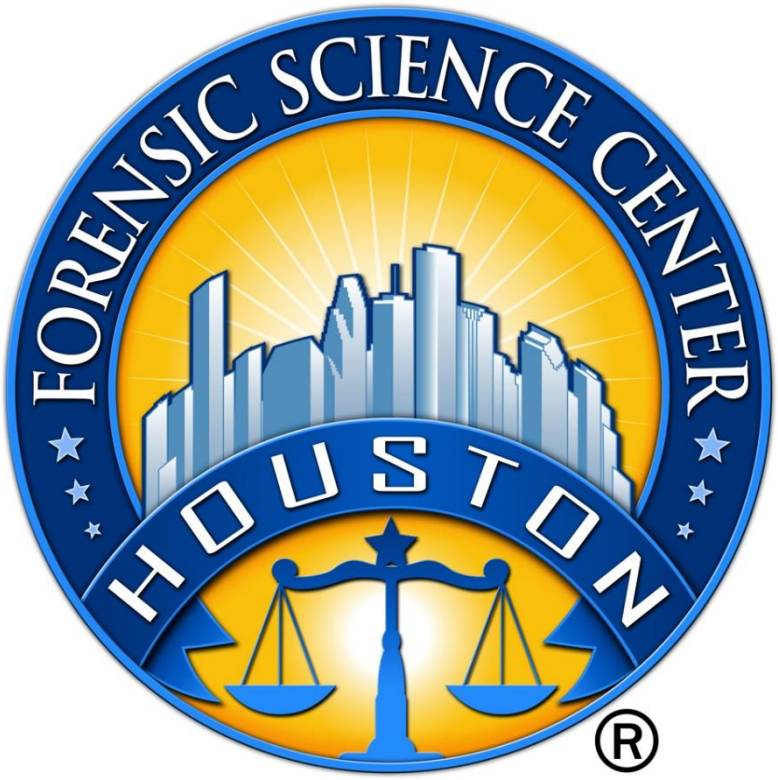

Forensic Biology
Biology SOP Manual

Forensic Biology Division

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1. Overview

1.1. The Biology Standard Operating Procedure (SOP) manual specifies procedures for routine examinations and analyses of biological evidence for human identification. It is approved for use in the Biology section of the laboratory. Within the scope of that purpose, it is intended to ensure effective and efficient use of the laboratory facilities for the benefit of all user agencies. It incorporates the quality assurance elements necessary to ensure the reliability and uniformity of analyses and reported conclusions.

1.2. Each approved revision of this manual is version-controlled and archived for retrieval by effective date. Only the current approved version shall be used for analysis.

1.3. Any deviation from accepted protocol requires approval as outlined in the quality manual and must be documented in the case file.

1.4. This SOP is only one part of the policies and procedures that govern all work performed by the Biology section. The other relevant documents include, but are not limited to, the following:

- Houston Forensic Science Center policies and procedures
- Quality Manual
- Health and Safety Manual
- Biology Training Manual

2. Facilities

2.1. The Biology section is designed to provide adequate space and setup to perform casework analysis. General facility requirements are described in the Quality Manual.

2.2. Work Areas

2.2.1. The Biology section has space for evidence examination. The tasks performed in this area can include: blood and semen fluid identification, swab collections, cutting of stains, portioning of swabs and cuttings, microscopy, and trace evidence collection.

2.3. Contamination

2.3.1. With the facility provided, setup and procedures are designed to minimize the potential for DNA contamination. Traffic in and through areas in which testing occurs shall be minimized.

2.4. Prevention and Decontamination

2.4.1. 10% bleach, or DNA Away where bleach may be harmful to instruments or equipment, shall be used to decontaminate utensils and workbenches. Other commercial decontaminants may be used only if they have been demonstrated to destroy or inactivate DNA.

2.4.2. To help prevent contamination, wear appropriate personal protective equipment (PPE) including disposable gloves, face masks, disposable lab coats, and hair coverings during all testing and reagent preparation (face masks and hair coverings are optional in post-amplification). Refer to the HFSC Health and Safety Manual for additional safety-related PPE requirements. Safety eye wear must be used when handling biological material that may pose the threat of splashing, such as liquid blood. Change gloves frequently and whenever gloves may have become contaminated. Discard gloves when leaving a work area, except when transporting samples or reagents. Centrifuge all liquid to the bottom of closed microcentrifuge tubes before opening. A de-capper or a clean Kimwipe may be used for opening microcentrifuge tubes. Use sterile, disposable pipet tips and microcentrifuge tubes. Use aerosol-resistant pipet tips while working with any sample that may be subsequently amplified. Change pipet tips between samples. Set up reagents and tools in work space in such a way that used tips do not cross over or near stock reagents or clean tubes/wells.

2.4.3. Clean work surfaces in the evidence examination areas thoroughly with a lab-approved decontaminant prior to and at the end of each evidence examination session. Use disposable bench paper whenever possible and change each time it comes in contact with evidence. Use a clean cutting surface such as weighing paper or piece of paper for evidence. Protect supplies of this paper from dust and other particulates or aerosols. Clean instruments (e.g., scalpels, scissors, forceps) with decontaminant between evidence samples. To prevent contamination of other standards or evidence, handle each piece of evidence one at a time. Evidence items shall be handled prior to reference/known samples. Liquid samples, such as blood standards, and wet exhibits shall be shall be air-dried in a way that prevents them from coming into contact with other evidence.

2.4.4. Wear gloves when cleaning glassware and plastics. In general, clean glassware/plastics with an appropriate soap, e.g., Liquinox or Alconox, and water. Rinse with deionized or distilled water and allow to air-dry inverted. Glassware should be autoclaved. Store glassware and plastic ware after being autoclaved or being cleaned by UV light.

2.5. Safety

2.5.1. There are biological and chemical hazards in the laboratory. Each lab employee is responsible for familiarity with the Health and Safety Manual. Any incident or condition that occurs in or under the control of the laboratory and that threatens the immediate or future health of any individual must be immediately brought to the attention of the section supervisor and a laboratory safety officer. Corrective action related to safety incidents will be defined by laboratory management.

3. Equipment Quality Control and Maintenance

3.1. Maintenance and Calibration

3.1.1. To provide and maintain the quality of the work conducted by the Biology Division, it is necessary to ensure laboratory equipment is in good working order. Routine quality control and maintenance accomplishes this. The calibration intervals listed below are generally considered to be the minimum appropriate in each case, provided that the equipment is of good quality and of proven stability and the laboratory has both the equipment capability and expertise to perform adequate internal checks. More frequent checks are acceptable. If there is any question concerning the reliability of an instrument or piece of equipment, a maintenance check must be performed immediately.

3.1.2. Full maintenance, calibration records, and repair activities shall be maintained and recorded in an equipment calibration and maintenance log. This log includes at a minimum: the date, activity, laboratory personnel performing or overseeing the activity, non-crime laboratory technician(s) performing or overseeing the activity, and a record of quality control checks performed to verify operation prior to returning a piece of equipment to casework use. The section supervisor and Quality Division are responsible for ensuring all systems are checked and maintained as required. Whenever practical, equipment that requires calibration shall be labeled with the date when last calibrated and the date or expiration criteria when recalibration is due.

3.1.3. Critical equipment is any piece of equipment that must be maintained in a proper working order to ensure the reliability of results produced. Some critical equipment and associated maintenance requirements can be found in the Quality Manual. Additional equipment and maintenance requirements are included here.

3.2. Equipment

3.2.1. **Ultrasonic Cleaner**

3.2.1.1. Ultrasonic cleaners use ultrasonic energy in the form of sound waves to create a mechanical “scrubbing” action to loosen debris from all surfaces that the solution touches.

3.2.1.2. This equipment’s condition is routinely maintained for the Biology section’s procedures. The water shall be clear and clean with no evidence of bacterial/fungal growth or rust. The following procedure should be performed as needed: Wash the inside of the ultrasonic cleaner with detergent and rinse well with water. Fill bath with the appropriate quantity of diH_2O water.

3.2.2. **Mini-centrifuges**

3.2.2.1. Mini-centrifuges are bench top, unrefrigerated centrifuges that have been designed for **quick spin-downs from tube walls and caps** of tubes. These mini-centrifuges are equipped with a circular, one sized rotor. The maximum speed is 6000 rpm. The relative centrifugal force can be determined as outlined in the manufacturer’s instructions, if required.

3.2.2.2. These centrifuges are primarily used for removing liquid from the cap of a microcentrifuge tube. They shall not be used for pelleting cellular material.

3.2.3. **Thermometers**

3.2.3.1. All thermometers must be either calibrated or their accuracy must be verified to ensure the temperature is being accurately measured.

3.2.3.2. NIST-traceable thermometers must be purchased or re-certified at least once every two years. A NIST-traceable thermometer certified for two years and used for conducting performance checks on equipment shall be performance checked yearly. A NIST-traceable thermometer certified for two years that is not used for conducting performance checks does not require the annual performance check and may be used until the certification expires. A NIST-traceable thermometer used beyond its certification date shall be recertified or be subject to the annual performance check requirements.

3.2.3.3. The minimum requirements of a performance check of a thermometer used for performing performance checks may be accomplished: (1) through certification by an outside vendor; or (2) in-house by the comparison of one or more temperature readings at various time intervals against another NIST-traceable thermometer.

3.2.3.4. Thermometers that are not NIST-traceable must be verified annually by comparison against a NIST-traceable thermometer. Any deviation from the NIST-traceable thermometer must be noted and all other readings adjusted by the same amount, and in the same direction for each thermometer. If the deviation is greater than 3°C, the thermometer must be serviced or replaced.

3.2.3.5. Handle thermometers carefully to avoid breakage. Thermometers may be wiped clean to facilitate easy and accurate readings. If any discontinuities are detected, repair or replace the thermometer.

3.2.3.6. The Temp@lert system may also be utilized for recording temperatures. Please refer to the Quality Manual for further information.

3.2.4. **Autoclaves**

3.2.4.1. Autoclaves are used to sterilize solutions, glassware, and instruments by subjecting them to high pressure and high heat simultaneously. Autoclaves also may be used to sterilize biohazard trash prior to discarding. Please consult the instructions on the outside of the unit for specific information on operation of the autoclave.

3.2.4.2. Whenever an item is autoclaved, a small piece of autoclave tape must be adhered to the item to verify correct functioning of the autoclave. Use proper sterilizer-loading procedures when placing materials in sterilizer chamber. Those include: all solid containers/instruments must be placed so that water or air is not trapped in them and sufficient space must be left between items to allow steam circulation. The temperature gauge must read 120^0C at the start and during the appropriate cycle.

3.2.4.3. If the autoclave is not functioning properly, a qualified service technician may repair the instrument.

3.2.5. **Alternate Light Source (ALS) Machines**

3.2.5.1. ALS machines are used to presumptively identify semen and other body fluids in forensic casework. The fan air filter, optical filters, and lenses shall be cleaned on a regular basis (approximately every 6 months), where applicable. Please consult the appropriate equipment manuals for more specific instructions on maintenance and operation of the ALS machines.

3.2.5.2. The LSV2 Leeds Spectral Vision system can be cleaned with a bleach and water solution or laboratory detergent cleaners. To clean the surface of the LSV2, wipe with a lint free, soft cloth slightly moistened with a diluted neutral detergent, for example, diluted bleach, surfactant.

3.2.5.2.1. *Important to note*: Always spray cleaning solution on a cloth, then clean components. Never spray bleach solution directly on the LSV2.

3.2.5.2.2. Parts that can be wiped down with this solution are as follows:

3.2.5.2.2.1. LSV2 Head Cover only

3.2.5.2.2.2. Touch Screen

3.2.5.2.2.3. Keyboard Cover

3.2.5.2.2.4. LSV2 Arm

3.2.5.2.2.5. LSV2 Base

3.2.5.2.3. DO NOT USE bleach/water solution on the following parts:

3.2.5.2.3.1. Inside LSV2 head

3.2.5.2.3.2. Inside vent holes

3.2.5.3. Leeds Forensic Systems, Inc. should be contacted for additional equipment maintenance.

3.1.1. **Swab Drying Box E025**

3.1.1.1. Swab drying boxes are used to dry swabs created in the lab during forensic casework. Before and after each use, the box must be wiped inside and out with a 10% bleach solution or other lab-approved decontaminant and dried with a soft cloth. Do not autoclave, immerse, or abrade the unit.

3.1.2. **Refrigerators and Freezers**

3.1.2.1. Reagents and evidence should be stored separately, either in separate cooling units or in separate space within those units. Maintenance will be performed on these units as needed.

3.1.3. **Pipettes**

3.1.3.1. Volumetric pipettes are used to accurately and precisely measure the volume of a solution to be delivered. The accuracy of the measurement is dependent upon the correct technique of the user as well as the proper maintenance and performance of the pipette. The pipette shall be able to provide accuracy, precision, repeatability and reproducibility over the full range of the pipette's capabilities. The Biology section is supplied with pipettes that cover the volume range from 0.1 - 1000 µl. Calibration shall be performed at least annually on these instruments. When a pipette is determined to be performing improperly, it will be returned to the

manufacturer, or another qualified repair technician, so that the problem may be identified and corrected.

3.1.3.2. Quality control data includes the type of pipette, volume range, model, and serial number.

3.1.3.3. **Procedure**

3.1.3.3.1. Set the volume of the pipette using the knob in the pipetting push-button.

3.1.3.3.2. Firmly attach the appropriate tip to the pipette.

3.1.3.3.3. Press the push-button to the first positive stop.

3.1.3.3.4. Holding the pipette vertically, immerse the tip into the sample liquid.

3.1.3.3.5. Release the push-button slowly and smoothly to aspirate the sample.

3.1.3.3.6. Pausing for a moment, withdraw the tip from the liquid.

3.1.3.3.7. Depending on the sample, it may be necessary to pre-rinse the tip before aspirating the sample.

3.1.3.3.8. To dispense the sample, place the tip into the transfer vessel and press the push-button to the first stop.

3.1.3.3.9. Pausing for a moment, press the push-button to the second stop to 'blowout' or expel any remaining liquid.

3.1.3.3.10. Slowly release the push-button.

3.1.3.3.11. Eject the tip by pressing the ejector button.

3.1.3.4. **Performance Verification/Maintenance**

3.1.3.4.1. Each pipette shall be externally calibrated and certified by an approved calibration vendor at least annually.

3.1.3.4.2. Internal pipette verifications should be performed about 6 months after the external calibration. If external calibrations occur more often than once every 6 months, the internal pipette verifications are not necessary.

3.1.3.4.3. Pipette performance verification must be documented.

3.1.3.4.4. To check for accuracy, room temperature deionized water shall be pipetted into a weighing vessel on an analytical balance.

3.1.3.4.5. The pipette shall be checked using 3-points over the full range with 5 replicates at each volume.

3.1.3.4.6. Each pipette shall fall within the verification tolerance ranges listed in Appendix A before it may be used for casework.

3.1.3.4.7. If a pipette fails a performance verification check, it shall be recalibrated. Calibrations are not performed in-house.

3.1.3.4.8. If an analyst believes that a pipette is not working properly they must:

3.1.3.4.8.1. Perform a pipette verification and if the pipette is not in proper working order:

3.1.3.4.8.2. Clearly mark the pipette "OUT OF SERVICE".

3.1.3.4.8.3. Inform the Technical Leader. No laboratory case work can be performed using the pipette until the problem is corrected.

3.1.3.4.8.4. Repair or send out the pipette for repairs

3.1.3.4.8.5. Verify that the pipette is working correctly and falls with-in the proper tolerances following routine repair, maintenance, or calibration.

3.1.3.4.8.6. Maintain the appropriate documentation and update the log book.

3.1.4. **Water Filtration System**

3.1.4.1. Water is used in reagents that are prepared in the laboratory. Therefore, it is necessary to ensure only high quality, reagent grade water is being utilized. Generally, 15MΩ-cm or greater deionized water is sufficient for the reagents utilized in our laboratory.

3.1.4.2. Water quality shall be checked on a weekly basis. Utilize the following procedure:

3.1.4.2.1. Turn on the deionized water.

3.1.4.2.2. Allow water to run for 1-2 minutes.

3.1.4.2.3. Record both the MΩ-cm reading and the temperature reading displayed on the resistivity monitor in the Water Quality logbook.

3.1.4.2.4. If reading is below 15MΩ-cm, call for service. A qualified service technician may repair the system. Label the system as being "out of service" until the appropriate service is performed and the filtration system is functioning properly.

3.1.5. **Balances**

3.1.5.1. Refer to the quality manual for information.

4. Quality Assurance

4.1. To provide and maintain the quality of the work conducted by the Biology section, it is necessary to identify certain reagents as critical. Critical reagents are those that require testing prior to use on evidentiary samples to prevent unnecessary loss of sample. All reagent preparations and quality control testing must be documented on the appropriate worksheet or log.

4.2. If any critical reagent does not pass all quality control checks, it cannot be utilized in casework. All inconsistencies shall be documented and reported to the Section Supervisor. Problems that cannot be resolved must be reported to the manufacturer.

4.3. Reagents and supplies that have passed their expiration dates may not be used on casework samples. Outdated reagents may be used for training purposes only, but must be clearly marked as such.

4.4. Chemical and Reagent Labels

4.4.1. Purchased chemicals and reagents shall be marked on the container with the date received and/or date opened. In general, follow the manufacturer's labeling to determine expiration dates of purchased chemicals and reagents. If no manufacturer expiration exists for a purchased reagent, it shall be considered expired 5 years from the date received and that 5-year expiration date from receipt shall be marked on the container.

4.4.2. Prepared reagent labels shall include the reagent name, initials of preparer, and expiration date. In general, most solutions prepared in the Biology section shall expire 1 year from the date of preparation. However, the expiration date of the overall reagent shall be no later than the expiration date of the individual reagent with the nearest expiration date (unless otherwise specified). Additional information may be documented in a reagent log. The lot number contains the date prepared and the initials of the preparer, e.g. mmddyyinitials.

4.5. Quality Control Checks

4.5.1. The following shall be tested with a positive and negative control to confirm that they are working properly each day used:

- Acid Phosphatase Spot Test
- Alternate Light Source
- Phenolphthalein test kit

4.5.2. The following shall be quality control checked to confirm they are working properly with each new lot received:

- ABAcard® HemaTrace® kits
- Phosphate Buffered Saline (1X)
- SERI Christmas Tree Stain
- SERATEC® PSA Semiquant test devices

4.5.3. **Acid Phosphatase (AP) Spot Test**

4.5.3.1. **Scope**

4.5.3.1.1. AP Spot Test solution undergoes a color change (Brentamine reaction) in the presence of acid phosphatase, which is found in highest concentration in semen. Instructions for use and interpretation are in the AP Spot Test procedure.

4.5.3.2. **Safety**

4.5.3.2.1. This reagent presents the following hazards: may cause cancer; irritant to eyes, respiratory system and skin; and corrosive (can cause burns). Wear gloves, lab coat, and mask during preparation and use. Broken skin must be covered.

4.5.3.3. **Equipment and Supplies**

- AP Spot Test premixed powder – purchased
- Darkened or foil wrapped container for storage
- Scale
- Sterile diH2O
- Timer
- Known semen sample
- Known non-semen sample

4.5.3.4. **Preparation**

4.5.3.4.1. Measure 10mL of sterile diH_2O in an appropriate container.

4.5.3.4.2. Add 0.26g of the purchased AP Spot Test premixed powder.

4.5.3.4.3. Mix until AP Spot Test premixed powder is completely dissolved in water.

- Note: Volume may be smaller or larger than 10mL, but the amount of AP Spot Test premixed powder added must be adjusted accordingly.

4.5.3.4.4. Prior to use each day, the prepared AP solution must be subjected to the appropriate in-house quality control test as outlined below:

4.5.3.4.4.1. A known semen sample shall be used as a positive control. This in-house-prepared sample is typically presented on filter paper.

4.5.3.4.4.2. An untreated piece of filter paper or swab moistened with sterile diH_2O is used as a negative control. Ideally, the negative control substrate mimics your sample substrate as closely as possible.

4.5.3.4.4.3. Apply ~ 1 drop of prepared AP solution to both controls.

4.5.3.4.4.4. Observe samples for 60 seconds for a color change.

4.5.3.4.4.5. The known semen sample must give a strong purple color change indicating a positive result.

4.5.3.4.4.6. The untreated filter paper or sterile swab must have no color change indicating a negative result.

- The results of each control test must be recorded on every applicable serology worksheet.

4.5.3.5. **Storage, Labeling, and Expiration**

4.5.3.5.1. Store prepared solution in a darkened or foil wrapped container. Label with reagent name, lot number, and expiration date. Reconstituted reagent is stable and sensitive for one day's use – make solution fresh daily. Store

purchased powder frozen. Purchased powder may be used on casework samples up to and including the expiration date stated on the bottle.

4.5.4. **Alternate Light Source**

4.5.4.1. **Scope**

4.5.4.1.1. An Alternate Light Source is specially designed for detection of forensic stains, fibers, and fingerprints. Instructions for use and interpretation are in the Alternate Light Source procedure.

4.5.4.2. **Safety**

4.5.4.2.1. This instrument presents the following hazards: exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. It is essential that proper eye protection is used. Avoid looking directly at the light source, reflected, or refracted light. Remove all unnecessary reflective surfaces from exam room. Wear goggles, gloves, lab coat, and other proper laboratory attire.

4.5.4.2.2. Mini-Crimescope MCS-400 ALS (Crimescope): If the bulb fails (suddenly stops emitting light), it may leak vapors for a few minutes. To be safe, immediately leave the room and let the unit run (fans) for 5-10 minutes until vapors are vented.

4.5.4.3. **Equipment and Supplies**

- Omnichrome OMNIPRINT 1000 Alternate Light Source set at 450 nm
- Mini-CrimeScope MCS-400 Alternate Light Source set at 455 nm
- Orange and/or yellow glasses
- Leeds Spectral Vision System (LSV2)
- Protective goggles
- Known semen sample
- Known non-semen sample

4.5.4.4. Prior to use each day, the ALS must be subjected to the appropriate in-house quality control test as outlined below:

4.5.4.4.1. Turn on power switch and ensure the fans are working.

4.5.4.4.2. Wear the proper glasses.

4.5.4.4.3. Turn on lamp switch.

4.5.4.4.4. Darken the examination room.

4.5.4.4.5. Direct the ALS at the samples.

4.5.4.4.6. The known semen sample must fluoresce indicating a positive result.

4.5.4.4.7. The known non-semen sample must not fluoresce indicating a negative result.

- When ready to shut down the ALS, turn off lamp switch and wait for the unit to cool down (~5 minutes). Then turn off the power switch.
- The results of each control test must be recorded on every applicable serology worksheet.

4.5.4.5. **Storage and Expiration**

4.5.4.5.1. Store equipment in a clean, dry atmosphere. The bulb should be replaced after 3-3.5 years of non-regular usage.

4.5.5. **Phenolphthalein Test Kit**

4.5.5.1. **Scope**

4.5.5.1.1. PHT Test Kit Solutions B and C are used for presumptive blood identification by oxidizing phenolphthalin (colorless in a basic solution such as the test reagent) to phenolphthalein (pink) in the presence of heme and hydrogen peroxide. Instructions for use and interpretation are in the phenolphthalein test kit procedure.

4.5.5.2. **Safety**

4.5.5.2.1. This reagent presents the following hazards:

4.5.5.2.2. Solution B: Flammable. Handle with care. Harmful or fatal if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Light sensitive! Avoid light, heat, sparks, and flame. Keep container tightly closed.

4.5.5.2.3. Solution C: Handle with care. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Avoid heat, sparks, and flame. Keep container tightly closed.

4.5.5.2.4. Wear gloves, lab coat, and mask during preparation and use. Broken skin must be covered.

4.5.5.3. **Equipment and Supplies**

- Solution B
- Solution C
- Sterile diH_2O
- Sterile cotton swab(s)
- Known blood sample
- Known non-blood sample

4.5.5.4. Prior to use each day, PHT Solutions B and C must be subjected to the appropriate in-house quality control test as outlined below:

4.5.5.4.1. Rub a cotton swab moistened with sterile diH_2O on a known bloodstain sample. Use this swab as a positive control.

4.5.5.4.2. Use an untreated cotton swab moistened with sterile diH_2O as a negative control.

4.5.5.4.3. Apply ~1 drop of Solution B to each swab.

4.5.5.4.4. Apply ~1 drop of Solution C to each swab.

4.5.5.4.5. Observe the swabs for a color change.

4.5.5.4.6. The cotton swab with the known bloodstain sample must give an immediate pink color change indicating a positive result.

4.5.5.4.7. The untreated cotton swab must have no color change indicating a negative result.

- The results of each control test must be recorded on every applicable serology worksheet.

4.5.5.5. **Storage, Labeling, and Expiration**

4.5.5.5.1. PHT test kits can be stored at room temperature. Each analyst should have an aliquot of Solutions B and C that shall be placed in amber bottles stored at room temperature or refrigerated. Each bottle shall be labeled with appropriate reagent name, lot numbers, and expiration dates. The kits may

be used on casework samples up to and including the expiration date stated on the bottles.

4.5.6. **ABAcard® HemaTrace® Test Devices**

4.5.6.1. **Scope**

4.5.6.1.1. ABAcard® HemaTrace® test kits are used to determine the presence of human blood in suspected blood stains. Instructions for use and interpretation are in the ABAcard® HemaTrace® procedure.

4.5.6.2. **Safety**

4.5.6.2.1. Wear gloves, lab coat, mask, and other proper laboratory attire. Broken skin must be covered.

4.5.6.3. **Equipment and Supplies**

- ABAcard® HemaTrace® test device(s)
- ABAcard® HemaTrace® extraction buffer
- Pipette and pipette tips
- Known blood sample
- Timer

4.5.6.4. Each new lot of ABAcard® HemaTrace® test kits must be subjected to the appropriate in-house quality control test as outlined below:

4.5.6.4.1. Make a $1{:}10^6$ dilution from a known human blood sample in extraction buffer provided in kit.

4.5.6.4.2. Add 150µl of dilution to sample well "S" of one test device to be used as a positive control.

4.5.6.4.3. Add 150 µl of extraction buffer from kit to sample well of second test device to be used as a negative control.

4.5.6.4.4. Read results up to 10 minutes from application of sample.

4.5.6.4.5. The $1{:}10^6$ dilution of known human blood must give a positive result. The extraction buffer provided in the kit must give a negative result.

4.5.6.5. **Storage, Labeling, and Expiration**

4.5.6.5.1. The test devices must be stored at room temperature (below 82ºF) and must remain in the sealed pouch until use. Label each test device with the name of the sample used. The test kits may be used on casework samples up to and including the expiration date stated on the box.

4.5.7. **Phosphate Buffered Saline (1X)**

4.5.7.1. **Scope**

4.5.7.1.1. PBS is used as an extraction agent for serological stains. Instructions for use and interpretation are in the Phosphate Buffered Saline procedure.

4.5.7.2. **Safety**

4.5.7.2.1. This reagent presents the following hazards: causes severe eye, respiratory tract and skin irritation. Avoid contact with skin and clothing. Wear gloves, lab coat, and other proper laboratory attire. Broken skin must be covered.

4.5.7.3. **Equipment and Supplies**

- OmniPur 10X PBS Premixed Powder
- diH_2O
- Graduated cylinder
- Two sterile containers for storage

4.5.7.4. **Preparation (10X)**

4.5.7.4.1. Measure 1 liter of diH_2O.

4.5.7.4.2. Add 100g of 10X PBS Premixed Powder.

4.5.7.4.3. Mix until all the powder is dissolved.

4.5.7.4.4. Autoclave solution.

4.5.7.5. **Preparation (1X)**

4.5.7.5.1. Measure 100mL of 10X PBS solution.

4.5.7.5.2. Measure 900 mL of sterile diH_2O.

4.5.7.5.3. Mix 10X PBS and sterile diH_2O.

- Each new lot of 1X PBS must be subjected to an appropriate in-house quality control test with the lot of SERATEC® PSA Semiquant test currently in use. See SERATEC® PSA Semiquant test quality control procedure for further detail.

4.5.7.6. **Storage, Labeling, and Expiration**

4.5.7.6.1. 1X and 10X PBS solutions can be stored at room temperature. Label each bottle with appropriate concentration, lot number and expiration date. Expiration for both PBS concentrations is one year from the date 10X PBS solution was prepared. Both concentrations of PBS may be used on casework samples up to this expiration date.

4.5.8. **SERI Christmas Tree Stain**

4.5.8.1. **Scope**

4.5.8.1.1. SERI Christmas Tree Stain is a Kernechtrot-Picroindigocarmine differential biological stain that assists in scanning slides for the presence of spermatozoa. The test consists of a two stain method which dyes sperm heads red, tails green and epithelium green or blue with red nuclei. Instructions for use and interpretation are in the SERI Christmas Tree Stain procedure.

4.5.8.2. **Safety**

4.5.8.2.1. This reagent presents the following hazards: May cause irritation upon contact. Toxic. Do not swallow. Do not inhale. Avoid contact with skin and eyes. Wear gloves and proper laboratory attire. Broken skin must be covered.

4.5.8.3. **Equipment and Supplies**

- Microscope slide
- Known semen sample
- Pipette and pipette tips
- Heat block
- Christmas Tree Stain - Solution A and Solution B
- Timer
- Tap water
- 95% ethanol

- Compound microscope

4.5.8.4. Each new lot of SERI Christmas Tree Stain must be subjected to the appropriate in-house quality control test as outlined below:

4.5.8.4.1. Pipette 10 µl of a neat in-house sample onto a glass slide. Fix smear by heating the slide on the heat block until dry.

4.5.8.4.2. Stain the slide.

4.5.8.4.2.1. Cover the sample area on the slide with Solution A for 10 minutes.

4.5.8.4.2.2. Wash with tap water by gentle flooding.

4.5.8.4.2.3. Cover the sample area with Solution B for 15 seconds.

4.5.8.4.2.4. Wash with tap water by gentle flooding.

4.5.8.4.2.5. Flood the slide with 95% ethanol and allow the slide to dry for at least 5 minutes on the heat block.

4.5.8.4.2.6. Examine the slide for the presence of spermatozoa at 1000X magnification. Sperm heads must be stained red and any observed tails must be stained green, indicating a positive result.

4.5.8.5. **Storage and Expiration**

4.5.8.5.1. The stain must be stored at room temperature. The stain may be used on casework samples up to and including the expiration date stated on the bottles.

4.5.9. **SERATEC® PSA Semiquant Test Devices**

4.5.9.1. **Scope**

4.5.9.1.1. SERATEC® PSA Semiquant test devices are used to indicate the possible presence of p30 in suspected semen stains. Instructions for use and interpretation are in SERATEC® PSA Semiquant test procedure.

4.5.9.2. **Safety**

4.5.9.2.1. Wear gloves, lab coat, mask, and other proper laboratory attire. Broken skin must be covered.

4.5.9.3. **Equipment and Supplies**

- SERATEC® PSA Semiquant test device(s)
- 1X PBS
- Pipette and pipette tips
- Known semen sample
- Timer

4.5.9.4. Each new lot of SERATEC® PSA Semiquant Test devices must be subjected to the appropriate in-house quality control test as outlined below:

4.5.9.4.1. Make a 1:10^5 dilution from a known semen sample in 1X PBS.

4.5.9.4.2. Add 120 µl of dilution to sample well of one test device to be used as a positive control.

4.5.9.4.3. Add 120 µl of 1X PBS to sample well of second test device to be used as a negative control.

4.5.9.4.4. Read results up to 10 minutes from application of sample.

4.5.9.4.5. The 1:10^5 dilution of known semen must give a positive result. The 1X PBS must give a negative result.

4.5.9.4.6. Mark the stock solution bottle of 1X PBS with all lot numbers of SERATEC® PSA Semiquant test devices quality control checked with that lot of 1X PBS.

4.5.9.5. **Storage, Labeling, and Expiration**

4.5.9.5.1. The test devices must be stored at room temperature and must remain in the sealed pouch until use. Label each test device with the name of the sample used. The test devices may be used on casework samples up to and including the expiration date stated on the sealed pouch.

4.6. Logbook

4.6.1. Reagent preparation logs shall include the lot number, the reagent name, the initials of preparer, and the expiration date. Quality control logs for ABAcard® HemaTrace® kits, Phosphate Buffered Saline (1X), SERI Christmas Tree Stain, and SERATEC® PSA Semiquant tests also contain the test date, signature of the analyst performing the quality control, a second reader signature if applicable, and date and any supporting documentation necessary to demonstrate the reagent passed all the tests.

4.7. Proficiency Testing

4.7.1. Proficiency testing and review follows the requirements of the quality manual. In addition, the Quality Division maintains a copy of the analysis documentation for each proficiency test. Proficiency tests are analyzed and interpreted according to standard operating procedures including technical review. Proficiency test participants must be notified of their final test results.

4.7.2. Analysts shall enter a proficiency test program within 6 months of being deemed competent on any portion of casework analysis. Proficiency testing shall include each technology to the full extent to which analysts and technicians participate in casework.

4.7.3. Proficiency work is to follow as closely as possible that of normal casework. If symbols are used in the reporting of data to the proficiency testing agency, they must be defined in the results submission form. It is also advisable to include comments in the comments section of the proficiency results form to explain certain results. For example, if a result is reported as inconclusive, the reason shall be presented in the comments section. When multiple tests for a particular fluid such as semen are conducted, and are not fully concordant, each of the tests and its respective result will be included in the comments section of the proficiency test results form. Analysts will continue to report proficiency test results as closely as possible to casework, but it should be noted that the flexibility permitted in reporting language (e.g., presumptively positive but no confirmatory testing conducted) does not generally exist within the proficiency test reporting options (e.g., positive, negative, inconclusive or not tested).

4.7.4. During the case file reviews, the proficiency results form (including the screening data and comments sections), along with the case file, shall be reviewed by the technical/administrative reviewers to ensure proper transcription of results by the author of the results form.

4.7.5. If performing quantification for proficiency samples, the analyst must create his/her own DNA standards.

4.7.6. Only one proficiency results form shall be completed for proficiency tests to ensure that only the most complete information is submitted to the proficiency testing provider in order to be included in the provider's published external summary report when submitting by fax, mail, or electronically. The screening analyst shall complete the proficiency test results form if the test does not proceed to DNA analysis; the DNA analyst shall complete the proficiency test results form if the test does proceed to DNA analysis.

5. Evidence Evaluation and Handling

5.1. The Biology section provides body fluid identification (semen and blood) and performs the collection and preservation of trace evidence and possible contact DNA evidence.

5.2. Case Acceptance and Evaluation

5.2.1. Refer to the Quality Manual or Evidence Handbook for procedures regarding the submission of evidence into the laboratory. Before a case is worked, the case and the requested examinations shall be evaluated. The examiner must be aware of the requested examinations, and when possible, the reason(s) for the requested analyses and the quality and quantity of the evidence.

5.2.2. To expedite casework, except for sexual assault kits, it is recommended that 5-10 items of evidence are initially screened for cases containing large volumes of evidence. Emphasis should be placed on those items believed to be of the most significant evidentiary value after consultation with the submitting party. Of the items screened, it is recommended that a maximum of 5 items of evidence continue on to DNA analysis initially. Additional items may be analyzed at a later time. Individual case needs may dictate that the initial quantity of examined items is greater than the recommended range presented above.

5.2.3. When submitted in conjunction with a sexual assault kit, clothing evidence does not typically have to be examined if the kit components yield sufficiently positive samples. ("Sufficiently positive" is relevant to the case information.) Clothing should be examined if the corresponding sexual assault evidence kit does not reveal any positive probative evidence, if there are multiple suspects, or there is a recent consensual sexual partner. Good judgment using the case information and/or the client's specific requests should always supersede this guideline.

5.3. Evidence Handling

5.3.1. It is not possible to anticipate every situation that may arise or to prescribe a specific course of action for every case; therefore, the examiner must exercise good judgment based on experience and common sense. In some cases, the manual offers guidelines for analysis that must be tempered with the experience of the examiner. However, any portion of a procedure not explicitly qualified as a guideline may not be modified for use in casework without prior written approval as outlined in the Quality Manual.

5.3.2. On a sexual assault case, for example, the analyst has a choice of using conventional serology for the detection of semen or quantitative PCR for the detection of male DNA. The evidence type may warrant one method over another. It may be more informative to detect actual spermatozoa on a non-intimate garment that may have been collected from a common area than to detect male DNA; alternatively, the detection of male DNA via quantitative PCR on an intimate swab may be more probative to a particular case.

5.3.3. When ample sample permits, the analyst should consider retesting when "inconclusive" results are obtained.

5.3.4. Please also refer to the Quality Manual for the Handling of Evidence.

5.4. Universal Precautions

5.4.1. Body fluids and extracts may contain infective agents. All analysts must routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when in contact with any body fluids. Gloves must be worn and changed after contact with each piece of evidence. Masks, lab coats, and protective eyewear shall be worn during procedures that anticipate contact with body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes. All analysts must take precautions to prevent injuries caused by scalpels or other sharp instruments or devices during procedures, when cleaning, or during disposal. After sharp instruments are used, they shall be placed in a puncture-resistant container for disposal.

5.5. Evidence Requiring Examination or Analysis from Other Forensic Sections

5.5.1. **Evidence Requiring Latent Print Examination**

5.5.1.1. Submitting parties may request that items be analyzed for latent prints as well as biological material. In general, biological evidence examinations precede any latent print examination. Appropriate steps must be taken, including consultation with the submitting party and/or a latent prints examiner, to ensure the appropriate course of action for the evidence presented. In some cases, it may be best if one type of examination is chosen over another. If an analyst finds a distinct print during examination, appropriate steps shall be taken to ensure the print is not destroyed and to inform a latent print examiner.

5.5.2. **Evidence Requiring Toxicology Analysis**

5.5.2.1. The Toxicology Section routinely reserves the second blood tube, when present, in case independent ethanol analysis is requested in a case. Because ethanol is a volatile, opening a tube can decrease the ethanol concentration. Therefore, Forensic Biology analysts may not open or process any blood tubes that may require toxicology analysis without first having acquired written instruction from a Toxicology analyst or the Toxicology Section Manager indicating which blood tube can be used by Forensic Biology. If the only reference material in a Forensic Biology case is whole blood, the Forensic Biology analyst must first verify that there is not an open Toxicology request before portioning the whole blood. If there is an open Toxicology request, the Forensic Biology analyst must consult with Toxicology personnel prior to opening any blood vials within that case. If the tube has already been opened by Toxicology, it becomes less critical for Forensic Biology to open it again. The important thing is to have Toxicology open the first tube first and to keep the second tube intact.

5.6. Storage of Evidence

5.6.1. Biological evidence must be properly stored to preserve biochemicals assayed in body fluid identifications and DNA typing for current and future analyses. Storage conditions for all types of evidence present must be considered so that the evidence is not compromised. In addition to the storage requirements detailed in the Quality Manual, the following procedures shall be followed:

5.6.2. Sexual assault kits may be stored in a cooler, freezer, or dry area at room temperature once received in the laboratory. After analyses, sexual assault kits may be stored at room temperature. Sexual assault kits that contain blood or urine specimens are ideally stored in a refrigerator.

5.6.3. Wet evidence must be dried upon receipt. Refrigerate or freeze liquid whole blood specimens until a sample is dried on an appropriate substrate; be careful when frozen blood has thawed, as it may expand and cause vial breakage. Portions of blood standards submitted in liquid form should be dried on stain cards within 30 days of receipt by the assigned analyst. Once the sample is in dried form, the liquid blood tube is placed back in its original packaging and returned to the submitting agency.

5.6.4. Cases containing small, dry items may be stored at room temperature or frozen depending on available space. Larger items such as clothing, bedding, weapons, and other physical evidence containing bloodstains shall be stored in a dry area at room temperature until examination.

5.7. Consumption of Evidence

5.7.1. The evidence's quality and quantity should be preserved as much as possible without sacrificing the quality of the analyses. Good judgment must be exercised to determine the smallest amount of sample that should be consumed for analysis that still provides accurate results. Whenever possible, at least half of the evidence sample should be preserved for possible re-analysis (for both additional screening and additional DNA analysis). When this is not possible, consumption of the evidence may be necessary. Refer to the quality manual for policy and procedures on consumption of evidence. All evidentiary hairs are treated individually and cannot proceed to DNA analysis without consumption permission.

5.8. Chain of Custody

5.8.1. Refer to the Quality Manual for chain of custody policies and procedures.

5.9. Reference Samples

5.9.1. Reference samples should be requested from suspects, complainants, witnesses, consensual sex partners, and/or other individuals for the purposes of elimination. Oral swabs are the preferred reference sample for the laboratory, but blood is certainly a viable source of reference material. Anecdotally, Sexual Assault Nurse Examiner (SANE) protocol dictates that evidentiary oral swabs are not only collected well before reference/known saliva swabs, but also after oral rinsing to help prevent possible foreign DNA from contaminating a reference/known saliva sample.

5.10. Potential for Contact DNA

5.10.1. Should contact DNA potentially be probative in a case, it is permissible for the examiner to bypass certain procedures that could possibly remove contact DNA that may be present. For example, an examiner may choose to skip the presumptive Acid Phosphatase press-out test and simply portion for the confirmatory microscopy examination to preserve potential contact DNA, if both penile and digital penetrations are

alleged. Examiners may also choose to skip serological analysis entirely to maximize the available DNA. Case records must clearly document when and why a routine procedure such as the acid phosphatase or phenolphthalein press-out test is not performed.

6. Alternate Light Source

6.1. Please refer to Section #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines of the Alternate Light Source.

6.2. Alternate light sources provide a non-destructive method for the visualization of body fluids and guide the analyst to focus on specific areas of an item of evidence for biological evidence collection and testing.

6.2.1. Certain molecules, such as those found in semen, saliva, and gunshot residue will fluoresce when exposed to certain wavelengths of light. These molecules absorb light at a specific wavelength and undergo electron excitation, emitting light back at a lower wavelength as their electrons return to the normal state. The difference between the excitation wavelength and the emitted wavelength is called the Stokes shift, named after George G. Stokes, who described the phenomenon of fluorescence.

6.2.2. Some dried semen stains on cloth are detectable visually because their color, off-white or yellow, is different from that of the material on which the semen has been deposited. However, on some substrates, semen stains are not readily visible. Under intense blue light (~450 nm), semen stains typically fluoresce. Blood absorbs light and does not fluoresce; fibers and other bodily fluids can typically fluoresce. Fluorescence only suggests the presence of semen; the test is therefore a presumptive test.

6.3. Omnichrome OMNIPRINT 1000 Alternate Light Source and Mini-CrimeScope MCS-400 Alternate Light Source

6.3.1 Scope

6.3.1.1 This laboratory uses the Omnichrome and Mini-CrimeScope lamps for detection of forensic semen stains. These lamps use a hand-held wand to provide intense light of specific wavelengths.

6.3.2 Safety

6.3.2.1 These instruments present the following hazards: exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. It is essential that proper eye protection is used. Avoid looking directly at the light source, reflected, or refracted light. Remove all unnecessary reflective surfaces from the exam room. Wear goggles, gloves, lab coat, and other proper laboratory attire.

6.3.3 Equipment, Materials, and Reagents

- Alternate light source (ALS) set at 450nm, 455 nm, or CSS (“Crime Scene Search”, which contains all wavelengths from 390-540 nm, but centered around 455 nm).
- Orange and/or yellow glasses

6.3.4 Procedure

6.3.4.1 Turn on power switch and ensure the fans are working.
6.3.4.2 Wear the proper glasses and darken the examination room.
6.3.4.3 Turn on the lamp switch.
6.3.4.4 Direct the ALS at the evidence samples.

6.3.4.5 Document areas of apparent fluorescence on the evidence.

- When ready to shut down the machine, turn off the lamp switch and wait for the unit to cool down (~5 minutes). Then turn off the power switch.

6.3.5 Interpretation

6.3.5.1 Fluorescence only suggests the presence of semen.

6.3.5.2 Substrate controls may be included in body fluid identification tests as appropriate.

6.3.5.3 It is possible that a blood stain may mask the ALS result of semen. Therefore any blood stains on items suspected of containing semen should also be subjected to acid phosphatase testing.

6.4. Leeds Spectral Vision System (LSV2) Alternate Light Source

6.4.1 Scope

6.4.1.1 The LSV2 Leeds Spectral Vision system is designed for evidence imaging and use as an Alternate Light Source tool.

6.4.1.2 The LSV2 takes advantage of the Stokes shift in order to provide high-contrast fluorescent images through the use of 5 filter barrier filters loaded into cassettes.

6.4.1.3 The LSV2 is primarily a fluorescence imaging device offering the LSV2 operating analyst two types of detection methods, **positive detection** and **negative detection**. Both methods of fluorescent imaging aim to maximize the fluorescence contrast between the sample or particulate and the background in which it rests.

6.4.1.3.1 In **positive detection**, the criminalist actively seeks to excite the sample or particulate and image the resulting light generated by the samples' Stoke shift.

6.4.1.3.2 In **negative detection**, the criminalist actively seeks to excite or image the background at exclusion of the sample and image the resulting light generated by the background's Stoke shift.

6.4.1.4 Semen, Saliva, Urine, and Sweat Detection

6.4.1.4.1 Bodily fluids (in this case defined as semen, saliva, urine, and sweat) will generally fluoresce when **excited with wavelengths of light from 300nm to 480nm**. The idea is to find the right excitation and barrier filter combination, to maximize the contrast of the suspected fluorescent bodily fluid and the background in which it rests. Based on this, listed below are general settings for imaging.

Bodily Fluid	Excitation	Barrier Filter	Fluorescence
Semen	455 nm	570 nm	Impressive and broad
Sweat	455 nm	550 nm	More subtle signal
Saliva	455 nm	570 nm	Relatively faint

6.4.1.5 Blood Detection

6.4.1.5.1 Blood is a bodily fluid that, unlike other bodily fluids previously mentioned, does not emit innate fluorescence usable for forensics. Instead of causing the blood to fluoresce and capturing the resulting emitted energy, it is much easier to negatively detect blood.

6.4.1.5.1.1 **Near IR Imaging - 850nm excitation** (near IR) with the **830nm Barrier filter**.

6.4.1.5.1.1.1 This takes advantage of blood's near IR absorptive properties. This light/filter setting combination allows a user to search for blood stains very effectively on dark/black fabrics. If the substrate that the blood is on absorbs IR light less effectively than hemoglobin, then blood stains will appear as dark stains on a lighter fabric.

6.4.1.5.1.2 **Violet Imaging – 405nm Excitation** (violet) with the **400nm (or clear) barrier filter**.

6.4.1.5.1.2.1 This technique takes advantage of blood's well defined 415nm adsorption peak. Similar to IR imaging of blood, if there is a significant difference between a fabric's violet light absorption efficiency, when compared to the hemoglobin's, blood stains will appear as dark stains on a lighter fabric.

6.4.1.6 Fluorescence or lack thereof only suggests the presence of semen, saliva, urine, sweat, or blood; the test is therefore a presumptive test.

6.4.2 **Safety**

6.4.2.1 Moving the LSV2 requires a minimum of two people.

6.4.2.2 LSV2 Head should never be removed unless instructed to do so by Leeds' personnel. Doing so will void warranty. Adhere to all precautions presented in the Leeds Spectral Vision System Instruction Manual, Revision 11/2015 or more current version.

6.4.2.3 When using ultraviolet (UV) light settings with the LSV2, take reasonable precautions to prevent UV exposure to eyes and skin. Leeds provides complimentary protective goggles rated to filter UV illumination up to 400 nm, protecting the retina against long-term exposure to high energy wavelengths. The LSV2, when used with UV illumination setting, emits a peak of 365 nm UV LED. Wear UV protective goggles, provided by Leeds, as well as lab coats and gloves to protect eyes and exposed skin from UV exposure.

6.4.3 **Equipment, Materials, and Reagents**

- Leeds Spectral Vision System (LSV2)
- Protective goggles

6.4.4 **Procedure**

6.4.4.1 Turn on the computer by pressing the power button as well as the green power switch.

6.4.4.2 From LSV2 High Definition Monitor, double click on the Leeds Vision Software Icon.
6.4.4.3 From Main Menu, select “Window”, then “Remote Window” to ensure image on remote touch screen is on.
6.4.4.4 Confirm Remote Touch Screen is on.
6.4.4.5 LSV2 System Set-Up
6.4.4.5.1 LSV2 head includes a level to adjust the position of the head. The built-in level is located on the right side of head handle.
6.4.4.5.2 Adjust multi-wavelength LED lights by holding onto the LSV2 head handle with one hand and adjusting each light individually.
6.4.4.5.3 Once the LSV2 arm is in the desired position, adjust the tension handle to hold the arm in place using LSV2 Arm Tension Adjustment handle.
6.4.4.6 Position the LSV2 Head
6.4.4.6.1 The LSV2 Head can be adjusted in three ways - Pitch, Yaw and Roll (or rotate).
6.4.4.6.1.1 Pitch
6.4.4.6.1.1.1 To adjust the pitch, locate the silver Pitch Home Locking Pin on the left side of the LSV2 head. Pull out the pin and rotate 90° to release head and allow for adjustments.
6.4.4.6.1.1.2 Adjust LSV2 head to desired position. Then lock the head into place using the LSV2 Head Positioning Lock handle, turning clockwise to tighten, and counter clockwise to loosen.
6.4.4.6.1.2 Yaw
6.4.4.6.1.2.1 To adjust the yaw, locate the Yaw Lock on right side of LSV2 head and loosen lock to allow LSV2 head to move side to side.
6.4.4.6.1.2.2 Adjust LSV2 head yaw left-to-right as desired.
6.4.4.6.1.2.3 Once LSV2 head is in desired position, tighten Yaw tension handle to keep head in position.
6.4.4.6.1.3 Roll (Or Rotate)
6.4.4.6.1.3.1 To adjust the LSV2 head Roll, locate the silver Roll Home Locking Pin located at the back of the black LSV2 head frame. Pull out the pin and rotate 90° to release the head and allow for adjustments.
6.4.4.6.1.3.2 Adjust LSV2 head to desired position. Lock the head into place using the LSV2 Head Positioning Lock handle, turning clockwise to tighten and counter clockwise to loosen.
6.4.4.7 The **LSV2 Controls/Metadata** panel position can be customized by navigating to “**Window**” and then choosing either “**Panels on Left”**, “**Panels on Right**”, or “**Hide Panels”** from the pulldown window. The Remote Window on the Touch Screen can be opened or closed from this window as well. In addition, the Live Image can be turned off and on by navigating to LiveImage, then selecting the desired live images for the Main Window and the Remote Window.
6.4.4.8 Main Window and File Save/Export
6.4.4.8.1 The main window is used to display both live and captured images. Images are managed through a tab system as is seen in the image below. Images

can be saved into a Leeds LVI file format or exported into standard image formats through the main window by clicking:

6.4.4.8.1.1 File, Save As

6.4.4.8.1.2 File, Export Raw or Annotated Images

6.4.4.8.2 Saving and Exporting Files

6.4.4.8.2.1 Unsaved images are marked with an asterisk " * ". Tabs can be selected by left clicking with mouse. Tabs can be closed by clicking on the "x".

6.4.4.8.2.2 To **save** an image in LVI file format, navigate to **File -> Save As**… (**Hot Key CTRL-S**) and name appropriately.

6.4.4.8.2.3 Image Metadata settings in LVI file formats can be quickly applied to the LSV2's hardware by clicking the "**Apply Settings**" button on the **Image Data** panel on captured images. **Presets** can be made from previously captured images.

6.4.4.8.2.4 To **export unannotated images** into standard formats such as Tiff, jpeg, BMP, and PNG, navigate to **File -> Export Raw Image**… and name appropriately (Figure 4) A metadata text file extension "(imagename).bmp.meta.xml" will be generated with the same name in the folder as the exported image.

6.4.4.8.2.5 To export images with annotations and measurements into standard image formats **File -> Export Annotated Image**

6.4.4.9 Camera and Hardware Control Panel

6.4.4.9.1 The Camera and Hardware Control Panel is used to control the camera, lens, presets, lights, and filters of the LSV2. This panel can be located on either the right or the left side of the image viewing window.

6.4.4.9.2 The Camera and Hardware Control Panel includes the controls for Image Capture, Overlay, Camera Control, Lens Control, Presets and Light Control.

6.4.4.10 Annotation and Measurement Toolbar

6.4.4.10.1 The annotation and measurement toolbar provides shortcuts to commonly used tools for image analysis and comparison, as follows:

Name	**Function**
Crosshair	Insert a crosshair, defining a point of interest, into the image.
Line	Insert a line between two points.
Text	Insert a text window.
Circle - Three Point	Insert a circle, based on three identified points on the circles circumference.
Circle – Two Point	Insert a circle, based on the center of the circle and the radius.
Polygon	Insert a polygon with a user defined number of points marking the outside perimeter.
Angle – Three Point	Insert an angle based on a point on one line, definition of the vertex, and a point on the second line.
Angle – Four Point	Identifies angle measurement using 4 points (2 points per line).
Calibration Line	Insert a line of known distance. This will calibrate all other measurements in the image as well. The line length must be defined and applied for calibrated measurements.
Magnify	Digital zoom on the image.
Link Images	Links two compared images so that when the field of view of one image is changed (i.e.

Unlink Images	increased zoom or change to display a different region of interest) that other image will change in the same way. In addition, if any annotation/drawing/positional magnification is added to one side of the comparison image, these markings will automatically appear on the other comparison image.

6.4.4.11 Camera

6.4.4.11.1 The Camera tab controls the LSV2's camera. There are two specific modes available – Manual exposure and Auto exposure.

6.4.4.11.2 **Manual exposure** gives direct control of the LSV2's exposure (or shutter time) settings to the user. The exposure can be adjusted by: clicking and dragging the slider, by clicking through a series of steps "–"and "+ "or by using the mouse wheel to change exposure settings one step at a time. Each step constitutes 1 micro second of exposure time. This step is similar for all Control panels in software.

6.4.4.11.2.1 **The longer the exposure the slower the systems frame rate (frames per second).** To compensate for this, gain control can be implemented. Gain increases sensitivity of the camera through electronic amplification. The **"Gain"** slider can be used in combination with the exposure slider to improve frame rate for screening of evidence. Higher gain settings can allow shorter exposure times to be used which in turn allows higher frame rates. ***Note: At high gain values, digital noise will be noticeable.***

6.4.4.11.3 **Auto Exposure** – Allows the LSV2's software to do the work of establishing the optimum exposure setting balanced by gain settings. The user controls a targeted intensity by using a user controlled **"Brightness"** slider.

6.4.4.11.3.1 When **"Auto Exposure"** mode is selected, the **"Brightness"** slider replaces the Shutter Time slider. The brightness slider functions very much the same as the shutter time slider allowing the user to indirectly set exposure settings with the system attempting to optimize the exposure depending on the brightness sliders position.

6.4.4.11.3.2 When the gain slider is used in auto exposure mode, the software will automatically reduce the exposure time to maintain the same light intensity, or brightness, target set by the user via the brightness slider. This gives the user the ability to quickly change between high frame rate screenings to taking very high quality images simply by adjusting the gain slider.

6.4.4.11.3.3 The **"White Balance"** button performs a whole image-area white balance. Each time the button is clicked the system averages a white balance. To set a white balance, the user can set a white target under the LSV2 head and click white balance a couple times until the desired white balance is reached.

6.4.4.11.3.4 Alternatively, the white balance can be set manually via **Tools -> Camera Settings**… and adjusting the **"Red"** and **"Blue"** White balance

sliders (scale of 0-255). A green slider is displayed in the absence of red and blue.

6.4.4.12 Lens

6.4.4.12.1 The Lens Tab allows the user to directly control the Focus, Aperture, Zoom, and Diopter (close up lens) settings. While the focus and zoom may be self-explanatory, the aperture and diopter settings are more involved.

6.4.4.12.1.1 The Aperture slider allows the user to control how much light is entering through the rear focal plane of the zoom optic. A fully wide open aperture (26500) lets the most light through and has the highest resolving power (higher Numerical Aperture) and light sensitivity. A closed aperture has higher contrast, lower resolving power (lower Numerical Aperture), and, importantly, provides a greater depth of focus.

6.4.4.12.1.2 The LSV2 employs three chromatically and flat field correct diopters or close up lenses to have the widest range of parfocal magnifications and working distances available. The four diopter selections are +3.5, +2, +1, and no diopter. Mousing over the diopter icons will display their respective optimum working distances.

6.4.4.12.1.2.1 The highest power diopter, the +3.5 diopter, allows the LSV2 to have a working distance of ~7.1 - 9 inches when selected.

6.4.4.12.1.2.2 The medium range diopter, the +2 diopter, gives the LSV2 a working distance of ~11.7 - 17.7 inches.

6.4.4.12.1.2.3 The lowest power diopter, the +1 diopter, presents a working distance of ~18.7 - 37.5 inches.

6.4.4.12.1.2.4 With No diopters engaged the LSV2 has a parfocal zoom with a working distance of ~39 inches to infinity.

6.4.4.12.1.2.5 *NOTE: Because a fluorescence intensity is strongly reduced as the distance increases between the sample and LSV2 imaging head, higher diopter settings (which allow closer working distances) will greatly increase the detection of faint fluorescent evidence.*

6.4.4.13 Presets

6.4.4.13.1 The Presets Tab - Leeds Vision Software, allows the user to save “Preset” options. Presets can be made from a Live Image or from a previously captured image. To Save a Preset:

6.4.4.13.1.1 Click “Save” under Preset menu.

6.4.4.13.1.2 Enter Preset “Label” in New Preset text window.

6.4.4.13.1.3 Select Options you prefer to save with your preset from the options list: Shutter Time, Light, Filter, Focus, Aperture, Zoom, Diopter, Gain, and White Balance

6.4.4.13.1.4 Click “OK” to save Preset.

6.4.4.14 Light

6.4.4.14.1 The Light tab - The LSV2 incorporates both a motorized LED based Alternative Light Source (ALS) and motorized filter cassette for excitation and filtration of fluorescent evidence.

6.4.4.14.2 For filtration of emitted light the LSV2 utilizes a 5-position barrier filter cartridge system.

6.4.4.14.3 The filter load-out can be exchanged for custom filters by removing the cartridge cover and replacing the cartridge with a new cartridge loaded with desired filters. As with the ALS system, specific labeling of each barrier filter can be edited via navigating to **Tools -> Filters** and entering the desired new label.

6.4.4.15 Comparison Images

6.4.4.15.1 Comparison Images – The LSV2 can take captured images and producing direct side-by-side comparison images. To perform a side-side-comparison of two captured images:

6.4.4.15.1.1 Select the captured image to be compared to.

6.4.4.15.1.2 Navigate to **Tools -> Compare To** to open the comparison selector tool.

6.4.4.15.1.3 In the comparison selector tool's dialogue choose the other image to be compared with. This will set up a side-by-side comparison between the two captured images with each side retaining its own respective applicable metadata and annotations.

6.4.4.15.1.4 The Link next to the Digital Zoom icon, when selected, will link both comparison images together for enhanced digital comparisons. Metadata displayed will be for the actively selected image in the comparison.

6.4.4.15.1.5 When the "Link" button is selected, drawing any annotation or measurement on one side of the image will simultaneously draw the same annotation or measurement on the comparison image in the same location.

6.4.4.15.1.6 When the "Link" button is selected, zooming in on one image simultaneously zooms in on the comparison image.

6.4.4.16 Turn power off to the base daily.

6.4.4.17 For troubleshooting, refer to the Leeds Spectral Vision System Instruction Manual, Revision 11/2015.

6.5 Interpretation

6.5.1.1 Fluorescence only suggests the presence of semen.

6.5.1.1.1 An item or stain shall be reported as positive when fluorescence is observed in any of the recommended filter combinations.

6.5.1.1.1.1 Positive results shall be documented with a printout of the image from at least one of the filter combinations.

6.5.1.1.2 An item or stain shall be reported as negative once all filter combinations have been examined and no fluorescence is observed.

6.5.1.2 Substrate controls may be included in body fluid identification tests as appropriate.

6.5.1.3 It is possible that a blood stain may mask the ALS result of semen, therefore any blood stains on items suspected of containing semen should also be subjected to acid phosphatase testing.

6.5.1.4 Recommendations:

Bodily Fluid	Recommended Light/Filter Combinations
Semen	Blue/orange, blue/dark orange, violet/dark orange, violet/orange, green/dark orange
Saliva	Blue/orange, blue/dark orange
Urine	Blue/orange, blue/dark orange, green/dark orange
Blood	IR, violet/orange, violet/clear, violet/dark orange

Fabrics	Comments
Denim	Absorbs light, fibers can fluoresce; stains tend to diffuse a lot, presumably due to large surface area
Polyester	During the validation, hot pink polyester fluoresced, causing the semen stain to show as a dark spot

6.6 References

6.6.1 Validation of the Leeds Spectral Vision System (LSV2, SN 486137) for the Detection of Bodily Fluids, prepared by Alicia Rairden and Tran Nguyen, Houston Forensic Science Center, September 22, 2016.

6.6.2 Performance Check of the Leeds Spectral Vision System (LSV2, SN 486945) for the Detection of Bodily Fluids, prepared by Alicia Rairden and Tran Nguyen, Houston Forensic Science Center, December 30, 2016.

6.6.3 Leeds Spectral Vision System Instruction Manual, Revision 11/2015.

6.6.4 Leeds Spectral Vision System Imaging Guide, Revision 10/2015.

6.6.5 Leeds Vision Software Guide Version 4.0, Revision 12/2014.

7 Blood Detection

7.1 Please refer to Section #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines of the Phenolphthalein Test Kit, and ABAcard® HemaTrace® Test Devices.

7.2 Many samples submitted to the Laboratory are tested for the presence of blood. Generally, a sample should be subjected to a presumptive phenolphthalein test. If positive, the sample may be subjected to a confirmatory test for the presence of human blood. Testing is typically discontinued on phenolphthalein negative samples.

7.3 Phenolphthalein Test Kit

7.3.1 Scope

7.3.1.1 Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. The test is exceedingly sensitive to minute traces of hemoglobin and its derivatives but can produce a false positive reaction in the presence of oxidizing substances. A color reaction only indicates the possible presence of blood; the test is therefore a presumptive test.

7.3.2 Safety

7.3.2.1 This reagent presents the following hazards:

7.3.2.1.1 Solution B: Flammable. Handle with care. Harmful or fatal if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Light sensitive! Avoid light, heat, sparks, and flame. Keep container tightly closed.

7.3.2.1.2 Solution C: Handle with care. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Avoid heat, sparks, and flame. Keep container tightly closed.

7.3.2.1.3 Wear gloves, lab coat, and mask during preparation and use. Broken skin must be covered.

7.3.3 Equipment, Materials, and Reagents

- Sterile diH_2O
- Sterile cotton swab(s) or filter paper
- Phenolphthalein Forensic Test reagents* - Solutions B and C

* Solution A is also included in the Phenolphthalein Forensic Test. However previous testing showed that using sterile diH_2O in place of Solution A did not affect the outcome of this test. Therefore, sterile diH_2O shall be used in place of Solution A.

7.3.4 Procedure

7.3.4.1 Moisten a sterile cotton swab or piece of filter paper with sterile diH_2O**.**

7.3.4.2 Rub or press down on the suspected bloodstain with a sterile cotton swab or filter paper to allow for any possible blood to transfer.

7.3.4.2.1 The application of sterile diH_2O is unnecessary if a portion of the suspected blood stain is tested directly.

7.3.4.3 Apply approximately 1 drop of Solution B to the swab, filter paper, or portion of sample being tested.

7.3.4.4 Observe briefly to identify color change. A green color is often observed when blood is present; any other color change must be documented.

7.3.4.5 Add approximately 1 drop of Solution C to the swab, filter paper, or portion of sample being tested.

7.3.4.6 Observe the swab or filter paper for an immediate pink color change indicating a positive reaction.

7.3.4.7 After interpretation, mark the items/areas tested with the results.

7.3.4.8 Discard the swab or filter paper by placing it in the biohazard trash.

7.3.5 Interpretation

7.3.5.1 Appearance of an immediate pink color change after the application of Solution C is a presumptive positive result for blood. No color change indicates a presumptive negative result for blood. A negative result indicates that blood is absent or below the detection threshold.

7.3.5.2 Substrate controls may be included in body fluid identification tests as appropriate.

7.4 ABAcard® HemaTrace® Test Devices

7.4.1 Scope

7.4.1.1 The ABAcard® HemaTrace® test is a qualitative detection method specifically designed for forensic identification of human blood. Heme is a part of the hemoglobin molecule that is characteristic of red blood cells found in blood. The ABAcard® HemaTrace® has been shown to detect as little as 0.05 µg/ml of hemoglobin in 10 minutes.

7.4.1.2 The sample is added to the sample well where any detectable human hemoglobin (hHb) present in the sample binds with mobile monoclonal antihuman Hb antibody which has an attached pink dye particle. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where an immobile polyclonal antihuman Hb antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody sandwich. Conjugated pink dye particles become visible in the area of immobilized antibody (in the test area: "T") when the hHb concentration in the sample exceeds 0.05 µg/ml. The resultant pink band indicates a positive result. As an internal positive control, hHb antibody-dye conjugates cannot bind to the antibody in the test area "T", but

are captured by an immobilized anti immunoglobulin antibody present in the control area "C" forming a complex. The captured pink dye particles form a band in the control area "C", indicating that the test has worked properly.

7.4.1.3 Hemoglobin from the Family Mustelidae (ferrets) and higher primates also gives a positive result. The examiner must evaluate the likelihood of blood from either of these sources being present when this test is used as, forensically, the practical implications of this cross reactivity is minimal.

7.4.1.4 The ABAcard® HemaTrace® test may be skipped if, in the analyst's opinion, consumption for human origin testing reduces the potential success of subsequent DNA analysis. Samples that are of limited size may be documented as Quantity Not Sufficient for Further Analysis ("QNS"). Only a presumptive screening result is reported but DNA analysis may be performed.

7.4.2 Equipment, Materials, and Reagents

- ABAcard® HemaTrace® Test device – one per sample
- ABAcard® HemaTrace® extraction buffer
- Pipettes and pipette tips
- Timer

7.4.3 Procedure

7.4.3.1 Allow the sample(s) to warm to room temperature if they have been refrigerated.

7.4.3.2 Extract body fluid by placing suspected bloodstain sample into the extraction buffer provided with the kit and allow to sit for 5-30 minutes. Aged samples may be less soluble and should be soaked closer to 30 minutes. The extract(s) may be placed at room temperature in an ultrasonic cleaner for 5-15 minutes. The analyst must exercise good judgment regarding the amount of sample to be portioned for each ABAcard® HemaTrace® test device, providing sufficient sample for subsequent DNA analysis. Stain intensity and size are a few characteristics that can be considered. Generally, up to approximately ¼ of a swab or up to approximately $(1\ cm)^2$ of a stain may be consumed for this analysis.

7.4.3.3 For each sample, unwrap and label an ABAcard® HemaTrace® test device.

7.4.3.4 Add approximately 150 µl of supernatant to the sample well "S" of the device.

7.4.3.5 Results may be read up to 10 minutes from application of the supernatant. Positive results can be seen as early as 2 minutes.

7.4.3.6 Record the ABAcard® HemaTrace® test results on the Serology Results worksheet.

7.4.3.7 Have a second qualified analyst examine the ABAcard® HemaTrace® test device and verify the documented test result(s) by initialing and dating the appropriate Serology Results worksheet.

7.4.4 Interpretation

7.4.4.1 There are two lines that appear on the test device:

7.4.4.1.1 **Control "C" line:** the presence of this line confirms the integrity of the test components and the test procedure. It is expected for all tests and must be

present. Absence of the "C" line is an invalid result and the test must be repeated if adequate sample remains.

7.4.4.1.2 **Test "T" line:** A line indicates the presence of human hemoglobin and shall be documented as a positive result. The absence of a line or a line that appears after 10 minutes is a negative result. A negative result indicates that blood is absent or below the detection threshold.

- Substrate controls may be included in body fluid identification tests as appropriate.
- Note: the presence of the "High Dose Hook Effect" may give false negative results due to the presence of a high concentration of human hemoglobin in the sample. When huge amounts of human Hb bind to the antibody to form an antigen-antibody complex but also free Hb migrates towards the test area "T", the antibody in the test area "T" is blocked by this free Hb. As a result, the mobile antigen-antibody complex with the pink color cannot bind to the antibody and the pink line cannot form in the test area "T", even though a lot of Hb is actually present. In such cases where human blood is strongly suspected, the extract may be retested using a 1:10 or 1:100-fold dilution.

7.5 References

7.5.1 Abacus Diagnostics, Inc. ABAcard® HemaTrace® For the Forensic Identification of Human Blood Technical Information Sheet.

7.5.2 Gaensslen, Robert E. 1983. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* U.S. Department of Justice, National Institute of Justice. Sections 10.3.2 and 10.3.3.

7.5.3 Lee, Henry C. 1982. Identification and grouping of bloodstains. *In*: Forensic Science Handbook, Volume 1. Richard Saferstein, ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 273.

7.5.4 Lytle, L. T. 1978. Chemiluminescence in the visualization of forensic bloodstains. Journal of Forensic Sciences 23(3): 550-562.

7.5.5 Saferstein, R. *Forensic Science Handbook: Identification and Grouping of Bloodstains*, (Prentice-Hall, Inc., 1982), p. 274.

7.5.6 Stoilovic, M. 1991. Detection of semen and blood stains using Polilight as a light source. *Forensic Science International* 51: 289-296.

8 Semen Detection

8.1 Please refer to Section #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines, of the Acid Phosphatase Spot Test, SERI Christmas Tree Stain, Phosphate Buffered Saline (1X), and SERATEC® PSA Semiquant test devices.

8.2 Many samples submitted to the laboratory are tested for the presence of semen. Generally, a sample should be subjected to a presumptive acid phosphatase test, followed by a confirmatory microscopic examination for the presence of spermatozoa. If the sample is not microscopically positive, a third test, a test for the presence of prostate-specific antigen, may be warranted and executed.

8.3 Alternative to the methods described below a sample may be subjected to Quantitative Male Detection. This process involves forwarding samples onto DNA analysis and then utilizing the quantitative step to detect the presence of male DNA.

8.4 Acid Phosphatase Spot Test

8.4.1 Scope

8.4.1.1 Acid phosphatase (AP) is found in relatively large quantities in semen and its presence is indicative of the possible presence of semen in a stain. In the following procedure, acid phosphatase is detected by a color-change reaction. Acid phosphatase liberates the phosphate from α-naphthyl phosphate, and the released naphthol combines with tetrazotized o-dianisidine to form a purple azo dye.

8.4.1.2 The AP test is semi-quantitative. A stronger reaction is more likely to indicate semen. However, because acid phosphatase occurs in other body fluids, most notably vaginal secretions, this is only a presumptive test. The presence of semen in the sample can subsequently be confirmed by the presence of spermatozoa.

8.4.2 Equipment, Materials, and Reagents

- Sterile diH_2O
- Sterile cotton swab(s) or filter paper
- AP Spot Test reagent
- Timer
- Non-porous surface
- Clips (optional)

8.4.3 AP Procedure for Swabs

- Note: Begin at step 1 when it is necessary to transfer the suspected semen sample from an item of evidence to a cotton swab or swabs for ease of testing. Otherwise, begin at step 3 following the procedure below:

8.4.3.1 Moisten cotton swab(s) with sterile diH_2O.

8.4.3.2 Swab the suspected semen stain from the evidence.

8.4.3.3 Moisten filter paper with sterile diH_2O.

8.4.3.4 Press the swabs firmly onto the filter paper.

8.4.3.5 Apply one or two drops of the AP Spot Test reagent to the filter paper.

8.4.3.6 Observe the treated filter paper for up to 60 seconds for a purple color change.

8.4.3.7 After interpretation, document the test results onto the serology worksheet(s).
8.4.3.8 Discard used filter paper by placing it in the biohazard trash.

8.4.4 AP Mapping Procedure

- This procedure can be used when larger items of evidence are presented.

8.4.4.1 Spread the clothing item, bedding, towel, or other item flat onto a non-porous surface (use clips if necessary).
8.4.4.2 Moisten filter paper with sterile diH_2O.
8.4.4.3 Lay the moist filter paper over the item or area of the item to be tested. Press the paper firmly against the item for approximately 30 seconds to 5 minutes to allow for any possible acid phosphatase to transfer to the paper.
8.4.4.4 Mark the position of the paper on the item.
8.4.4.5 Remove the filter paper.
8.4.4.6 Spray or apply drop(s) of AP test reagent to the filter paper.
8.4.4.7 Observe the treated filter paper for up to 60 seconds for a color change.
8.4.4.8 After interpretation, mark the tested areas with results.
8.4.4.9 Discard used filter paper by placing it in the biohazard trash.

8.4.5 Interpretation

8.4.5.1 A color change to purple takes place within 60 seconds, the result only indicates the possible presence of semen; the test is therefore a presumptive test.
8.4.5.2 Grade the reaction according to the time it takes for the color to appear as follows:

- 0-15 seconds: 4+
- 16-30 seconds: 3+
- 31-45 seconds: 2+
- 46-60 seconds: 1+
- No change, or color change after more than 60 seconds: negative

8.4.5.3 A negative result indicates acid phosphatase is absent or below the detection threshold.
8.4.5.4 Unclear or uncertain results should be reported as “inconclusive”.
8.4.5.5 Substrate controls may be included in body fluid identification tests as appropriate.

8.5 Body Fluid Extraction

8.5.1 Scope

8.5.1.1 Possible body fluid stains are removed from the substrate and dissolved in PBS for testing. The extract is used to collect cellular debris from a stain for spermatozoa examination and can also be used for p30 testing.
8.5.1.2 Microscopic examination for spermatozoa or p30 testing may be skipped if, in the analyst’s opinion, consumption for these tests reduces the potential success of subsequent DNA analysis. Samples that are of limited size may be documented as Quantity Not Sufficient for Further Analysis (“QNS”). Only a presumptive screening result is reported but DNA analysis may be performed.

8.5.2 Equipment, Materials, and Reagents

- Microcentrifuge tube(s)

- Forceps (optional)
- Scalpel and/or scissors
- Mini-centrifuge
- 1X PBS
- Pipette and pipette tips
- Ultrasonic cleaner

8.5.3 Procedure

8.5.3.1 Add sample to a microcentrifuge tube. The analyst must exercise good judgment regarding the amount of sample to be portioned for stain extraction, providing sufficient sample for DNA analysis. Stain intensity and size are a few characteristics that can be considered. Generally, up to approximately ¼ of a swab or up to approximately $(1\ cm)^2$ of a stain may be consumed for this procedure.

8.5.3.2 Add approximately 200 µl of 1X PBS to the sample. More PBS may be required to ensure the sample is fully submerged.

8.5.3.3 The extract(s) may be placed at room temperature in an ultrasonic cleaner for 5-15 minutes. Alternatively, the extracts may be soaked for 30 minutes - 24 hours in refrigerated storage.

8.5.3.4 Centrifuge the extract(s) briefly.

8.5.3.5 The extract, containing any cellular debris, is used for microscopic spermatozoa examination and may also be used for p30 testing.

8.5.3.6 Store the extract refrigerated, when necessary, for up to 24 hours. Beyond 24 hours the extract shall be stored frozen.

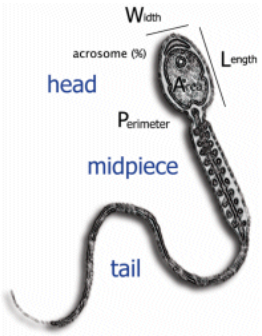
- Substrate controls may be included in body fluid identification tests as appropriate.

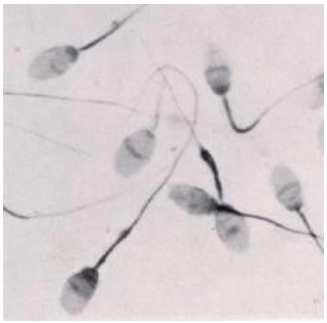
8.6 Microscopic Spermatozoa Examination

8.6.1 Scope

8.6.1.1 Spermatozoa (sperm) detected on an evidence sample confirm the presence of semen; the test is therefore a confirmatory test. This procedure uses the SERI Christmas Tree Stain which is a Kernechtrot-Picroindigocarmine differential biological stain that assists in scanning slides for the presence of spermatozoa. The test consists of a two stain method which dyes the tip of sperm heads pink, the bottom of sperm heads red, the tails green or blue and any epithelium green with red nuclei.

8.6.1.2 Human sperm are flagellated with a total length of about 50 µm. The sperm cell head generally is oval, flattened at the anterior end, with dimensions about 4.6 µm x 2.6 µm x 1.5µm.





8.6.1.3 Identifying characteristics for spermatozoa:

- Size (head is approximately 4.6 µm x 2.6 µm x 1.5µm)
- Morphology (oval to round or teardrop-shaped structure)
- Acrosomal cap at tip of sperm (unstained or lightly stained)
- Staining (dark pink to red head, with unstained or lightly stained acrosomal cap; tails green or blue, if present)
- Presence of a flagella or tail (may not be present)

8.6.2 Equipment, Materials, and Reagents

- Microscope slide
- Body fluid extract
- Pipette and pipette tips
- Heat block
- Christmas Tree Stain - Solution A and Solution B
- Timer
- Tap water
- 95% ethanol
- Compound microscope
- Immersion oil

8.6.3 Procedure

- If slide has already been prepared, proceed to 7.6.3.3.

8.6.3.1 Pipette 10 µl of the supernatant from the body fluid extract onto a glass slide. Fix smear by heating the slide on the heat block for approximately 5 minutes.

8.6.3.2 Stain the slide.

8.6.3.2.1 Cover the sample area on the slide with Solution A for 10 minutes.
8.6.3.2.2 Wash with tap water by gentle flooding.
8.6.3.2.3 Cover the sample area with Solution B for 15 seconds.
8.6.3.2.4 Wash with tap water by gentle flooding.
8.6.3.2.5 Flood the slide with 95% ethanol and allow the slide to dry for at least 5 minutes on the heat block.

8.6.3.3 Examine the slide for the presence of spermatozoa at 1000X magnification.

8.6.3.4 Document the approximate number of sperm seen on the slide. Once the count exceeds 25 spermatozoa observed, the documented count can be "25+", ">25", or "TNTC". When only one spermatozoon is observed, the slide must be verified by a second qualified analyst who must initial and date the examination documentation. Additionally, a second slide must be created and stained following steps 1-3 above using the same supernatant, when possible. If only one spermatozoon is again observed, the slide must be verified by a second qualified analyst who must initial and date the examination documentation; this sample may be reported as positive for the presence of spermatozoa. If more than one spermatozoon is observed on the second slide, then the sample may be reported as positive without verification. If the second slide is negative for the presence of spermatozoa, the sample shall be reported as inconclusive for the presence of spermatozoa.

8.6.3.5 If too much cellular material and/or debris is potentially obscuring any spermatozoa on the slide for the analyst to make a determination about the presence or absence of sperm, the following procedure may be performed:

8.6.3.5.1 Centrifuge the sample to pellet any cells. Remove and save supernatant for p30 analysis.

8.6.3.5.2 Wash the cell pellet 1x with 500µl of sterile diH_2O or 1X PBS, pipetting the pellet up and down or vortexing.

8.6.3.5.3 Centrifuge again to pellet any cells.

8.6.3.5.4 Re-suspend in 50 to 300µl of sterile diH_2O or 1X PBS, again by pipetting up and down or vortexing.

8.6.3.5.4.1 Use the new suspension to make another slide, repeating steps 2-5. Examination documentation must reflect the creation of multiple slides.

8.6.4 Interpretation

8.6.4.1 Sperm heads stain red and the tails stain green by this procedure. A minimum of two spermatozoa must be observed to report the sample as positive for the presence of spermatozoa. A search of the entire slide is required before negative results for spermatozoa can be reported. If the slide contains too much cellular material or debris for the analyst to determine the presence or absence of sperm, the analyst may then report the result as "inconclusive". Alternatively, if sperm-like objects are observed, but one or more of the identifying characteristics are lacking, the slide may be reported as "inconclusive". Finally, if only one spermatozoon is observed, the slide shall be reported as "inconclusive".

8.6.4.2 It is recommended that "inconclusive" samples proceed to DNA analysis, along with positive samples.

8.7 SERATEC® PSA (p30) Semiquant Test

8.7.1 Scope

8.7.1.1 The cells that line the ducts of the prostate make a glycoprotein known as p30 or prostate-specific antigen (PSA). The protein is secreted into seminal fluid to a concentration of approximately 0.2 - 3.0 mg/ml, and its detection may indicate the presence of semen. PSA may be found at very low concentrations in vaginal fluid (0.4-1.25 ng/ml) and is therefore a presumptive test.

8.7.1.2 The SERATEC® PSA Semiquant test is a detection method specifically designed for forensic identification of p30. This test is used qualitatively only in this laboratory. The test is capable of detecting p30 in a concentration range of at least 2ng/ml - 100µg/ml PSA.

8.7.1.3 SERATEC® PSA Semiquant test contains two monoclonal murine anti-PSA antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane. The upstream control region and the region of the internal standard (between control and test region) contain immobilized polyclonal goat anti-mouse antibodies. The amount of antibody at the internal standard is adjusted to a color intensity of the line, which is equal to the color intensity of the test line at a PSA concentration of 4 ng/. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad with the dried and gold-labeled second monoclonal murine anti-PSA antibody. PSA at the sample binds to the remobilized gold-labeled antibody and form a PSA-gold-labeled-anti-PSA-antibody complex.

8.7.1.4 Through the capillary effect of the membrane, the reaction mixture including the complex mixture is carried upwards with the fluid. In any case, the colored gold-labeled anti-PSA-antibody binds to the anti-mouse-antibody at the control region and the region of the internal standard, thus developing two red lines (one at the control region and one at the region of the internal standard). These two lines are independent of the existence of PSA in the sample and indicate only the correct execution of the test.

8.7.1.5 If the sample contains PSA, the PSA-gold-labeled- anti-PSA-antibody complex binds to the immobilized monoclonal antibody of the test result region that recognizes another epitope on the PSA molecule (sandwich complex). The binding is indicated by the formation of an additional line. Thus a PSA positive sample shows three colored lines in the result window.

8.7.2 Equipment, Materials, and Reagents

- SERATEC® PSA Semiquant test device – one per sample
- Body fluid extract
- Pipettes and pipette tips
- Timer

8.7.3 Procedure

8.7.3.1 Allow previously extracted sample(s) to warm to room temperature if they have been refrigerated.

8.7.3.2 For each sample, unwrap and label a SERATEC® PSA Semiquant test device.

8.7.3.3 Add approximately 120 µl of the supernatant to the sample well of the device.

8.7.3.4 Results may be read up to 10 minutes from application of the supernatant.

8.7.3.5 Record the SERATEC® PSA Semiquant test results on the Serology Results worksheet.

8.7.3.6 Have a second qualified analyst examine the SERATEC® PSA Semiquant test device and verify the documented test result(s) by initialing and dating the appropriate Serology Results worksheet.

8.7.4 Interpretation

8.7.4.1 There are three lines that appear in the test device "result window":

8.7.4.1.1 **Control "C" line:** the presence of this line confirms the integrity of the test components and the test procedure. It is expected for all tests and must be present. Absence of the "C" line is an invalid result and the test must be repeated if adequate sample remains.

8.7.4.1.2 **Internal Standard line:** The intensity of this line correlates with a p30 concentration of approximately 4 ng/ml. It is expected for all tests and must be present. The absence of the internal standard line is also an invalid result and the test must be repeated if adequate sample remains.

8.7.4.1.3 **Test "T" line:** A line indicates the presence of semen and shall be documented as a positive result. The absence of a line or a line that appears after 10 minutes is a negative result or p30 concentration is below the detection limit.

8.7.4.2 Samples that produce a negative result but show strong positive acid phosphatase activity must be diluted to an appropriate dilution and be retested to ensure the High Dose Hook Effect is not occurring (see SOP #6.3 ABAcard® HemaTrace® Test Devices).

8.8 References

8.8.1 http://www.micropticsl.com/eng/products/sperm_analysis_sca_morphology.html. Accessed July 21, 2012.

8.8.2 http://www.historyforkids.org/scienceforkids/biology/cells/transportation2.htm. Accessed July 21, 2012.

8.8.3 Edwin L. Jones, Jr. (2005). The Identification of Semen and Other Body Fluids. In R. Saferstein (Ed.), *Forensic Science Handbook, Volume II*. (pp. 329-399). Mt. Laurel, New Jersey: Pearson/Prentice Hall.

8.8.4 Baechtel, S. F. 1988. The identification and individualization of semen stains. *In*: Forensic Science Handbook, Volume 2. Richard Saferstein, ed. Prentice-hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 349.

8.8.5 Gaensslen, Robert E. 1983. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* U.S. Department of Justice, National Institute of Justice. Sections 10.3.2 and 10.3.3

8.8.6 Kind, Stuart S. 1957. The use of acid phosphatase in searching for seminal stains. Journal of Criminal Law, Criminology, and Police Science. 47(5):597-600

8.8.7 Omniprint™ 1000A-110 Operating Instructions

8.8.8 SERATEC® PSA Semiquant Test. *In-vitro diagnostic test for professional forensic use for the detection of seminal fluid by the semi-quantitative determination of PSA (Prostate-specific antigen).* Product insert, revised June, 2011.

8.8.9 Smith, Charles. 1979. An acid phosphatase reagent for use in searching large areas for seminal stains. Unpublished

8.8.10 Stoilovic, M. 1991. Detection of semen and blood stains using Polilight as a light source. *Forensic Science International* 51: 289-296.

8.8.11 Stone, I. C. 1972. Staining of spermatozoa with Kernechtrot and picroindigocarmine for microscopical identification. Document CIL No. 2, Southwestern Inst. Forensic Sci., Criminal Investigation Laboratory (USA).

Trace Observation/Collection

9.1 Scope

9.1.1 This procedure may be used when necessary for the preservation of trace evidence prior to forensic biology analysis. Examples of typical trace evidence in criminal cases include fingerprints, glove prints, hairs, cosmetics, plant fibers, mineral fibers, synthetic fibers, glass, paint chips, soils, botanical materials, gunshot residue, explosives residue, and volatile hydrocarbons (fire debris). However, the reporting of trace may be limited to apparent hairs and fibers.

9.1.2 The Locard Exchange Principle presumes that when a person comes into contact with an object or person, a cross-transfer of material, sometimes trace evidence, occurs.

9.1.2.1 When practicable, trace evidence shall be secured upon initial observation. An example of when this is not practicable is clothing or bedding that is saturated in trace evidence.

9.1.3 Trace collection room doors should remain closed at all times. The rooms are vacuumed and the floors damp mopped when in use for the detection of trace material. Lab coats are provided in the trace rooms for use during analysis. Lab coats used in other areas of the laboratory shall not be taken into the trace rooms due to possible contamination. For any collection method used, tables and tools shall be cleaned with bleach solution (or other lab-approved cleaning solution) before each use to remove any extraneous debris. At a minimum, tools shall be cleaned and new bench paper used for each item when multiple items are received packaged together.

9.1.4 Generally, for any method used, a clean sheet of butcher paper is used on the table for each evidence package opened.

9.1.5 Evidence from victim(s) and suspect(s) shall be examined in separate rooms to prevent cross contamination. The examiner shall change lab coats between the examination of items from victim(s) and suspect(s). If separate rooms are not available, items from victim(s) and suspect(s) can be processed in the same room after a period of 7 days.

9.2 Procedure

9.2.1 Equipment, Materials, and Reagents

- Butcher paper
- Tweezers
- Plastic zipper bags
- Clear packing tape or trace evidence lifters

9.2.2 There are several options available for ensuring that trace evidence is properly collected/stored/preserved. These are as follows:

9.2.2.1 Visual examination: locate and remove trace evidence from the evidence item with tweezers. Recovered trace shall be placed in a suitable labeled container (zipper bags or on tape) to prevent loss or contamination of the sample.

9.2.2.2 Tape Lifting: clear tape is pressed over the area potentially bearing trace evidence. This application is repeated until all areas of interest are covered. New tape must be used once the tape in current use loses its stickiness. Tape(s) shall be placed on

the inside surface of a clean cut open zipper bag or a sheet protector. The bag/protector shall be labeled as to its contents.

9.2.2.3 Retaining in butcher paper: Trace evidence may also be preserved by retaining the evidence item in butcher paper. In some instances, however, it may be most appropriate to repackage the item using the original packaging. For example, a swab with an embedded hair or fiber may be repackaged in the original swab carton. The method of packaging selected shall prevent damage, deterioration, and/or loss of the trace evidence. The evidence item may only be re-opened in a clean trace environment to prevent contamination and/or loss.

9.2.3 Place all collected trace evidence in the original evidence container with the item from which it was collected.

9.2.4 The observation or collection of trace evidence must be documented on the appropriate examination documentation. Examiners shall not make any attempt to identify on their worksheets what was collected (i.e. hair, fiber, etc.) unless they also possess current training in the disciplines of Trace Evidence. However, it is permissible for sample descriptions to include adjectives such as “apparent” or “-like” when describing trace evidence (e.g., hair-like or apparent fibers).

9.3 Reference

9.3.1 Saferstein, R. (2007). *Criminalistics: An Introduction to Forensic Science, Ninth Edition.* Upper Saddle River, New Jersey: Pearson/Prentice Hall.

10 Contact DNA Collection

10.1 Scope

10.1.1 This procedure is used for the collection of contact DNA evidence prior to forensic biology analysis. This laboratory does not currently "screen" for the presence of contact DNA through a method such as microscopic examination for the presence of epithelial cells. Contact DNA is generally very limited in nature and consumption during screening may further reduce the potential success during DNA analysis. For this reason, when an item is suspected of containing contact DNA, efforts to collect possible contact DNA should be made. These samples may then be submitted for DNA analysis.

10.2 Equipment, Materials, and Reagents

- Sterile cotton swabs
- Sterile reagent grade water
- Swab drying box (optional)

10.3 Procedure

10.3.1 Moisten swab(s) with sterile diH_2O.

10.3.2 Swab the suspected area(s) on the evidence with the tip(s) of the cotton swab(s). Efforts to concentrate the possible contact DNA on the tip of the swab should be made. This serves to increase chances of DNA success not only by concentrating the possible DNA but also in the absence of visible staining, later portioning will more likely capture DNA if the correct area of the swab is selected for portioning. As the area to be swabbed increases in size, so too may the number of swabs needed to adequately collect the evidence.

10.3.3 The wet swab may be followed by a dry swab on non-absorbent surfaces.

10.3.4 Avoid any suspected semen or blood stains. These sources of biological material generally contain more DNA and potentially "mask" any lower level contact DNA. These stains may be collected separately.

10.3.5 Allow swab(s) to completely dry in a swab drying box for 30 minutes. Alternatively, the swab(s) may be air dried overnight in a secure and clean environment.

- Sometimes, such as in the case of fingernail scrapings and clippings, suspected bloodstains cannot always be avoided. In such instances, the analyst shall look for any visible red/brown staining and document accordingly prior to swabbing the item. It may be appropriate to forgo blood or semen detection to maximize potential contact DNA for DNA analysis.

11 Portioning for DNA Analysis

11.1 Scope

11.1.1 After serological analysis, any evidence to be forwarded on for DNA analysis may be portioned. Please refer to Section 5 (Evidence Evaluation and Handling) for guidelines on general evidence handling and the consumption of evidence in particular. These are guidelines only; good judgment and case specifics may warrant a divergence from what is prescribed below.

11.2 Equipment, Materials, and Reagents

- Scalpel or scissors
- Microcentrifuge tube(s)
- Weighing paper or butcher paper

11.3 Procedure

NOTE: Make every effort to preserve at least half of the sample for re-analysis. If this is unavoidable, refer to the Quality Manual for further instructions.

11.3.1 Set the evidence onto paper.
11.3.2 Portion the evidence according to the chart below:

Swabs

Results	Portions
Acid Phosphatase (+) or P30 (+) only, no spermatozoa	~ ½ of total swab(s)
<10 spermatozoa	~ ½ of total swab(s)
≥ 10 spermatozoa	Remainder of swab cut for p30 or micro
Phenolphthalein	Up to ~ ½ of total swab(s)
HemaTrace (+)	~ ½ remainder of swab cut for HemaTrace
QNS or Quantitative Male Detection	~ ½ of total swab(s)
Contact	~ ½ of total swab(s)
Bloodstain card	~ 0.5 cm^2 from center of stain
Known saliva swabs	~ ½ of 1 swab

Clothing

Results	Portions
<10 spermatozoa or p30 (+) only or Quantitative Male Detection	~ 1 cm^2 cutting

≥ 10 spermatozoa	~ 0.5 cm^2 cutting
HemaTrace (+)	~ 0.5 – 1 cm^2 cutting
QNS	~ ½ of the stain

11.3.3 Place portion into a microcentrifuge tube.
11.3.4 Label microcentrifuge tube with case number, item number, and initials.

12 Case Records

12.1 Examination Documentation

12.1.1 The notes and other documentation must support the conclusions of the examiner. The laboratory report must communicate both the analytical results and the conclusions of the examiner, conveying the essence of what he or she would say if asked for an expert opinion in court. Decisions may be made by police officers, attorneys, and the courts based on the report alone without examiner clarification, so the report must be able to stand alone. Some case record requirements can be found in the Quality Manual; any additional case record requirements are included here.

12.1.2 Refer to the quality manual for chain-of-custody policies and procedures, documentation of chain-of-custody, and documentation required in all laboratory case records.

12.1.3 Documentation must be in such a form that another qualified examiner or supervisor, in the absence of the primary examiner, can evaluate what was done and interpret the data. The case reviewer shall be able to determine from the notes that sufficient testing, relevant testing, and correct methods of testing were used. To this end, all documentation of procedures, standards and controls used, observations made, results of tests performed, charts, graphs, photographs, sketches, etc. that are used to support the examiner's conclusions must be preserved as a record. Observations, data, and calculations shall be recorded at the time they are made; the date of an in-house photograph shall be included on the photograph or associated examination documentation. Examination records shall be of a permanent nature. If a written examination record is created (or if original observations are made) on non-traditional media (for example: sticky notes, paper towels, gloves, etc.), then either the original media shall be retained or an electronic scan/picture of the original shall be retained in the case record. Once an electronic scan/picture is created, the original hardcopy may be destroyed. Examination documentation shall reflect the name and/or initials of the individual who performed the work.

12.1.4 Appropriate worksheets must be completed during analysis. Examination documents must have notes that help in the identification of the item of evidence. A written description may suffice for some items, whereas others may need a drawing, sketch, or photograph.

12.1.5 If an item is submitted for immediate analysis, such as a mobile phone that needs to be swabbed for possible contact DNA and immediately returned to the submitting party, it is not necessary for the evidence to be sealed and it may not be possible to establish a proper seal and/or to be marked with a unique identifier. However, case record documentation must include identifying information that permits a later identification of the item handled. For example, in the absence of analyst markings on the actual item of evidence, a photograph of the mobile phone's serial number, along with a photo of the mobile phone can enable later identification of the actual item handled.

12.1.6 Items collected at autopsy do not always include the name of the complainant, as it may be unknown at the time of the autopsy. If morgue evidence is received without the name of the complainant, the evidence must be described using the Medico-Legal

number (ML#) and/or "unknown". Alternatively, if written notice is provided by the investigator or other case agent, the complainant name provided may be used in the evidence descriptions in conjunction with the ML#. This written notice must be maintained in the case file and may be referred to in the report.

12.1.7 Each examination worksheet(s) shall include the following: initials, case number, controls, dates of analyses, item names/numbers/quantity, results, and disposition. Items that are not tested or opened are described to the extent to which they can be. A written description may suffice for some items, whereas others may need a drawing or photograph. Descriptions of evidence shall be clear and sufficient to permit the later identification of an item. They may include any of the following: location and size of tears, cuts, and holes; missing buttons; soiling condition; degree of degradation; type of material, significant foreign material, etc. Significant stains must be drawn or documented in a manner which clearly demonstrates the location, relationship to other stains, reactions to screening tests, etc. Abbreviations may be used; see section 12 for a listing of approved abbreviations.

12.1.8 If a control is collected, the location of the control is documented in reference to the stain. The type of material or fabric the stain is on may be significant.

12.2 Reports

12.2.1 For some items, it may be useful to report what area of the item was tested. Also include trace examination, whether collected or observed. Instructions must be included in the report as to requesting trace analysis if needed. Note if any items listed were not analyzed.

12.2.2 It may be appropriate to include in the report a request for the submission of known samples from any of the following: suspects, complainants, witnesses, consensual sex partners, and/or other individuals for the purposes of elimination.

12.2.3 Although interpretation of individual tests is discussed in the appropriate protocols, analysis results must be considered together to ensure that conclusions take into account all reasonable possibilities. During analysis, the examiner must continuously monitor results for problems and inconsistencies that may ultimately affect final conclusions. Therefore, careful review of notes and results by the examiner must be undertaken before conclusions are drawn. A supervisor or qualified examiner may assist in developing appropriate conclusions. Slightly different situations may lead to different conclusions. When in doubt, consult with other qualified examiners and/or the supervisor.

12.2.4 Reports shall include the disposition of all items of evidence, whether they are retained within the laboratory or returned to the client.

12.3 Examination Counts

12.3.1 Information related to the number of examinations performed during each analysis can be documented on the "Serology Examination Count Sheet" in LIMS. This information can be used by laboratory management to accurately capture metrics such as laboratory capacity, volume of specific requests, and sectional productivity.

The following is a summary of how totals for this form should be calculated:

Category	How to Calculate
Number of Items	Number of items listed on the report
ALS Exams	Number of items viewed with ALS (excluding controls)
AP	Number of swabs/stains tested (excluding controls)
Sperm searches	Number of slides viewed
p30	Number of cards tested
PHT	Number of swabs/stains tested (excluding controls)
HemaTrace	Number of cards tested
Trace collection/observation	Number of items with trace collected or trace observed
Bloodstain cards	Number of bloodstain cards made
Swabbings/cuttings	Number of cuttings or swabs collected
Portions for DNA	Number of portions created
Total examinations	Total of examinations – LIMS calculates

13 Abbreviations

13.1 In addition to standard scientific abbreviations and those found in the dictionary, the following abbreviations are defined here and acceptable for use on case work documentation. Non-standard scientific abbreviations, those not found in the dictionary, and abbreviations not contained on this list must be defined on each examination document on which it is used.

Ø	none	FNSC	fingernail scrapings/clippings	POSS	Possible
~	approximately	FTC	found to contain	PR	HPD Property Room
ALS	alternate light source	GSR	gunshot residue	PR#	payroll number
AP	acid phosphatase	HPS	hospital patient sticker	PREP	preparation
APP	apparent	HRS	hours	PT	patient
AR	administrative review	HT	HemaTrace®	Q	questioned sample
BC	barcode	INC	inconclusive	QNS	quantity not sufficient (for further analysis)
BSC	bloodstain card	INC #	Incident Number	R/B	reddish/brown
BUC	buccal	IPC	Internal positive or PCR control	REP	representative
c̄	containing	IT	item	REC'D	received
CAPT	captain	K	known standard sample	RMS	Records Management System
COMM	communication	KBS	known buccal swabs	RPR	return to property room
COMPL	complainant	KSS	Known saliva swabs	S/SEC	second
CONT'D	continued	LG	large	SA/SAK	sexual assault/sexual assault kit
CS	Controlled Substances	LIMS	Laboratory Information Management System	S-CELLS	sperm cells
CSP	Consensual sex partner	LT	lieutenant	SCRN	Screening
CTRL	control	MED	medium	SF	sperm fraction
D	depleted	MICRO	sperm search by microscopy	SGT	sergeant
DOB	date of birth	MFG	manufacturer	SM	small
diH_2O	deionized water	N/A	not applicable	ST	stain
DISP	disposition	NEG	negative	STC	said to contain
ECD	excessive cellular debris	NSO	no stains observed	SUPPL	supplement
E-CELLS	epithelial cells	NTC	no testing conducted	SUSP	suspect

EF	epithelial fraction	NTO	no trace observed	SWIFS	Southwestern Institute of Forensic Sciences
EMS	Evidence Management System	NSTO	no significant trace observed	TEMP	temperature
ENV	envelope	NVB	no visible bloodstain	TNTC	too numerous to count
EtOH	ethanol	OBS	observed	TOX	Toxicology
EVID	evidence	OFTC	opened, found to contain	TR	technical review
EXP	expiration	OLO	online offense system	UCC	Unfired cartridge casing
F	frozen	PBS	phosphate buffered saline	UNK	unknown
FA	Firearms	PG/PP	page/pages	VAG	vaginal
FCC	Fired cartridge casing	PHT	phenolphthalein	W/	with
FCN	Forensic case number	PKGD/ PKG'D	packaged	WIF	walk-in freezer
FNS	fingernail scrapings	POS/+	positive	YOA	years of age

Appendix A

Volumetric Pipette Range	Percent	Check Volume (uL)	Acceptable Check (uL) Average needs to be between these values	
0.5-10	5	2.5	2.375	2.625
	3	7.5	7.275	7.725
	3	10	9.7	13
1-10	5	2.5	2.375	2.375
	3	7.5	7.275	7.725
	3	10	9.7	10.3
5-50	3	12.5	12.125	12.875
	3	37.5	36.375	38.625
	3	50	48.5	51.5
2-20	3	5	4.85	5.15
	3	15	14.55	15.45
	3	20	19.4	20.6
10-100	3	25	24.25	25.75
	3	75	72.75	77.25
	3	100	97	103
20-100	3	25	24.25	25.75
	3	75	72.75	77.25
	3	100	97	103
20-200	3	50	48.5	51.5
	3	150	145.5	154.5
	3	200	194.0	206
40-200	3	50	48.5	52
	3	150	145.5	154.5
	3	200	194.0	206
50-200	3	50	48.5	51.5
	3	150	145.5	154.5
	3	200	194	206
100-1000	3	250	242.5	257.5
	3	750	727.5	772.5
	3	1000	970	1030
200-1000	3	250	242.5	257.5
	3	750	727.5	772.5
	3	1000	970	1030