5 mL microcentrifuge tubes

6.7.3 Procedure

1. Specimen cuttings of approximately 3-7 mm² (cloth) and approximately ¼ to 1/3 of a cotton tipped applicator are placed in 1.5 mL polypropylene tubes. Adjust cutting size to thickness of material.
2. Add approximately 200-300 µL of RSID-Universal Buffer to each sample and allow one to two hours for extraction at room temperature.
3. Remove the RSID-Semen cassette from the sealed pouch.
4. Label the cassette with the appropriate item number and case number.
5. Combine extract aliquot (max of 20 µL) with RSID-Universal Buffer to bring test sample to a total volume of 100 µL.
6. Add the test sample to the sample well.
7. Read results at 10 minutes.
8. No results are valid past 10 minutes.

6.7.4 Controls

1. Positive Control: A positive control is run when new lot numbers are obtained. All results are recorded in the Quality Control of Critical Reagents Logbook and indicated on the Semen Exam worksheet.
2. Negative Control: A negative (reagent) control is required daily. The results are recorded on the Semen Exam worksheet. The positive reaction of a negative control renders the test inconclusive.

6.7.5 Interpretation

1. *Positive results:* Two red lines, one in the test area “T”, and one in the control area “C”, indicate the test result is positive.

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2. *Negative results:* Only one red line in the control area “C” indicates the test result is negative. This may indicate (a) no detectable semenogelin is present or (b) “High Dose Hook Effect” presence of which may give false negative results due to the presence of high concentration of semenogelin in the sample containing large amounts of human semen.
3. *Invalid:* No red line visible in the control area “C” indicated the test is inconclusive.

6.8 Alternate Light Source (ALS) Examination

6.8.1 Purpose

The alternate light source (e.g. Omni Print 1000 and Crime-Lite) is a tool to be used to collect trace evidence, make possible semen stains, saliva stains, urine stains, and other body fluids on physical evidence visible. It should not be considered an alternative to the chemical tests.

6.8.2 Method

Examine the evidence in a dark area.

Seminal fluid, saliva, sweat, urine: These stains may fluoresce with the following combination of glasses and wavelengths.

450 nm with the yellow glasses (seminal stains)

530 nm with the red or orange glasses.

485 nm with red or orange glasses

Systematically scan the light over the evidence looking for the stains or other evidence that may be of forensic value.

Mark the stains observed using ALS so that they may be located in normal lighting conditions.

If no stains of interest are visible with the alternate light source, additional search methods (such as targeted swabbings, quadrant swabbings, regularly spaced cuttings, or filter paper presses) in conjunction with the appropriate testing methods (e.g. phenolphthalein, AP testing, etc.) may be required for a thorough examination depending on the evidence, case, and other factors as determined by the analyst.

6.8.3 Cautions

- Semen stains will not always fluoresce; lack of fluorescence does not mean semen is not present. Blood/semen mixtures may not fluoresce.

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- Other non-biological stains such as beverages, food products, and detergents may fluoresce in a similar manner as body fluids and it may be difficult to distinguish stains of forensic interest from background fluorescence.

6.8.4 Safety Hazard

Never look directly into the light or allow beams to bounce off surfaces into your eyes or the eyes of other persons in the vicinity. Goggles should be worn to view evidence.

6.9 Preparation and Use of Phenolphthalein Presumptive Test Reagent

6.9.1 Principle

This oxidation test for the presumptive identification of blood is based on the catalytic activity of the heme group of hemoglobin.

6.9.2 Materials

1. Phenolphthalein
2. Sodium or Potassium Hydroxide
3. Zinc, granular
4. Distilled water
5. Glass boiling beads
6. Ethanol, 95%
7. 3% Hydrogen Peroxide (see Miscellaneous Preparations and Procedures)
8. Cotton applicator swabs.

6.9.3 Reagent Preparation

1. Place 2gm phenolphthalein, 20gm NaOH (or KOH), 20gm zinc granules and 100ml distilled water in a 500ml round bottomed flask. Secure to a reflux apparatus. (This solution is 2% phenolphthalein)
2. Reflux 2 to 4 hours, or until resulting solution is colorless (if rheostat is used, set at about 80) to reduce the phenolphthalein to phenolphthalin.
3. Store stock solution refrigerated over zinc granules in an amber container.
4. The working solution is prepared as a 1:5 dilution with ethanol.
5. Store working solution refrigerated over zinc granules in an amber container when not in use.

6.9.4 Procedure

1. Sample a questioned stain by lightly rubbing with a cotton swab moistened with distilled water.
2. Add a single drop of the phenolphthalin working solution to the swab and observe to detect any oxidative contaminants which may be present.
3. Add a single drop of 3% hydrogen peroxide and observe carefully to detect any pink color which usually develops immediately.

6.9.5 Controls

1. Positive Control: A known blood sample is tested utilizing the method described above. Test is considered positive as indicated in interpretation section below.
2. Negative Control: A negative control is tested utilizing the method described above. A swabbing is taken from a control sample and tested with the above method.

6.9.6 Interpretation

1. Appearance of the distinct pink color on the swab is indicative of the presence of blood. A pink color forming after one minute should not be considered as a positive result, as auto-oxidation can occur in air and light.
2. Prior to use on questioned stains in casework, the working solution should be tested against both positive and negative controls.

6.10 Preparation and Use of Takayama Confirmatory Test Reagent

6.10.1 Principle

Confirmation of the presence of hemoglobin heme in suspected blood stains is accomplished by preparation of the pyridine hemochromogen derivative as microscopic water-insoluble crystals.

6.10.2 Materials

1. Saturate D-glucose solution (See Miscellaneous Preparations and Procedures)
2. Sodium Hydroxide solution, 10% (See Miscellaneous Preparations and Procedures)
3. Pyridine
4. Distilled water

6.10.3 Reagent Preparation

1. In a clean, amber-colored dropper bottle, mix 15 drops saturated D-glucose solution, 15 drops of 10% sodium hydroxide solution, 16 drops of pyridine and 30 drops of distilled water (1:1:1:2).
2. This solution is made as needed and expires 7 days from the date prepared.
3. Reagent reliability is checked daily upon use by running it against a positive control of known blood and negative control of blank cloth or swab.

6.10.4 Procedure

1. A small portion (thread, scraping, cutting, etc) of the suspected stain is placed on a slide and covered with a fragment of a cover slip.
2. The reagent is allowed to flow under the cover slip, covering the questioned material. Avoid excess reagent.
3. The slide is placed on a slide warmer or the oven (70 to 80⁰C for approximately 20 to 30 seconds). The development of a pink color in and around the sample usually accompanies the reaction.

6.10.5 Controls

1. Positive Control: Before using the Takayama reagent, a positive control must be confirmed each day.
2. Negative Control: Before using the Takayama reagent a negative control test is performed each day. Any crystal formation, indicative of Takayama crystals identified in this control renders the interpretation inconclusive.

6.10.6 Interpretation

1. A positive test is indicated by the observation of pink feathery crystals of pyridine ferroprotoporphyrin. The use of 100 x magnification is usually adequate but higher magnification may be helpful in some cases.
2. Formation of the pyridine ferroprotoporphyrin (Takayama crystals) confirm the presence of heme in the stain .
3. Both high magnification and focusing to view through the depth of the sample are often helpful to locate small crystals of those which are hidden within the fibers of a fabric sample.

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4. Extended periods of time may be required after heating to allow for crystal development may also be enhanced by short periods (five to 10 minutes) of refrigeration at 4⁰C or freezing at -20⁰C
5. Crystal morphology may be altered slightly in decomposing blood (plates and/or needles)

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7 INSTRUMENT/EQUIPMENT CALIBRATION MAINTENANCE

- Water Baths – Temperature is checked monthly (37 °C, +/- 5 °)
- Refrigerator/Freezer – Temperature is checked monthly
 - Refrigerator (0-10°C, +/- 5 °)
 - Freezer (-14 to -26 °C)

Appropriate logs will be maintained in the section.

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8 PROFICIENCY TESTING PROGRAM

Proficiency Testing is performed according to the laboratory Quality Manual (Section 8).

Each Analyst must complete at least one proficiency test annually.

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9 CASE RECORDS

9.1 Documentation

The Serology Unit uses the documentation of case records set forth in the laboratory Quality Manual (Section 9).

The date the case is started is recorded in the notes or on the case worksheet. Dates of analysis are documented in the notes or on the documentation generated by the instrument. The ending date for work is considered the date recorded in Justice Trax as “Draft Completed”.

9.2 Release of Information

Information is released in accordance with the laboratory Quality Manual (Section 9.2.2)

9.3 Technical and Administrative Reviews

Reviews are conducted in accordance with the laboratory Quality Manual (Section 9.3)

10 TESTIMONY REVIEW

Testimony reviews will be conducted according to the guidelines in the laboratory Quality Manual (Section 10).

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11 AUDITS

Audits for the section will be conducted according to guidelines in the laboratory Quality Manual (Section 11).

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12 COMPLAINTS

Complaints will be handled in compliance with the overall Quality Manual for the Laboratory (Section 12).

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13 SAFETY

All safety protocol and information is contained in a separate manual. This safety manual covers general laboratory safety.

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