



BOISE POLICE DEPARTMENT CRIME LABORATORY



Biology Screening Technical Manual Version 7.0

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Authority: Quality Assurance/Lab Manager, Lab Supervisor, Technical Leader



BIOLOGY SCREENING

Technical Manual | Effective Date 12/8/25 | Revision #7
Boise Police Department Crime Laboratory

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1. Introduction

1.1. Background

- 1.1.1. Forensic serology is the branch of forensic sciences dealing with the identification and characterization of biological, evidentiary samples — such as blood, semen, saliva, sweat, breast milk, and any other bodily fluids. Screening is performed before DNA analysis, which an external laboratory conducts. Forensic DNA analysis involves several steps, including extraction, quantification, amplification, and separation, with a focus on specific chromosomal locations that vary widely between individuals. These specific locations are used to generate a DNA profile from evidentiary items or known source samples.
- 1.1.2. Biology screening involves visually examining the item for indications of biological fluids and performing testing to determine whether specific body fluids that may contain DNA are present.
- 1.1.3. Some items of evidence are analyzed for sampling for touch DNA. The analyst collects possible skin cells, sweat, or any other fluid with DNA from areas that may have been



handled or came into physical contact with an individual, despite visible staining not being present.

1.2. Scope

- 1.2.1. This manual shall serve as a guide to performing scientific examinations of physical evidence or crime scenes for the presence of blood, semen, and saliva using visual examination, presumptive screening, and confirmatory testing. The primary purpose of these procedures is to ensure quality and efficiency by establishing documentation and collection procedures utilized by the Lab.
- 1.2.2. This manual has aspects that are focused on biology screening in the lab, but many methods outlined in this manual may be used in the field. Refer to the BPD Crime Scene Technical Manual for specific methods and chemicals that may be used on scene. It is expected that certain lab requirements may not be appropriate for field use (e.g., lab coats, butcher paper on the bench top, labeling the item after analysis), and analysts should follow guidelines outlined in the BPD Crime Scene Technical Manual when appropriate.
- 1.2.3. The analyst is responsible for the preservation and possible collection of evidentiary materials that may be on the item or area being requested to be processed. The analyst must remain cognizant of other probative evidence that may be on each item in addition to what is being requested.
- 1.2.4. The procedures presented are to be used in conjunction with all applicable laboratory policies, good laboratory practice, and proper scientific methodology.
- 1.2.5. Only the procedures, formulas, and/or processes that are found in this manual are approved for use. All other procedures, formulas, and/or processes not outlined here must be validated as per the Quality Assurance Manual.
- 1.2.6. This manual is not designed to be an all-inclusive collection of procedures but should serve as a guide to situations most often encountered and as a sound framework upon which to build. It is expected that deviations in the methodology will occur, and it is at the discretion of the individual analyst to exercise sound judgment in working on a case. Analysts should seek guidance and/or approval from the Technical Leader and/or Lab Supervisor should they encounter a situation outside their experience, or requirements outlined in this manual.

2. References

- BPD Policy and Procedures Manual
- BPD Crime Lab Health and Safety Procedures Technical Manual
- BPD Crime Lab Evidence Processing and Latent Print Technical Manual
- BPD Crime Lab Quality Assurance Manual

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- BPD Crime Lab Crime Scene Investigation Technical Manual
- NIST OSAC Subcommittee: Biological Methods at <https://www.nist.gov/topics/forensic-science/osac-biological-methods-subcommittee> Omnigo LIMS Manual
- uQA-2 Verbiage Guidance Document
- US Department of Justice Uniform Language for Testimony and Reports for Forensic Serological Examinations (Effective: 12.12.22)
- Las Vegas Metropolitan Police Department Forensic Laboratory Biology/DNA Detail Procedures Manual (November 2019)
- Washington State Patrol Biochemical Analysis Procedures (May 2019)
- Ada County Sheriff's Office – Biology Screening Analytical Method Version 1.0
- Idaho State Police Forensic Services Biology DNA Casework Methods Rev 11 and DNA Quality Manual Rev 10
- College/University-level Chemistry and Biology textbooks
- Operation and maintenance manuals for each instrument

3. Responsibilities

3.1. Crime Lab Supervisor

- 3.1.1. Overall responsibility for ensuring that personnel adhere to established methods, safety practices, and laboratory policies and procedures
- 3.1.2. May assign casework based on priority, crime type, crime date, court date, or other factors at their discretion or the discretion of the QA/Lab Manager or Chief Administrative Officer

3.2. Technical Leader

- 3.2.1. Oversee validation and approval of all methods used
- 3.2.2. In the absence of the Supervisor, may assign casework and initiate, suspend, and authorize resumption of casework
- 3.2.3. Assists with resolving technical differences of opinion
- 3.2.4. Follows all requirements of an analyst

3.3. Analyst

- 3.3.1. Responsible for adherence to established methods, safety practices, and laboratory policies and procedures
- 3.3.2. Duties may include, but are not limited to (as appropriately authorized):
 - The examination of articles for the presence of biological evidence
 - The collection of swabs, cuttings, or other methods for biological evidence preservation and future testing
 - The use of presumptive and confirmatory tests
 - Issuing reports of examination activities



4. General Safety

4.1. Personnel Safety

Personnel shall comply with all Departmental and Laboratory Section occupational safety requirements, as delineated in the Safety and Security Section A, 6.00 of the City of Boise's Employee Policy Handbook, the Boise Police Department's Policy & Procedures Manual, the Boise Crime Laboratory's Safety and Security Procedures Manual, the IAI Safety Guidelines, and the BPD Crime Laboratory's online MSDS library. This includes, but is not limited to:

- Personal protective equipment
- Chemical handling and storage
- Contamination prevention
- Emergency procedures

4.2. Equipment and Supplies

Personnel shall conform to the manufacturer's requirements and recommendations and/or training.

5. Request for Analysis

5.1. See BPD Crime Lab's Evidence Processing and Latent Print Technical Manual

6. Evidence Submission, Collection, Control, and Disposition

6.1. See BPD Crime Lab's Evidence Processing and Latent Print Technical Manual

7. Technical Requirements

7.1. General

7.1.1. A positive and negative quality control (QC) check shall be performed on commercially prepared tests/kits and on prepared biology screening reagents, before use in casework, to ensure the reagent, chemical, or test is performing as expected.

7.1.1.1. The results of QC checks shall be documented in the case technical record.

7.1.1.2. If the test fails to perform as expected during the control check, perform two additional tests. Both additional tests must have correct results before using the chemical/reagent for casework. If the chemical/reagent fails to perform as expected a second time during the control check, immediately discontinue the use of the chemical/reagents, notify the Technical Leader and/or Lab Supervisor, and contact the manufacturer if applicable.

7.1.2. Reagents shall be quality-checked after they are made and before casework. A new stock solution is quality-checked at the time the first working solutions are made from it. The QC results of reagents and stock solutions are documented in the QA-23 Reagent Log.

7.1.3. All chemicals and reagents shall be labeled and tracked. See the BPD's Crime Lab Health and Safety Procedures Manual for requirements.



- 7.1.4. The lot number for each chemical/reagent used in processing will be recorded in the case technical record.

7.2. Accommodation and Environmental Conditions

- 7.2.1. Standard laboratory safety protocols shall be followed. Refer to the BPD Crime Lab's Health and Safety Procedures Manual.
- 7.2.2. Gloves, a mask, and a lab coat shall be worn during forensic biology screening in the lab. At a minimum, gloves should be changed between the handling of different items of evidence and whenever there is a possibility that transfer may occur between different stains and/or areas of interest on a single item.
- 7.2.3. Appropriate eye protection shall be worn whenever a hazard to the eyes exists. This includes chemical exposures and alternate light sources (UV, laser, etc.).
- 7.2.4. Examination utensils (e.g., forceps, scissors, measuring devices, etc.), used in processing, shall be disinfected between items of evidence.
 - 7.2.4.1. A ~10% dilute bleach solution (or a solution that will remove/degrade the DNA). Dilute bleach solutions for utensil decontamination will be prepared on the day of use.
 - 7.2.4.2. Wiping the utensil with a clean disposable tissue is strongly recommended. Serrated tools should not be used for sampling biological evidence.
- 7.2.5. When using aerosol-type chemicals, proper engineering controls, work practice controls, and PPE must be employed to avoid chemical exposure.
- 7.2.6. Diluted bleach (10%), stabilized bleach solution (e.g., Dispatch), or a solution that will remove/degrade the DNA, shall be used to clean all exposed work areas and any tools/resources used during examination and/or analysis (e.g., reusable scales or rulers used during photo documentation). At a minimum, decontamination of all exposed work areas should be performed before the initiation of screening, between the screening of different items of evidence, and at the conclusion of all daily screening activities. Reagent bottles/test kits may interact with bleach/dispatch solution, and therefore, it is recommended that they be decontaminated with an alcohol solution (ethanol).

Disposable bench (butcher) paper will be placed on top of decontaminated lab work surfaces before screening. Additional bench paper shall be layered on top of the base bench paper for the purposes of catching potential trace evidence that may come off during analysis. This bench paper will be changed between the examination of different items of evidence. Work with only one item at a time to avoid sample mix-up and/or contamination.



- 7.2.7. Place each item of evidence on a new sheet of paper (i.e., Kimwipe, butcher paper, etc.). Exhibits packaged together in a single (innermost) wrapping may be examined on the same sheet of paper.
- 7.2.8. Evidence items shall be handled in a manner to prevent loss, alteration, contamination, or mixing.
- 7.2.9. Crime scene samples shall be handled at a different time or in a different space from standards/known samples.

8. Physical Evidence Examination

Several techniques at an analyst's disposal can be used to thoroughly examine physical evidence for biological fluids. An appropriately thorough examination may require inspecting the front, back, outside, and inside of the item, using a multitude of techniques. The method(s) chosen shall be based upon the training and experience of the analyst and will depend on the evidence substrate, the condition of the evidence, the targeted biological fluid(s), the request for analysis, and case-specific considerations as to what is probative. The analyst's decisions must pass the scrutiny of the peer review process.

Unless circumstances dictate otherwise, examinations for biological stains should be done after collection and/or preservation of trace evidence and advanced bloodstain pattern analysis, but before collection and/or preservation of latent print and or other discipline examination (firearms, ignitable liquids, etc.).

8.1. Documentation

Documentation is extremely important to provide a detailed record of events, aid in report writing, assist with testimony, and allow for independent review by other experts. Any consultation with an analyst regarding evidence examination on a specific case shall be described in the technical record. Include the name and date of consultation. In general, the following applies.

- 8.1.1. Documentation may include a combination of notetaking, photography, and diagramming.
- 8.1.2. Documentation shall take place as the analysis is occurring.
 - 8.1.2.1. Conclusions cannot be documented solely in the photolog because a photolog is not done concurrently with the observation. If the only form of documentation is photographic, then ensure that the conclusion is included in the photograph. For example, a statement may be written on a sticky note to provide context, such as "suspected blood."
- 8.1.3. All original documentation shall be retained.
- 8.1.4. The technical record shall include the following:
 - 8.1.4.1. Type of item and quantity (e.g., two black and white Nike shoes, two swabs)
 - 8.1.4.2. A general description of the item (e.g., color, size, brand, and designs on clothing, bedding, towels, swab with red/brown staining, etc.)



- 8.1.4.3. Condition of the item (e.g., cleanliness, damage, holes, buttons missing, hooks bent, etc.). If the item is a swab, this may not apply.
- 8.1.4.4. The approximate location of any targeted biological stains/substance areas seen and their visual appearance, as applicable.
- 8.1.4.5. If any stains/substance areas consistent with blood, semen, and/or saliva are incidentally observed but not the targeted substance, the analyst may document that in the technical record. The analyst may need to consult with the requester on a case-by-case basis. Document communication in the technical record.
- 8.1.5. Document the quality control and/or performance check results in the technical record. See subsections of each chemical for specific positive/negative controls. Quality control and/or performance checks shall be done before use on evidence. For tests that require extraction, a shorter extraction time may be used for the positive quality control check due to the high concentration of the reference samples. If a positive quality control fails, wait for the recommended extraction time and re-test.
- 8.1.6. Document the test results in the technical record (positive (+), negative (-) or inconclusive (INC)). If the only form of documentation of the test results is photographic, then ensure that the conclusion is included in the photograph. For example, a statement may be written on a sticky note to provide context, such as "TMB = + for blood" or "TMB = inc for blood – flash of blue..."
 - 8.1.6.1. The reasoning for an inconclusive test result shall be documented in the technical record.
 - 8.1.6.2. If the final conclusion, after examining and/or testing an item for the targeted substance(s), is "inconclusive," then a detailed explanation must be included in the report.

8.2. Method

- 8.2.1. Lay out the evidence on a clean piece of bench paper or Kimwipe.
- 8.2.2. Describe the evidence in the technical record. (See 8.1.4.)
- 8.2.3. Examine the evidence for the targeted biological stains/substances. Examine all surfaces of the evidence that are relevant.
 - 8.2.3.1. Various lighting conditions (e.g., oblique, backlighting, ambient, alternate light source) and magnification may be used in general evidence examination to enhance the observation of biological fluids and to help locate minute, faint, or low-contrast stains.
- 8.2.4. Document the visual appearance of any targeted biological stains/substances found and their approximate location in the technical record.
 - 8.2.4.1. This may be recorded using words, diagrams, photographs, or a combination thereof.

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- 8.2.5. Not all biological stains are visible to the unaided eye. Use of an alternate light source and/or IR may be performed depending upon the compatibility of the substrate. Additionally, the analyst may choose to conduct general surface rubbings with a swab or filter paper. Document this in the technical record.
- 8.2.6. In general, the following processes may be used when testing a sample. Special circumstances dependent on stain type are listed later in this manual.
- 8.2.6.1. Use a swab, a pre-moistened filter paper, or take a small cutting (~2-3mm²) from the targeted area of the evidence. Testing sample size may vary. If using a commercially purchased kit/test, follow the provided recommendations.
- 8.2.6.2. Test the sample with the reagent appropriate for the targeted biological fluid.
- 8.2.6.3. Record the area tested and the results in the technical record. The method used for sampling (e.g., swab, cutting, or filter paper) shall be recorded in the technical record if the sample method is a cutting or filter paper method. Include the approximate size of the cutting if using a cutting. Otherwise, the default sample method is swabbing. Blood enhancement reagents (e.g., Bluestar®, Amido Black) do not have a sample method as they are applied directly to the item/surface.
- 8.2.6.4. Repeat the above steps until all locations requiring testing have been sampled and tested.
- 8.2.7. Mark the evidence with the item number and analyst's initials upon completion of processing in the lab so that it can be identified in court. The evidence shall be marked in a way that it is clear which item corresponds to which item number. All markings shall be made in such a manner that the evidentiary value of the item is not compromised.
- 8.2.7.1. Marking the proximal container or exterior packaging would be appropriate in the following circumstances. If the analyst marks something other than the item of evidence, document it in the technical record for clarification.
- The evidence item does not lend itself to marking. Examples: challenging substrate, size of item, swab samples.
 - Analyst recommends that the entire item be submitted to another laboratory for analysis, and marking the item would compromise further testing.
 - Non-destructive methods are requested by the customer.
- 8.2.7.2. It is not necessary to mark items of evidence that are received but not examined.
- 8.2.7.3. Do not mark evidence after processing in the field.

8.3. Bloodstain pattern analysis

If bloodstain pattern analysis is relevant, preserve the evidence photographically and/or refer the evidence to a qualified analyst to perform Bloodstain Pattern Analysis and/or Reconstruction **before** using destructive methods such as swabbing for presumptive tests.

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- 8.3.1. The significance of some patterns or the recognition that bloodstain pattern analysis is relevant may not be realized until all processing is complete, or all the information gathered. Thus, the accurate documentation of the bloodstain patterns through notes, diagrams, and photographs becomes imperative.
- 8.3.2. Documenting the bloodstain patterns must be accomplished in a way that the orientation, location, size, and position can readily be determined.
- 8.3.3. The location of bloodstains or patterns on an evidence item shall be recorded. This can be accomplished through notes, photographs, sketches, or a combination thereof.
- 8.3.4. When documenting bloodstain patterns, the analyst shall record the physical characteristics of the stain or pattern such as stain color, apparent concentration, size of stains, number of stains, distribution of stains, saturation of stain on fabric substrates (i.e., on the surface or penetrates the weave), clarity, sharpness, and symmetry of stain edges, and any other observable characteristics that assist in the determination of spatter vs. transfer. This can be accomplished through notes, photographs, sketches, or a combination thereof.

8.4. Consumption Issues

- 8.4.1. If a stain is small and/or dilute and sampling for chemical testing may consume the evidence, then precautions must be taken to conserve it. If consumption issues are recognized, an analyst may choose to take smaller cuttings than recommended, select a swabbing or filter paper technique instead, or omit presumptive testing.
 - 8.4.1.1. If an analyst opts to omit all testing entirely, the report shall recommend that the item be submitted to an approved laboratory that performs DNA analysis.

8.5. Cautions/Safety

- Wear a mask and use clean gloves to handle the packaging for swabs that are used for DNA sample collection. Avoid touching the exposed swab head to anything other than the item being swabbed.
- Avoid talking over evidence during examination.
- Do not lay an item of evidence directly on top of its external packaging and avoid placing external packaging on a surface that will come into direct contact with an item.

9. Other Evidence Encountered – Trace, Drug, and Latent Print Evidence

Trace, drug, and latent print evidence may be encountered during the processing of biological evidence. If the submitting agency did not request the collection and/or analysis of trace evidence, drug, and/or latent print evidence, but such evidence is incidentally observed during the requested examination, the evidence may be collected for preservation purposes. Consideration of preservation is case-dependent. The analyst should consult the submitting agency or requester to discuss specific analyses needed, or what may be



possible, on evidence from violent crime cases. Consultation with the requestor shall be documented in the communication record.

9.1. Trace and Drug Evidence

For trace collection procedures, refer to the BPD Evidence Processing and Latent Print Technical Manual. For drug collection/repackaging procedures, refer to the BPD Quality Assurance Manual.

9.1.1. Hairs and Fibers Identification

When hairs and/or fibers are observed, documentation that they are present may be sufficient (i.e., using terms such as possible hair, possible fiber, hair-like, fiber-like). Macroscopic and microscopic observations of hairs may be used to determine whether the sample is appropriate for DNA analysis. This type of examination is on a case-by-case basis and at analyst discretion.

To evaluate the suitability of hair for DNA analysis, examine the entire sample using a stereomicroscope. Examination may allow for the differentiation between apparent hairs and apparent fibers, and whether there is adhered cellular material and/or a root that may be suitable for DNA analysis.

The results of the stereomicroscopic examination will be documented in the technical record. It is recommended that the microscopic image with a scale bar be included in the technical record. If the result is that the sample includes a hair with adhering cellular material and/or a root, the report shall include a statement that recommends the item be submitted to an approved lab for DNA analysis.

9.1.2.1. Basic Hair/Root Identification:

Examine a sample using a stereomicroscope to determine if a hair has a root.

Analyst will make the following interpretations:

1. Sample is an apparent hair with adhering cellular material and/or a root. Sample may be suitable for DNA analysis.
2. Sample is an apparent hair and is unable to determine if there is a root. Provide reasoning (e.g., covered in substance or debris). Sample may be suitable for DNA analysis.
3. Sample is an apparent hair without either adhering cellular material or a root. Sample may be unsuitable for DNA analysis. Other testing approaches, such as examination by a Materials Analysis hair analyst or alternative DNA testing technologies, should be considered and discussed with the requester/lead investigator as appropriate.
4. It is inconclusive whether the sample is hair. Provide reasoning.
5. If the sample is not hair, indicate whether it is a possible fiber.
6. If the sample is not a hair or fiber (e.g., botanical debris), describe the appearance.



Characteristics observed and used to determine the above interpretations should be noted in the technical record and could include: Color, general morphology, length, root shape, pigment distribution, and any scale patterns/structures

9.2. Latent Print Evidence

If latent print processing is requested and apparent latent print evidence is observed during the examination of an item for biological stains, consideration must be given as to which evidence may be more probative and/or which should be preserved first. During this analysis, if the expected biology processing examinations have the potential to alter or destroy latent print evidence, care should be taken to avoid swabbing any areas that may be processed for latent print evidence. If the analyst is not authorized in latent print processing, consultation with an analyst authorized in the latent print discipline shall be done before the continuation of processing.

See the BPD Evidence Processing and Latent Print Technical Manual for detailed information.

10. Biological Evidence Collection Methods

For additional collection methods for items not covered below, refer to the Crime Scene Investigation Technical Manual.

10.1. Wet Blood

Absorb a sample from the stain onto a sterile swab, saturating the swab, and allow the swab to dry in a swab box.

- Small biological stain: Collect the entire sample with one swab, concentrating the stain on the tip of the swab.
- Large biological stain: Collect the sample with up to two swabs, when practical.

10.2. Dry Blood

Collect the item, cut out the stained area, or collect the stained area onto a sterile swab(s) moistened with distilled water (dependent on the size of the entire item) and place into a swab box.

- Small biological stain: Collect the entire sample with one moistened swab, concentrating the stain on the tip of the swab.
- Large biological stain: Collect the sample with up to two moistened swabs when practical.

10.3. Semen and Saliva

Follow the above protocols for small or large, dry, or wet stains.

10.4. Latent Blood

Chemical enhancement (Bluestar®, Amido Black, Acid Yellow 7, Hungarian Red, Leucocrystal Violet) helps visualize and enhance bloodstains, including latent bloody shoe and fingerprint evidence; however, some of these processes can degrade DNA. It is strongly recommended to collect a sample of apparent blood prior to processing, regardless of chemical used. See BPD Evidence Processing and Latent Print Technical Manual for Amido Black, Acid Yellow 7, Hungarian Red, and Leucocrystal Violet procedures.



10.5. Possible DNA Material from a Drinking Receptacle/Other Items with a Mouth Rim

Evidence with mouth rims may be swabbed for possible DNA. Collect a sample from the mouth rim with a minimum of one moistened swab. Based on the size of the area to be swabbed, up to two swabs can be used. Concentrate the sample on the tip of the swab(s). If liquid is present in a drinking receptacle, a sample of the liquid may be collected after swabbing. Discard the remaining liquid.

10.6. Envelope Seals, Postage Stamps, and other Adhesive Surfaces

When encountering a surface that may contain a body fluid, such as saliva, the area may be sampled as follows.

- Collect a sample from the adhesive side of the seal, stamp back, or other applicable area (if accessible) with a minimum of one moistened swab. Based on the size of the area to be swabbed, up to two swabs can be used. Concentrate the sample on the tip of the swab(s).
- When the adhesive is stuck to another surface, attempt to release the adhesive through methods described in the Evidence Processing and Latent Print Technical Manual.
- When swabbing is not feasible, use a sterile scalpel to cut a small sample from the target area of the item. Place the sample into a coin envelope, or other appropriately sized breathable packaging, prior to packaging into a paper envelope. *Consideration must be made if also processing the item for latent impressions.

10.7. Urine

Wet or dry urine on bedding materials, diapers, clothing, etc., generally contains limited forensic biology value. If a urine sample or evidence item with urine stain(s) is collected, ensure the item is frozen when possible.

10.8. Feces

Feces generally contain limited forensic biology value. If a fecal sample appears to contain potentially probative blood or semen, the suspected blood or semen samples should be collected immediately to prevent further contamination by the fecal matter. Swab the blood or semen, attempting to minimize fecal contamination on the swabs. If DNA analysis is needed on fecal matter (example: missing child case), the evidence may be sampled as follows.

- Swab the exterior of the feces (for rectal skin cells). Follow the above protocols for dry or wet stains.
- Typically, feces as a whole do not require collection.

10.9. Touch DNA Evidence (handle, surface, etc.)

Touch DNA may be found on items that were touched or handled. Many factors affect whether touch DNA may be found on an item, and include, but are not limited to, the DNA source, shedder status, handling time, handling intensity, surface type, temperature, humidity, UV, and recovery methods. Touch DNA can be challenging to work with due to low quantities of DNA and has high intra- and inter-person variability. Touch DNA is not a visible stain/substance.

To preserve touch DNA, collect the item of evidence, if possible, or take a swabbing of the item using the following protocols.



- With a sterile swab moistened with sterile water, use the entire surface of the swab to apply vigorous pressure. If the area is large, use two sterile swabs with sterile water collected simultaneously and placed in the same swab box. The technical record shall indicate evidence was collected for the preservation of possible touch DNA.

10.10. Firearms Touch DNA Evidence

Swabbing for touch DNA is case-dependent. Avoiding the visible ridge detail and/or areas that appear to be stained with blood, follow the above touch DNA collection method. Of note, additional swabs may be used for collection depending on the amount of staining transferred to the swab. Swab the firearm, focusing on the following areas (dependent on substrate). Consider all textured areas, buttons, and levers. If the swab sample may be submitted to a specific laboratory for touch DNA analysis (such as ISPFs), the analyst may follow firearm touch DNA collection recommendations from that laboratory. Example areas below (not an all-inclusive list):

- Revolvers – trigger, hammer, cylinder release, ridged surfaces on the cylinder, and grip
- Semiautomatic handguns – trigger, slide serrations, hammer, and grip
- Weapon Magazines – baseplate of the magazine and the magazine follower, and feed lips
- Rifles and Shotguns – trigger, stock, and forestock

10.11. Wearer DNA

Wearer DNA may be found on items worn by an individual, often through the transfer of epithelial cells from the skin, sweat, or other body fluids to the item. Consider sending the entire item to an external laboratory that performs DNA analysis.

10.11.1. If the item is nonporous (e.g., eyeglasses) and is being processed for latent impressions, a swab sample may be collected before latent processing.

10.11.1.1. With one or two sterile swabs (depending on the size of the area) moistened with distilled water, use the entire surface of the swab to apply vigorous pressure to the surface. Place the swab(s) in a swab box.

10.12. General Surface Rubbings

When the substance is not visible using various lighting conditions, the analyst may collect general surface rubbings based on the case scenario.

10.12.1. With one or two sterile swabs (depending on the size of the area) moistened with distilled water, use the entire surface of the swab to apply vigorous pressure to the surface. Place the swab(s) in a swab box.

10.13. Collecting Testing Extracts

At the analyst's discretion, testing extract(s) may be collected. It is recommended to retain the extract if there is a limited sample remaining for DNA testing (e.g., a Stain swabbed with two swabs, one swab used for confirmatory testing with positive results. Only one swab remains for future DNA analysis). Alternatively, the analyst may retain extracts on a case-by-case basis.

10.13.1. Extracts from the same case may be collected as one item number if the same extraction buffer was used.



- 10.13.2. Before collecting extracts, it is recommended that the evidence sample (swab/cutting) be removed from the centrifuge tube, placed in a separate, clearly labeled centrifuge tube, and collected. A centrifuge should be utilized to spin down the liquid prior to collecting the extraction liquid in one tube and the evidence sample in a separate tube. See centrifuge procedure for further.
- 10.13.3. If collecting extracts, a negative control (minimum of 300 µl of the extraction buffer) shall be retained and clearly labeled. Package with the extracts.
- 10.13.4. Packaging:
- Ensure the centrifuge cap(s) are tight.
 - Ensure each centrifuge tube is uniquely labeled and the unique identifier documented in the technical record.
 - Place centrifuge tubes in clear plastic recloseable bag(s).
 - Place the bag(s) in paper packaging.
 - Store frozen. This should occur as soon as possible.

10.14. Special Circumstances (fire evidence/scenes)

Extreme heat, in close proximity to the fire, and the presence of soot can negatively impact the sensitivity of biology screening tests, substance collection, and DNA analysis. Consider using deionized water or 70% isopropanol to remove the soot before testing and/or collection. Several methods are available, including scraping, brushing, and wiping. Wiping is the preferred method.

Procedure for the wipe method:

1. Saturate the wipe with deionized water or 70% isopropanol.
2. Wring out excess liquid.
3. Rub a wipe across the surface
4. Stop wiping when the wipe ceases to turn black from the sample's surface.

11. Biological Evidence Testing Methods

Numerous screening methods, presumptive, and/or confirmatory tests are available for use with blood, semen, and saliva at the crime scene and/or on evidence from a scene.

For fire evidence/scenes, see the Special Circumstances (fire evidence) section.

11.1. Alternate Light Source (ALS)

An alternate light source is a specialized light that combines powerful illumination with the ability to select wavelengths of light. Certain combinations of wavelength and colored goggles/glasses/filters may enable the visualization of physical evidence such as biological fluids (blood, semen, and saliva). Biological fluids may fluoresce or absorb light, appearing darker (blood). ALS should not be considered an alternative to chemical presumptive tests when possible biological fluids are visible.

11.1.1. Method

1. An examination using an ALS should be conducted in a darkened environment.

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2. Follow the manufacturer's recommendations for proper operation of the ALS.
3. Select the wavelength of light and goggles/glasses/filter appropriate for the biological fluid targeted and document in the technical record. When using the Crime-lite 82S, Violet (395-425nm) is a good starting point for bloodstains. UV (350-380nm) is a good starting point for other body fluids.
4. Examine evidence/surface and systematically scan for areas of fluorescence/absorption as appropriate.
5. Circle or otherwise document areas of fluorescence/absorption so they may be sampled and/or located later for follow-up testing, and document the locations of those areas in the notes. The locations where areas of fluorescence/absorption were noted may be recorded using words, diagrams, photographs, or a combination thereof.

11.1.2. Interpretations

Fluorescence/absorption may indicate the presence of a biological fluid. However, further analysis shall be performed before the presence of a biological fluid is determined. The absence of fluorescence/absorption does not confirm the absence of a biological fluid.

11.1.3. Cautions/Safety

- See the BPD Crime Lab's Health and Safety Procedures Manual
- Several other materials may also fluoresce, such as stains from food, drink, cosmetics, laundry detergent, and/or the substrate material itself.
- Not all semen stains will fluoresce; it depends on the stain and other factors.
- Blood serum and semen stains mixed with blood may fluoresce.

11.1.4. Infrared (IR)

Bloodstains on dark-colored fabrics/surfaces, such as black fabrics and denim, can be difficult to visualize. Infrared lighting/photography can be used to aid the analyst in locating these stains. Dark fabric/surfaces and bloodstains will absorb most of the light in the visible spectrum, making it difficult to distinguish the stains from the background. Infrared lighting (700-900nm) causes some dark fabric/surfaces to reflect visible and infrared wavelengths of light. If present, a bloodstain on the fabric/surface will typically still absorb both wavelengths of light. This will result in the surface appearing white or gray, while the bloodstains will still appear dark in color. If a substrate does not reflect IR, IR light may still provide enough contrast between the substrate and bloodstains to visualize stains.

An infrared camera is a DSLR camera that has been converted to detect and measure infrared light. The Crime-lite Auto also has IR capabilities. Alternatively, a video camera with night-time viewing capabilities may also be used to view infrared. Either may be used in conjunction with an IR light emitter, such as an alternate light source. Images and/or videos recorded by an IR camera/video camera are black and white because infrared light is longer in wavelength than the colors of the visible spectrum. This also means IR light cannot be seen by the naked eye and can only be observed



using an IR-sensitive camera/video camera. IR should not be considered an alternative to chemical presumptive tests when possible biological fluids are visible.

11.1.4.1. Method

1. An examination using IR can be conducted in various lighting conditions.
2. Select equipment to be used for viewing the substrate (DSLR IR camera or Crime-lite AUTO). Follow the manufacturer's recommendations for proper operation of each.
 - a. The DSLR camera's white balance should remain on "PRE" when in manual mode. Viewing needs to be done through "Live view".
3. Examine evidence/substrate and systematically scan for areas of absorption as appropriate.
4. Circle or otherwise document areas of absorption so they may be sampled and/or located later for follow-up testing, and document the locations of those areas in the notes. The locations where areas of absorption were noted may be recorded using words, diagrams, photographs, or a combination thereof.

11.1.4.2. Interpretations

Absorption may indicate the presence of a biological fluid. However, further analysis shall be performed before the presence of a biological fluid is determined. The absence of an absorption result does not confirm the absence of a biological fluid.

11.1.4.3. Cautions/Safety

- See ALS cautions/safety
- Several other materials may also absorb light, such as inks, oils, charred material, paint, GSR, and/or the substrate material itself.

11.1.5. Crime-lite AUTO

The Crime-lite AUTO is an all-in-one portable forensic imaging system that allows the analyst to search surfaces using UV, visible, and IR wavelengths and capture photographs with the built-in camera. The Crime-lite AUTO combines ALS, IR, photography, and video capabilities into a single system. The Crime-lite AUTO can be used to visualize evidence such as biological fluids (semen, saliva, and blood) with the same principles as this manual's ALS and IR sections. Physical evidence may fluoresce or absorb light, appearing darker. This device should not be considered an alternative to chemical presumptive tests when possible biological fluids are visible.

11.1.5.1. Method

1. An examination using this unit should be conducted in a darkened environment.
2. Follow the manufacturer's recommendations for the proper operation of the unit.
3. Examine the evidence/surface. Use the wavelength or combination of wavelengths most appropriate for the surface type and targeted evidence. The unit can automatically select the corresponding filter to optimize visualization. Manual control of filter selection is also available.
4. Systematically scan for areas of fluorescence/absorption as appropriate.



5. Circle or otherwise document areas of fluorescence/absorption so they may be sampled and/or located later for follow-up testing, and document the locations of those areas in the notes. The locations where areas of fluorescence/absorption were noted shall be photographed, videoed, and/or documented in the technical record. Wavelengths used should be documented in the technical record.

11.1.5.2. Interpretations

- See ALS interpretations

11.1.5.3. Cautions/Safety

- See ALS and IR cautions/safety

11.1.5.4. Digital Imaging

The unit can enhance/process images within the software. All requirements outlined in the Digital Imaging section of this manual and the BPD Evidence Processing and Latent Print Technical manual shall be considered.

11.1.5.5. [Discover Fingerprint Imaging System](#)

Used in conjunction with the Crime-lite AUTO.

- 11.1.5.5.1. See BPD Crime Lab's Evidence Processing and Latent Print Technical Manual

11.2. Presumptive Blood

After locating a suspected bloodstain or area in question, the stain may be tested with a preliminary chemical color test to determine if blood is present. Not every stain may need to be tested based on the case specifics or at the analyst's discretion. If testing was requested, the reason for not testing or deviating from the request shall be documented in the technical record.

It is under the analyst's discretion, and dependent upon the case scenario, whether to use a confirmation test for human blood following the results of the presumptive test. If the results indicate a presumptive positive for blood and the laboratory was requested to determine if it is of human origin, an RSID™ test shall be performed and documented.

The following presumptive test is not species-specific and will produce a positive reaction with animal blood.

11.2.1. Tetramethylbenzidine (TMB) Test

TMB is a catalytic test that is based on the peroxidase-like activity of hemoglobin.

Hemoglobin has the ability to cleave oxygen molecules from hydrogen peroxide (H₂O₂) and catalyze the reaction from the reduced form of 3,3',5,5'-tetramethylbenzidine to the oxidized blue-green colored product, thus indicating the presence of blood (heme).



A commercially available tetramethylbenzidine (TMB) presumptive blood test kit can also be used. Use of TMB follows the procedures outlined in the instructions that accompany the TMB reagent kit. If making your reagent, follow the procedure outlined below.

11.2.1.1. Reagents

- Weigh out 0.2 grams of TMB
- Add to a 50ml beaker
- Measure out 10 ml of glacial acetic acid and add to the TMB
- Mix thoroughly until the TMB is dissolved
- Store refrigerated – expiration 1 week

Hydrogen Peroxide (H₂O₂) 3%

- Measure out 10 ml of 30% hydrogen peroxide
- Add 90 ml of deionized water
- May be stored at room temperature or refrigerated – expiration date one year.

11.2.1.2. Method

1. Take a small cutting, swabbing, or filter paper rubbing of the targeted stain.
2. Place 1 drop of TMB reagent on the collected sample.
3. Place 1 drop of 3% H₂O₂ on the sample
4. Interpret and document the results in the technical record.

11.2.1.3. Interpretation

- Positive: A positive reaction is indicated by the sample turning a deep blue color within 5-10 seconds.
 - On a white substrate, the developed color appears as a blue/green.
 - On a red substrate, the developed color appears more like a deep blue or indigo.
 - A very weak reaction from a brown stain is an indication that the stain could be aged or diluted, and the heme molecules are less likely to be removed from the substrate.
- Negative: An absence of color change, especially on repeated tests, is an indication that there is no or insufficient hemoglobin present.
- Inconclusive: A color change that is suspected to be from substrate contamination, a positive reaction that occurs with the addition of TMB reagent only, or other circumstances that lead the analyst to be cautious about a positive or negative interpretation.



Note: the analyst shall document the observation(s) that led them to make an inconclusive determination in their technical record

11.2.1.4. Cautions/Safety

- Do not place reagents directly on the evidence.
- It should be noted that the activity of the 3% hydrogen peroxide solution decreases over time. Caution should be used when using hydrogen peroxide that is older than 6 months.
- To optimize the efficacy of hydrogen peroxide, analysts should store the working solution in a refrigerator/cooler when not in use and limit the reagents' exposure to air and light.
- In the absence of blood, the two reagents will begin to react with each other, within a period of 15-20 seconds, and will produce an interference color, which may appear to be a positive result.
- False positives
 - Substances other than blood may yield positive reactions.
 - Examples include but are not limited to catalysts, and fruits and vegetables such as grapes, garlic, and horseradish.
- False negatives
 - It is not recommended to use TMB on items previously processed with Bluestar®. Negative results obtained with the TMB test after Bluestar® application should be interpreted with caution, as Bluestar® may interfere.

11.2.1.5. Controls

- Positive: A known blood stain.
- Negative: Unstained swab, fabric, filter paper, or empty spot well.
- A positive and negative control will be run before use on samples.

11.3. Chemical Blood Enhancement

A blood-enhancing chemical may be used for the detection and visualization of blood that has been altered (cleaned up), enhancement of impression evidence or stains, and as a presumptive test for an unknown stain. Typically, a blood enhancement technique is conducted after all other analyses are complete. Refer to the Latent Blood section for additional information regarding enhancing impression evidence.

11.3.1. Bluestar® Test

Bluestar® is a catalytic blood test that gives a positive reaction in the presence of blood due to the peroxidase activity of hemoglobin. A positive reaction is a chemiluminescence that is observable in a dark environment. Bluestar® is most useful when blood is suspected



but is not visible, and may be used to help locate non-visible blood stains. Bluestar® is an ideal reagent to locate non-visible blood or enhance suspected blood that is on black or dark colored porous and non-porous substrates. More specific presumptive and/or confirmatory blood tests may be subsequently used on Bluestar® positive areas/stains. Use of Bluestar® follows the procedures outlined in the instructions that accompany the Bluestar® kit.

11.3.1.1. Supplies

Working Solution (125 ml is generally sufficient to cover a 250 sq. ft. area)

- The working solution is prepared before each use. For optimal results, this solution should be used within 3 hours after mixing.
- Bluestar® Forensic tablet box holds two tubes; one contains beige tablets (containing Sodium Hydroxide), and one contains white tablets (containing Hydrogen Peroxide – Urea)
- Open the spray bottle (mister); add 125 mL (4 fl. oz) of distilled water.
- Take a white tablet out of the white-top tube and close the tube immediately. Take a beige tablet out of the red-top tube and close the tube immediately. Ensure caps are not switched.
- Add the pair of tablets to the distilled water. If you need more working solution, use 125 ml (4 fl. oz) per pair of tablets.
- Close the spray bottle firmly.
- Allow about 1 or 2 minutes for complete dissolution and mixing of the chemicals, swirling gently with a circular motion of your hand. Do NOT shake the container upside down.

The camera and tripod should be set up before the application.

11.3.1.2. Method

1. Determine the area to be sprayed with Bluestar®. Total darkness is not required. Natural low-intensity diffused light is preferred. Allow eyes time to adjust to the lighting condition.
2. Spray lightly, horizontally ahead of you, at least 50 cm (2') away from the target, in a side-to-side sweeping motion. Do not point towards the ground.
3. When documenting via photography, photograph chemiluminescence immediately after application.
4. Additional application may be necessary; however, use caution to avoid saturation and dilution of the target.
5. Circle or otherwise mark areas of chemiluminescence so they may be sampled and/or located for follow-up testing, and document the



locations of those areas in the notes. The locations where areas of chemiluminescence were noted may be recorded using words, diagrams, photographs, or a combination thereof.

11.3.1.3. Photography

Photographing the chemiluminescence reaction is recommended but not required. This is on a case-by-case basis and at the analyst's discretion. It is challenging to capture the chemiluminescence, and specific lighting conditions are necessary. Digital enhancement of Bluestar® captured images may be needed.

Photography settings: Utilize standard low-light photographic techniques for ISO, aperture setting, shutter speed, and subject lighting. The Bluestar® technical sheet has photography setting recommendations.

Note that the chemiluminescence reaction with Bluestar® can be bright enough to be photographed without significantly darkening the room; however, longer exposure times will typically be necessary to assure quality, so use of a tripod is essential. It may be necessary to cover windows and doors. Outdoor scenes should be photographed at night with as few lights illuminated as possible.

11.3.1.4. Interpretation

- Positive: Blue chemiluminescence is observed when sprayed with Bluestar®. See Cautions/Safety (false positives).
- Negative: No chemiluminescence observed.
- Inconclusive: Whitish chemiluminescence or reaction that has a fast initial burst of color that rapidly diminishes. See Cautions/Safety (false positives).

11.3.1.5. Cautions/Safety

- All other analysis and search options should be considered before the Bluestar® application.
- Spraying of Bluestar® dilutes bloodstains. If possible, sample stains prior to the use of Bluestar®.
- Bluestar® reagent is primarily water and will cause dried bloodstains to dissolve. Any pattern evidence (e.g., shoeprints, fingerprints, impact spatter, etc.) will be negatively affected. If pattern evidence is located, Bluestar® use should be immediately stopped and the evidence and scene assessed to determine the best method to use before proceeding.



- When photographing, photographic equipment should be set up and ready for use before the Bluestar® application.
- Use in a ventilated area when possible.
- Wear appropriate PPE when spraying.
- False positives
 - Substances other than blood may yield positive reactions.
 - Examples include but are not limited to the presence of certain household detergents, chlorine, some paints and varnishes, copper, certain iron-metabolizing plants, and soils containing iron.
 - False positives are easily identifiable because their color, brightness, and duration differ from those of the typical reaction with blood. False positives may result in a whitish chemiluminescence or one that has a fast, initial burst of color that rapidly diminishes.
- Any samples collected after Bluestar® processing should be thoroughly dried before packaging.
- Unused mixed Bluestar® should be disposed of in a sink under running water. Do not store mixed Bluestar® as it may cause the bottle to swell and leak.
- A presumptive blood test, such as Tetramethylbenzidine, used after Bluestar® application, can result in a false negative. A confirmatory blood test should be considered instead.
 - If sending an item previously processed with Bluestar® to an external DNA laboratory, it is recommended that analysts communicate the following: The sample was collected after Bluestar® application, presumptive testing may yield a false negative result, and request that the item be forwarded for DNA analysis.

11.3.1.6. Controls

- Positive: Known blood stain on a substrate
- Negative: Unstained substrate
- A positive and negative control will be run before use on samples

11.4. Confirmatory for Human Blood



11.4.1. RSID™ - Blood (Rapid Stain Identification of Human Blood)

This RSID™ - Blood test is an immunochromatographic test specific for human blood. It uses dual monoclonal antibodies specific for human Glycophorin A Antigen, which is abundantly and specifically expressed in red blood cells. No cross-reaction has been observed with other body fluids. No cross-reactivity has been observed with other species of animal blood. No interference has been observed with stains that have been treated with Bluestar®. No high-dose hook effect.

Note: Using RSID™ – Universal Buffer, a single extract can be used to test for three body fluids (blood, semen, and saliva). If testing for more than one body fluid using the RSID™ test, refer to the RSID™ – Universal Buffer Technical Information and Protocol Sheet. If there are multiple universal buffer options due to multiple RSID™ test kits being utilized, select one ensuring that it is not expired.

Use of RSID™ - Blood follows the procedures outlined in the instructions that accompany the RSID™ - Blood kit

11.4.1.1. Supplies

- RSID™ cassette
- RSID™ – Universal Buffer

Note: Ensure RSID™ – Universal Buffer is at room temperature before extraction.

- Centrifuge (optional)

11.4.1.2. Method

1. Label RSID™ test device (e.g., centrifuge tube) for each sample with the item # or other unique identifying mark, including controls. Repeat the procedure below for each sample. If the extract may be collected, a spin filter basket may be used for extraction. Follow the centrifuge procedure.
2. Extract the swab in 200-300 µl of the RSID™ extraction buffer for 1-2 hours.
 - a. Alternatively, a cutting of a swab or filter paper rubbing may be used. Sufficient RSID™- Universal Buffer should be added to easily cover the sample.
 - b. Stains on fabric or porous substrates should be sampled by taking a cutting (≈ 20 mm²) of the item. Depending on the stain's appearance/size, a smaller cutting size than recommended may be used. The cutting should be extracted in 100 µL of RSID™- Universal Buffer for 1-2 hours.



3. A general guideline of a maximum of 10% of extract, up to a maximum of 20 μL should be run. The remainder of the extract can be preserved (frozen) for DNA analysis if necessary. The buffer provided is STR-free and contains a DNA stabilizer. The provided buffers do not interfere with extraction or amplification.
 - a. See section on Collecting Testing Extracts.
4. Remove the cassette from the foil pouch. Discard silica gel desiccant.
5. Label a cassette for each sample with the item # or other unique identifying mark, including controls.
6. Combine extract aliquot (max of 20 μL) with RSID™-Universal Buffer to bring the test sample to a total volume of 100 μL .
7. Add diluted sample RSID™-Universal Buffer to the sample window.
 - a. Alternatively, you may add 100 μL of the sample to the sample window. This will have little to no effect on the sensitivity or specificity of the test; however, any problems encountered by using a concentrated sample may be avoided if the extraction is diluted in RSID™-Universal Buffer, as described above.
8. At 10 minutes, score and record results as shown in the Scoring Diagram in the RSID™ kit.

Note: Assays should be performed at room temperature.

11.4.1.3. Interpretation

- Positive: The formation of red lines in the test “T” and control “C” regions of the membrane at exactly 10 minutes.
- Negative: The formation of a red line in the control “C” region only at exactly 10 minutes.
- Invalid: The formation of a red line only in the test “T” region indicates a failed test. The test is invalid and should be repeated with a new test. This may occur if the sample is too thick and does not migrate up the membrane; diluting the sample may correct the problem. If an additional invalid test is obtained, no conclusion can be made, resulting in test failure. Recommend submitting the evidence for DNA analysis, since no conclusion can be made.
- Inconclusive: Shadow band, also referred to as ghost band, is a hazy, barely visible link (so faint that the color of the line is indistinct) that appears at the test line near the 10-minute time window. This is a rare phenomenon, but analysts must be aware of it.
 - When possible, seek a second opinion from a trained analyst. Document in the technical record.

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- Often, there is a disagreement between analysts that a band is present at all.
 - If there is enough sample, consider running the sample.
 - If unable to determine the result of the test(s), report the result as inconclusive.
 - The test card results, for both the casework and the positive and negative control, shall be documented in the technical record.
- 11.4.1.4. Cautions/Safety
- Do not use buffers or cassettes after the printed expiration date.
 - The detection limit, used as suggested, is < 1 µL of human blood. Undiluted blood should not be used.
 - Under standard laboratory testing, users will not observe false negative results due to high dose hook effects.
- 11.4.1.5. Controls
- Positive: Known human blood
 - Negative: RSID™ –Universal buffer
 - A positive and negative control will be run before use on samples.

11.5. Presumptive Semen

Examination of items of evidence for the presence and identification of human semen is routinely performed in Forensic Biology using visual examination, screening, presumptive testing, and confirmatory testing for identification.

If the results indicate a presumptive positive for semen, an RSID™ - Semen test may be performed, dependent upon the circumstances of the case.

11.5.1. Acid Phosphatase (AP) Test

Acid phosphatase (AP) is an enzyme found at elevated levels in semen. When the Brentamine reagent is applied to the test sample, a purple color forms in the presence of AP activity. The AP enzyme cleaves phosphate from sodium.

This presumptive test is used to indicate the presence of semen; it does not confirm the presence of semen since the enzyme can be found in lower concentrations in other body fluids (e.g., vaginal secretions and fecal material) as well as certain plants and fungi. Use of AP follows the procedures outlined in the instructions that accompany the AP reagent kit.

If making your own reagent, follow the procedure outlined below.

11.5.1.1. Reagents

The reagent volume may be reduced as long as the ratio of chemicals remains consistent.

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- Acid Phosphatase Stock Solution (A)
 - Fast Blue B 1g
 - Sodium acetate 20g
 - Distilled water 100ml
 - Glacial acetic acid, ≥99.5% 10ml
 - Combine and refrigerate – expiration 6 months
- Acid Phosphatase Stock Solution (B)
 - 1-naphthyl phosphate sodium salt, ≥98% titration 0.4g
 - Distilled water 5 ml
 - Combine and refrigerate – expiration 6 months
- Acid Phosphatase Working Solution (Brentamine reagent)
 - Solution A 10 ml
 - Solution B 1 ml
 - Distilled 89 ml
 - Combine and refrigerate – expiration 2 weeks

It is recommended that the prepared reagents be retested with positive and negative controls approximately every 2 hours. If you observe a loss of sensitivity in the reagents, make up new reagent(s) and document the control test results in the technical record.

11.5.1.2. Spot Test Reagent

1. The AP spot test working solution must be made fresh daily.
2. Add 0.26 grams of the AP Spot Test to 10 ml of deionized water. The reagent volume may be reduced if the ratio of AP Spot Test to deionized water remains constant.
3. Mix the solution thoroughly.
 - a. Place the AP working solution in an amber-colored bottle to minimize light exposure.
 - b. Cap the AP working solution when not in use to minimize oxidation.
4. Wait 10 minutes before using this reagent.

11.5.1.3. Methods

Individual Stains

1. Moisten a piece of filter paper or swab with distilled water.
2. Press or gently rub the filter paper or swab on the targeted stain.
3. Apply 1-2 drops of Brentamine reagent to the paper or swab and allow 3 minutes for color development.



- a. Old and/or desiccated samples may require closer to 3 minutes for color development.
4. Test result and approximate location(s) of stain(s) shall be documented in the case file.

Mapping (whole garments, bed sheets, underwear crotch panels, etc.)

1. Cut filter paper to appropriate lengths. Moisten the filter paper with distilled water using a spray bottle and blot.
2. Lay the moist filter paper over the area of interest and press onto the item for a minimum of 30-60 seconds.

Note: Additional filter paper, a glass or plastic plate, and weight (i.e., books or bricks) on the paper may be useful at this step. Large areas may require two or more filter paper overlays laid side by side to map efficiently. If multiple sheets are used, they should be marked in a manner that facilitates their orientation to one another and relates positive test reactions to specific areas of the evidence item.

3. After pressing, hang the filter paper in a chemical fume hood and spray with Brentamine reagent. Allow 3 minutes for color development.

Note: Brentamine reagent may also be applied dropwise to the filter paper to avoid aerosolizing the reagent. The drop-wise application does not require a chemical fume hood.

4. Test result and approximate location(s) of stain(s) shall be documented in the case file.

11.5.1.4. Interpretation

- Positive: A purple or faint purple color change immediately to within 3 minutes. See Cautions/Safety
- Negative: The absence of a purple color change
- Inconclusive: A purple or faint purple color change occurring after 3 minutes. Reddish purple or pink color change within 3 minutes. See Caution/Safety

*The timelines listed above are guidelines only. Old and/or desiccated samples may require closer to 3 minutes of reaction time for color development due to a weak semen stain or from AP in other body fluids (such as vaginal acid phosphatase).

Items with negative or inconclusive results may be submitted to an approved laboratory that performs DNA analysis. The determination is left

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to the analyst's judgement and should be based on training and experience and bearing in mind the circumstances of the case.

11.5.1.5. Cautions/Safety

- When interpreting AP results, make sure to compare the color of the reaction for the tested sample to the controls. If the color change is faint but the purple is the same shade as the positive control, this is a positive result. Other body fluids may give a slow reddish-purple or pink color change, which should not be considered a positive reaction.
- If the color of the stained substrate is similar to the color of the positive control, an unstained area of the substrate should be tested as well.
- False positives
 - AP can be found in lower concentrations in other body fluids (e.g., vaginal secretions and fecal material) as well as certain plants and fungi
- o-dianisidine (Fast Blue B) is a possible carcinogen and should be handled cautiously.
- To optimize efficacy, store the reagents in a refrigerator/cooler when not in use and limit the reagents' exposure to air and light.

11.5.1.6. Controls

- Positive: Known human semen
- Negative: Unstained swab, fabric, filter paper, or empty spot well.
- A positive and negative control will be run before use on samples

11.6. Confirmatory Semen

11.6.1. RSID™ - Semen (Rapid Stain Identification of Human Semen)

The RSID™ - Semen test is an immunochromatographic test specific for human semen. It uses dual monoclonal antibodies to detect the presence of semenogelin, which is a protein produced by the seminal vesicles and is responsible for coagulum post ejaculation of semen. No cross-reaction has been observed in other body fluids. No cross-reactivity has been observed with other species of animal semen.

Use of RSID™ - Semen follows the procedures outlined in the instructions that accompany the RSID™ - Semen kit.

Note: Using RSID™ – Universal Buffer, a single extract can be used to test for three body fluids (blood, semen, and saliva). If testing for more than one body fluid using the RSID™ test, refer to the RSID™ – Universal Buffer Technical Information and Protocol Sheet. If



there are multiple universal buffer options due to multiple RSID™ test kits being utilized, select one ensuring that it is not expired.

11.6.1.1. Supplies

- RSID™ cassette
- RSID™ – Universal Buffer
- Centrifuge (optional)

Note: Ensure RSID™ – Universal Buffer is at room temperature before extraction.

11.6.1.2. Method

1. Label RSID™ test device (e.g., centrifuge tube) for each sample with the item # or other unique identifying mark, including controls. Repeat the procedure below for each sample. If the extract may be collected, a spin filter basket may be used for extraction. Follow the centrifuge procedure.
2. Extract a swab in 300-400 µl of the RSID™ extraction buffer; shake for 10 seconds, longer incubation times are optional.
 - a. Alternatively, a cutting of a swab or filter paper rubbing may be used. Sufficient RSID™- Universal Buffer should be added to easily cover the sample.
 - b. Stains on fabric or porous substrates should be sampled by taking a cutting (≈ 20 mm²) of the item. Depending on the stain's appearance/size, a smaller cutting size than recommended may be used. The cutting should be extracted in 100 µL of RSID™-Universal Buffer for 10 seconds with shaking, or longer.
3. A general guideline of a maximum of 10% of extract, up to a maximum of 20 µL should be run. The remainder of the extract can be preserved (frozen) for DNA analysis if necessary. The buffer provided is STR-free and contains a DNA stabilizer. The provided buffers do not interfere with extraction or amplification.
 - a. See section on Collecting Testing Extracts.
4. Remove the cassette from the foil pouch. Discard silica gel desiccant.
5. Label a cassette for each sample with the item # or other unique identifying mark, including controls.
6. Combine extract aliquot (max of 20 µl) with RSID™-Universal Buffer to bring test sample to a total volume of 100 µl.
7. Add diluted sample RSID™-Universal Buffer to the sample window.

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- a. Alternatively, you may add 100 µl of the sample to the sample window. This will have little to no effect on the sensitivity or specificity of the test; however, any problems encountered by the high dose hook effect or using a concentrated sample may be avoided if the extraction is diluted in RSID™-Universal Buffer, as described above.

8. At 10 minutes, score and record results as shown in the Scoring Diagram in the RSID™ kit.

Note: Assays should be performed at room temperature.

11.6.1.3. Interpretation

- Positive: The formation of red lines in the test “T” and control “C” regions of the membrane at exactly 10 minutes.
- Negative: No formation of a red line in the control “C” region at exactly 10 minutes. See Cautions/Safety.
- Invalid: The formation of a red line only in the test “T” region indicates a failed test. The test is invalid and should be repeated with a new test. This may occur if the sample is too thick and does not migrate up the membrane; diluting the sample may correct the problem. If an additional invalid test is obtained, no conclusion can be made, resulting in test failure. Recommend submitting the evidence for DNA analysis, since no conclusion can be made.
- Inconclusive: Shadow band, also referred to as ghost band, is a hazy, barely visible link (so faint that the color of the line is indistinct) that appears at the test line near the 10-minute time window. This is a rare phenomenon, but analysts must be aware of it.
 - When possible, seek a second opinion from a trained analyst. Document in the technical record.
 - Often, there is a disagreement between analysts that a band is present at all.
 - If there is enough sample, consider running the sample.
 - If unable to determine the result of the test(s), report the result as inconclusive.
- The test card results, for both the casework and the positive and negative control, shall be documented in the technical record.

11.6.1.4. Cautions/Safety

- Do not use buffers or cassettes after the printed expiration date.



- The detection limit, used as suggested, is 1 µL of human semen. Undiluted semen should not be used.
- Weak positive/False negative
 - A weak positive or false negative may occur when very high levels of target antigen are present in the tested sample, causing a high-dose hook effect. If you suspect that this may have occurred, you should dilute the extract (1:10 fold dilution with RSID™ Universal Buffer) and retest.

11.6.1.5. Controls

- Positive: Known human semen
- Negative: RSID™-Universal buffer
- A positive and negative control will be run before use on samples.

11.7. Presumptive Saliva

Saliva is difficult to conclusively identify because of the lack of sufficient amounts of detectable substances specifically unique to saliva. While it is not unique to saliva, the enzyme α -amylase provides an important parameter for saliva stain identification. Other body fluids that may contain α -amylase include the following: pancreatic juice, fecal material, urine, blood, and semen. Methods for testing α -amylase depend on the enzyme's ability to hydrolyze starch. Tests to distinguish between salivary and pancreatic amylase exist but are not commonly used in the field today.

11.7.1. RSID™ – Saliva (Rapid Stain Identification of Human Saliva)

The RSID™ – Saliva test is an immunochromatographic test that has been demonstrated to detect levels of human α -amylase (an enzyme found in human saliva that aids in the digestion of dietary starches). No high-dose hook effect.

It is a presumptive test because α -amylase can be found in other body fluids and the saliva of a gorilla.

Use of RSID™ – Saliva follows the procedures outlined in the instructions that accompany the RSID™ – Saliva kit.

Note: Using RSID™ – Universal Buffer, a single extract can be used to test for three body fluids (blood, semen, and saliva). If testing for more than one body fluid using the RSID™ test, refer to the RSID™ – Universal Buffer Technical Information and Protocol Sheet. If there are multiple universal buffer options due to multiple RSID™ test kits being utilized, select one ensuring that it is not expired.

11.7.1.1. Supplies

- RSID™ cassette

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- RSID™ – Universal Buffer
- Centrifuge (optional)

Note: Ensure RSID™ – Universal Buffer is at room temperature before extraction.

11.7.1.2. Method

1. Label RSID™ test device (e.g., centrifuge tube) for each sample with the item # or other unique identifying mark, including controls. Repeat the procedure below for each sample. If the extract may be collected, a spin filter basket may be used for extraction. Follow the centrifuge procedure.
2. Extract a swab in 300-400 µl of the RSID™ – Universal Buffer: shake for 10 seconds; longer incubation times are optional.
 - a. Alternatively, a cutting of a swab or filter paper rubbing may be used. Sufficient RSID™- Universal Buffer should be added to easily cover the sample.
 - b. Stains on fabric or porous substrates should be sampled by taking a cutting ($\approx 20 \text{ mm}^2$) of the item. Depending on the stain's appearance/size, a smaller cutting size than recommended may be used. The cutting should be extracted in 100 µL of RSID™- Universal Buffer for 10 seconds or longer.
3. A general guideline of a maximum of 10% of the extract, up to a maximum of 20 µL should be run. The remainder of the extract can be preserved (frozen) for DNA analysis if necessary. The buffer provided is STR-free and contains a DNA stabilizer. The provided buffers do not interfere with extraction or amplification.
 - a. See the section on Collecting Testing Extracts.
4. Remove the cassette from the foil pouch. Discard silica gel desiccant.
5. Label a cassette for each sample with the item # or other unique identifying mark, including controls.
6. Combine extract aliquot (max of 20 µl) with RSID™- Universal Buffer to bring the test sample to a total volume of 100 µl.
7. Add diluted sample RSID™- Universal Buffer to the sample window.
 - a. Alternatively, you may add 100 µl of the sample to the sample window. This will have little to no effect on the sensitivity or specificity of the test; however, any problems encountered by using a concentrated sample may be avoided if the extraction is diluted in RSID™-Universal Buffer, as described above.



8. At 10 minutes, score and record results as shown in the Scoring Diagram in the RSID™ kit.

Note: Assays should be performed at room temperature.

11.7.1.3. Interpretation

- Positive: The formation of red lines in the test “T” and control “C” regions of the membrane at exactly 10 minutes. See Cautions/Safety.
- Negative: The formation of a red line in the control “C” region only at exactly 10 minutes.
- Invalid: The formation of a red line only in the test “T” region indicates a failed test. The test is invalid and should be repeated with a new test. If an additional invalid test is obtained, no conclusion can be made, resulting in test failure. Recommend submitting the evidence for DNA analysis, since no conclusion can be made.
- Inconclusive: Shadow band, also referred to as ghost band, is a hazy, barely visible link (so faint that the color of the line is indistinct) that appears at the test line near the 10-minute time window. This is a rare phenomenon, but analysts must be aware of it.
 - When possible, seek a second opinion from a trained analyst. Document in the technical record.
 - Often, there is a disagreement between analysts that a band is present at all.
 - If there is enough sample, consider running the sample.
 - If unable to determine the result of the test(s), report the result as inconclusive.
- The test card results, for both the casework and the positive and negative control, shall be documented in the technical record.

11.7.1.4. Cautions/Safety

- The detection limit, used as suggested, is 10 nL of human saliva. Undiluted saliva should NOT be used.
- Under standard laboratory testing, users will not observe false negative results due to high dose hook effects.
- False positives
 - Substances other than blood may yield positive reactions.
 - Examples include human fecal samples, breast milk, and fresh urine. The α -amylase in urine is not stable and is no longer detectable if the stain is stored for several days.
 - Saliva from a gorilla has produced positive results with this test.



- False negatives
 - Studies have shown that α -amylase may vary significantly in concentration between individuals and may at times be at similar concentrations as those found in other body fluids. Therefore, a negative test does not indicate the absence of saliva.

11.7.1.5. Controls

- Positive: known human saliva
- Negative: RSID™-Universal Buffer
- A positive and negative control will be run before use on samples.

12. Supplies

See the BPD Crime Lab's Quality Assurance Manual for purchasing requirements. See the BPD Crime Lab's Health and Safety Manual for chemical intake requirements.

12.1. Chemicals/Reagents

Reagents made for biology screening testing shall not be used after the expiration date. Chemicals used for biology screening testing shall not be used after the expiration date unless they have undergone sensitivity testing and been approved for use by the Technical Leader and/or the Quality Assurance and Laboratory Manager. If a purchased reagent kit does not have an expiration date, the kit will be given an expiration date of one year. This may be extended to two years if it has undergone sensitivity testing and has been approved for use by the Technical Leader and/or the Quality Assurance and Lab Manager.

Sensitivity testing shall be documented in QA-22 Chemical Log. Expiration dates and approval for expiration date extension shall be documented in the chemical log and noted on the chemical container/test kit.

12.2. RSID™ field kits

An alternative to purchasing separate RSID™ field kits is to generate a field kit from the RSID™ lab kit. Use the following procedure:

- Pipette 750 μ L of universal buffer into sterile centrifuge tubes.
 - Place centrifuge tubes in a recloseable bag. Ensure the bag is properly labeled with all chemical intake information.
 - These may be stored at room temperature or with the scene response chemicals.
 - If less universal buffer is needed for testing, the excess may be removed from the centrifuge tube by the analyst before extraction. 750 μ L universal buffer is the pre-portioned amount currently used in the purchased RSID™ field.
- Place cassettes and sterile transfer pipettes in a separate bag with all chemical intake information.
 - If using Cole Parmer 3mL transfer pipettes, 3 drops equal $\sim$ 100 μ L. If using a different transfer pipette, the drop size must be measured before use.
- Follow the RSID™ field kit technical sheet for crime scene testing.



- Note: Lab kits and Field kits must be clearly designated. Once RSID™ supplies go to the scene, they shall not be used in the lab.

13. Equipment

See the BPD Crime Lab's Evidence Processing and Latent Print Technical Manual or Crime Scene Technical Manual for equipment not listed below.

13.1. Centrifuge

13.1.1. Operation/Procedure

The centrifuge may be used for sample extraction. Usage should be considered specifically if the extracts may be collected for possible future DNA analysis. Refer to the user manual(s) for operation and follow the procedure below.

1. Extract the sample following the test's extraction procedure.
2. Transfer the evidence sample (swab tip or cutting) from the centrifuge tube into a spin filter basket. The spin filter basket should be placed in the same centrifuge tube. Ensure the cap is tight.
3. Spin in the centrifuge for approximately 1 minute or until the extraction liquid has been removed from the sample.
4. Remove the spin filter basket and place it into a separate centrifuge tube for collection. Ensure the tube is labeled appropriately.
5. Use the extraction liquid for testing.
 - a. The remaining extract can be collected. Ensure the tube is labeled appropriately.

Note: Alternatively, after extraction and testing, the contents (extraction liquid and evidence sample) may be transferred into a spin filter basket/centrifuge tube and spun down. Follow the applicable steps above.

See the section on Collecting Testing Extracts.

13.1.2. Calibration/Performance Check

Performance checks are not required

13.1.3. Maintenance

Maintenance should be per the manufacturer's recommendations. Refer to the user manual(s) for preventative maintenance and routine cleaning.

13.2. Micropipettes

13.2.1. Operation

Refer to the user manual(s) for operation

13.2.2. Calibration/Performance Check

Pipettes were factory-calibrated before receipt. Performance checks are not required. Measurements are approximate.

13.2.3. Maintenance



Maintenance should be in accordance with the manufacturer's recommendations. Refer to the user manual(s) for preventative maintenance and routine cleaning.

13.3. Vortex

13.3.1. Operation

The vortex may be used if mixing is required in reagents, chemicals, or sample preparation. Use with caution if mixing should be "gentle". Refer to the user manual(s) for operation.

13.3.2. Calibration/Performance Check

Performance checks are not required

13.3.3. Maintenance

Maintenance should be in accordance with the manufacturer's recommendations. Refer to the user manual(s) for preventative maintenance and routine cleaning.

14. Digital Imaging

Digital imaging technology is used to preserve, document, and analyze evidence that has the potential to be of evidentiary value. Images generated for documentation and/or examination will be captured, stored, and documented in the history/chain of custody within LIMS. Images generated for orientation and documentation will also be retained in the case record.

14.1. Image Capture

Orientation images utilized to document the location of stains on a surface may be captured in a compressed format.

Examination-quality images are to be captured in uncompressed formats at a calibrated resolution exceeding 1000ppi unless circumstances prevent capturing the image at that resolution. Anything not meeting this standard must be documented. Examination-quality images should include a metric scale on the same plane as the targeted area. Examination quality would be needed for bloodstain images for reconstruction/analysis.

Orientation images utilized to document the location of stains on a surface may be captured in a compressed format.

14.2. Digital Image Storage

Original digital image files will be retained in the Crime Lab's LIMS, which is a secure database.

Examination-quality images shall be saved in the same format in which they were captured. The unaltered saved images will be considered the original images.

14.3. Image Enhancement/Processing

Enhancement work shall be done on a copy of an original image and not to the original image itself. Image enhancement techniques employed by an analyst must be explainable and within the scope of their training.



The processing steps for enhancements of examination quality images shall be documented in the case record (which is captured electronically and retained by LIMS and Photoshop). The resultant enhanced image(s) will also be retained in LIMS.

15. Traceability

- 15.1. Known blood, semen, and saliva reference materials used to verify the accuracy of detection tests, reagents, and techniques shall be assigned a unique lot number. Synthetic Blood from an authorized supplier or one's own known source of blood will suffice for the positive check of blood tests.
- 15.2. The donor of the source may be identified; however, if the individual wishes to remain anonymous or is unknown, a general designation of the source may be used instead (e.g., lab staff-1, lab staff-2, etc.).
- 15.3. Reference materials are subjected to the tests, reagents, and techniques for which they are used as a positive control in casework, and an accurate positive result must be obtained.

16. Sampling

Sampling is not applicable to the Biology Screening section.

In general, serological examinations involve the collection and preservation of substances or materials for DNA testing. Each sample selected is treated independently of other samples collected. The results are reported for the specific sample tested.

Every effort is made to retain a portion of the collected substance or material for future independent testing.

17. Handling of Test Items

See BPD Crime Lab's Quality Assurance Manual

18. Quality Assurance

See BPD Crime Lab's Quality Assurance Manual

19. Reporting

See the BPD Crime Lab's Quality Assurance Manual for detailed information. The report and/or analysis worksheets shall contain the following for all evidence:

- 19.1. Description of the evidence. The level of description is left to the analyst's discretion; however, the description should be sufficient to distinguish between items listed in the same report, if possible.
- 19.2. Location of biological stains, areas sampled for biological evidence, and/or apparent trace/latent evidence found.

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- 19.2.1. If it is not possible to determine the exterior and interior surfaces of an item (e.g., condom), then the results should be documented concerning how the item was submitted.
- 19.2.2. The results/conclusions of each test or series of tests shall be documented for each item of examined evidence.
- 19.2.3. The report shall be clear that the results/conclusions are for only the samples or evidence tested.
- 19.2.4. Multiple swabs that are collected from the same source at the same time (e.g., two swabs used on a large stain, touch DNA samples, etc.) can be considered a single sample.
- 19.2.5. If a located stain is small and/or dilute and sampling for chemical testing may consume the evidence, and the analyst opts to omit presumptive testing entirely, the report shall state "Not analyzed further due to consumption issues" and a note that recommends the item be submitted to an approved laboratory that performs DNA analysis.
- 19.2.6. See [Appendix A](#) for report wording guidelines that shall be used to aid the analyst in generating the final report as outlined above, taking into consideration the facts of the case and the analyst's training and experience.

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20. Terms and Definitions

ACID PHOSPHATASE (AP)

An enzyme found at elevated levels in semen and lower levels in some other body fluids (including vaginal fluid and saliva).

AP TEST

A presumptive test for semen.

ALTERNATE LIGHT SOURCE (ALS)

An instrument that delivers a high-intensity light of specific wavelengths. Different types of evidence, such as semen or fibers, may fluoresce during exposure to this light. Other types of evidence, such as bloodstains, may absorb light.

AMYLASE

An enzyme that breaks down starch found in high concentrations in saliva and lower concentrations in some other body fluids (including feces and semen).

BIOLOGICAL FLUIDS

Fluids that have human or animal origin, most commonly encountered at crime scenes (e.g., blood, mucus, perspiration, saliva, semen, vaginal fluid, urine, or biological material such as tissue, muscle, fecal material, etc.).

BLOOD

Body fluid that circulates through the body, delivering nutrients and removing metabolic waste. It is composed of solid components such as red blood cells, white blood cells, platelets, and a liquid component called plasma.

BLUESTAR® TEST

A chemical enhancement test for blood.

BODY FLUIDS

Blood, semen, saliva, vaginal secretions, urine, mucous, perspiration, cerebrospinal fluid, synovial fluids, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, and other fluids that come from the human body.

BUCCAL SWAB

A swab from the inner cheek lining. It is typically used as a known standard from an individual.



BUFFER

A solution that resists a change in pH.

CHEMILUMINESCENCE

The emission of light during a chemical reaction that does not produce significant quantities of heat.

CLEAN/SANITIZE

The process of removing biological and/or chemical contaminants from tools and/or equipment (e.g., using a mixture of 10-percent household bleach and water).

CONFIRMATORY TEST

A test that is specific for a biological material or substance of interest and that is used for the conclusive identification of a biological fluid; this usually refers to a serological or microscopic test for the detection of a particular biological fluid (e.g., blood or semen), often after a positive presumptive test for a biological fluid.

CONTAMINATION

The unintentional introduction of exogenous materials or substances into a test sample.

DNA (DEOXYRIBONUCLEIC ACID)

The genetic material of organisms, usually double-stranded, composed of two complementary chains of nucleotides in the form of a double helix; a class of nucleic acids characterized by the presence of the sugar deoxyribose, and the four bases adenine, cytosine, guanine, and thymine. DNA can be found from skin contact (shed skin cells - touch DNA) or in hair, bones, teeth, saliva, blood, semen, and other biological fluids.

ENZYME

A protein that may speed up a specific biochemical reaction without being changed or consumed during the process.

EXAMINATION QUALITY PHOTOGRAPHS

Close-up photographs taken at 90 degrees (when possible) in a non-compressed format (e.g., RAW) containing a scale, taken of specific items in a manner to capture maximum detail, so they can be used later in a scientific comparison.

FALSE NEGATIVE

A test result that erroneously indicates that a particular substance is not present, when in fact the targeted substance is present. This may occur when the amount of the specimen is so small as to go undetected. Also see high-dose hook effect.



FALSE POSITIVE

A test result that erroneously indicates that a particular targeted substance is present, when in fact, it is not the true target substance of the test.

FLAVINS

A component of semen that is responsible for the yellow color and fluoresces when viewed with an alternate light source.

FLUORESCENCE

The property of absorbing light of shorter wavelength and emitting light of longer wavelength.

HEME

Iron-containing compound of the porphyrin class that forms the non-protein part of hemoglobin and some other biological molecules. Acts as the catalyst in the peroxidase activity tests of TMB and BlueStar.

HEMOGLOBIN

Protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs. Its molecule comprises four subunits, each containing an iron atom bound to a heme group.

HIGH DOSE HOOK EFFECT

Refers to a decrease in test line intensity with some immunochromatographic strip tests when very high levels of target antigen are present in the tested sample. Under these conditions, the unbound target antigen can reach the test line before the antibody-bound antigen, occupying the test line antibody site and possibly resulting in a weak positive or false negative result.

INDICATED

'Indicated' for the presence of blood, semen, or saliva when using a serological test is an analyst's conclusion that blood, semen, or saliva may be present in a tested sample. The basis for an 'indicated' conclusion is the interpretation of a positive result from an appropriate presumptive serological test. A presumptive positive serological test result for blood, semen, or saliva does not confirm the presence of either substance.

INFRARED (IR)

Infrared energy, or low-frequency energy, has wavelengths longer than those of the visible spectrum and is most useful for identifying organic compounds, such as human body fluids or various flammables and explosives. The molecules of these organic compounds, when exposed to this infrared energy, produce unique spectral data that can be compared to forensic samples. In this way, the composition of the samples can be



identified. This technique is known as infrared spectroscopy or IR. IR consists of longer wavelengths ranging from 700 nanometers to 15,0000 nanometers.

LATENT PRINT

Although this refers specifically to an impression that is not readily visible or needs to be developed by physical or chemical processes, it can generically refer to any unintentional deposit of friction ridge detail. For this manual, the term “latent print” is used as a generic term for a friction ridge skin impression that is unknown.

LIGHT

Light is measured as a wavelength and visible light is generally expressed in nanometers. The human eye is sensitive to wavelengths from 400 to 700 nanometers. Different colors of light have different wavelengths, ranging from violet on the low end and red on the high end.

LUMINESCENCE

The emission of light by processes that derive energy from non-thermal sources, such as chemical and biochemical, and not by incandescence.

NEGATIVE CONTROL

A sample tested before use on questioned samples, which contains none of the substances tested for.

OBLIQUE LIGHT

Light that is positioned at a low angle of incidence relative to the surface being photographed or visualized. It is also referred to as side lighting.

OCCULT BLOOD

Blood that is present in amounts too small to be seen and can be detected only by chemical analysis or microscopic examination.

OXIDATIVE

Relating to the process or result of oxidizing or being oxidized.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Specialized clothing or equipment (such as disposable gloves, masks, eye protection, and lab coats) utilized to provide a barrier to keep biological or chemical hazards from contacting the skin, eyes, and mucous membranes and to avoid contamination of the items. General work clothes (e.g., uniforms, pants, shirts, etc.) that are not intended to function as protection against a hazard are not considered to be personal protective equipment.



PEROXIDASE

A catalase typically found in plant cells that prevents the buildup of peroxide.

PLATELETS (THROMBOCYTES)

Cells carried in the blood that are involved in clot formation.

POSITIVE CONTROL

A known sample tested before the questioned samples, which should produce a known positive reaction with the test, indicating the test is working correctly.

PRESUMPTIVE TEST

A screening test that indicates the presence of a material of interest, although the test result does not constitute the identification of that material.

REAGENT

A substance used because of its chemical or biological activity or because it takes part in or brings about a particular chemical or biological reaction.

RED BLOOD CELLS (ERYTHROCYTES)

Non-nucleated cells in blood that contain hemoglobin and carry oxygen to and aid in the removal of carbon dioxide from tissues.

RSID™ - BLOOD (RAPID STAIN IDENTIFICATION OF HUMAN BLOOD)

A test for human Glycophorin A Antigen; confirmatory for human blood when preceded by a positive presumptive test for blood.

RSID™- SALIVA (RAPID STAIN IDENTIFICATION OF HUMAN SALIVA)

A test for human α -amylase; presumptive test for the presence of saliva. Alpha-amylase is present in high concentrations in saliva and in lower concentrations in other body fluids.

RSID™- SEMEN (RAPID STAIN IDENTIFICATION OF HUMAN SEMEN)

A test for semenogelin, a protein produced by the seminal vesicles and is responsible for the coagulum associated with ejaculate; confirmatory for human semen when preceded by a positive presumptive test for semen.

SALIVA

A thin, colorless fluid that contains amylase and is secreted by several glands in the mouth.



SAMPLING SELECTION

A practice of selecting items to test, or portions of items to test, based on training, experience, and competence. In sample selection, there is no assumption about homogeneity.

SEMEN

Composition of fluid secretions from a variety of glands (seminal fluid) and spermatozoa. Seminal fluid may be present without spermatozoa.

SEMINAL FLUID

Organic fluid secreted by the gonads.

SENSITIVITY

The lowest concentration of a given substance in a biological specimen that can be detected.

SPECIFICITY

The degree to which a reagent will identify one substance to the exclusion of all others.

SPERMATOZOA

Male reproductive cells. Cellular component of semen.

TETRAMETHYLBENZIDINE (TMB)

A presumptive test for the presence of blood.

TOUCH DNA

A small amount of DNA left behind after handling or touching an item that is not visible to the eye. Has a low quantity of DNA and is considered a heterogeneous trace deposit.

TRACE EVIDENCE

Physical evidence resulting from the transfer of small quantities of materials (e.g., hair, textile fibers, paint chips, glass fragments, gunshot residue particles).

WHITE BLOOD CELLS (LYMPHOCYTES, NEUTROPHILS, BASOPHILS, EOSINOPHILS, AND MONOCYTES)

Nucleated cells involved in fighting infections, immunity, and antibody production.



21. Appendix A: Report Writing Guidelines

Presumptive test statement:

When reporting the results of presumptive screening tests only, the following statement shall be issued (based upon the test used): “Presumptive tests for [blood, semen, and/or saliva] provide an indication, but not a confirmation, of the presence or absence of [blood, semen, and/or saliva].”

Insufficient quantity and quality statement:

If the final result is a negative or inconclusive on a presumptive or confirmatory test, and the analyst believes that this may be a result of an insufficient quantity or quality of blood, semen, and/or saliva, the analyst may report, “An insufficient quantity or quality of [blood, semen, and/or saliva] can limit the ability of both [presumptive and/or confirmatory tests] to detect [blood, semen, and/or saliva],” which provides support for the collection of the substance.

False positive statement:

When reporting the results of a positive presumptive test and there are observations to suggest that the substance may not be the target substance but rather a substance listed under false positives for that test, a false positive statement should be included: “Presumptive testing indicated the presence of [blood, semen, and/or saliva], however this result must be interpreted with caution as [substances other than blood, semen, and or saliva OR specially false positive(s) that lead you to include this statement]] may cause a false positive result”.

Note: In the latent print processing report and evidence processing report, the substances in these statements may be generalized to “biological fluids” or similar due to the limitation of the LIM system.

Minor variations in reporting statements may be necessary for the flow of the report, specifically in CSI reports; however, the following underlined words are required. If report writing scenarios occur that are not covered below, consult with the Technical Leader and/or the Quality Assurance/Laboratory Manager before issuing the report.

The guidelines below are for the final conclusions of screening and/or testing methods. It should be noted that each method’s results are documented in the technical record, while the overall final result shall be reported for each crime scene area searched or evidence item examined.



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Analysis Results	Report Wording
Examination	
Positive examination results for [blood, semen, and/or saliva] with an [alternate light source, visual and/or stereomicroscopic examination]	A statement that (a) suspected [blood, semen, and/or saliva] stain(s) was/were observed.
Negative examination results for [blood, semen, and/or saliva] with an [alternate light source, visual and/or stereomicroscopic examination]	A statement that no suspected [blood, semen, and/or saliva]stain(s) was/were observed.
Inconclusive examination results for [blood, semen, and/or saliva] with an [alternate light source, visual and/or stereomicroscopic examination]	A statement that an <u>inconclusive</u> result for <u>[blood, semen, and/or saliva]</u> was obtained during examination with various lighting conditions. The reason(s) why the result was inconclusive must also be included in the report (i.e., substrate not compatible). An example could be when using IR to search for blood on a black shirt, and the shirt isn't IR compatible.
Blood	
Positive TMB and/or Bluestar® test	A statement that <u>presumptive testing indicated</u> the presence of <u>blood</u>. Include presumptive test statement. May include a false positive statement.
Negative TMB and/or Bluestar® test	A statement that a <u>negative</u> result with a <u>presumptive test</u> for <u>blood</u> was obtained. If a negative TMB result is obtained after latent print processing, the report must qualify the result (i.e., A <u>negative</u> result with a <u>presumptive test</u> for <u>blood</u> was obtained; however, this <u>result must be interpreted with caution, as certain latent print processing techniques may interfere with presumptive blood testing</u>). Include presumptive test statement.

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	May include an insufficient quantity and quality statement.
Inconclusive TMB and/or Bluestar® test	A statement that an <u>inconclusive</u> result with a <u>presumptive test</u> for <u>blood</u> was obtained. No determination can be made regarding the presence or absence of blood on the item. The reason(s) why the result was inconclusive must also be included in the report (i.e., slow reaction). Include presumptive test statement. May include an insufficient quantity and quality statement.
Positive TMB and/or Bluestar® test and a positive RSID™ -Blood test	A statement that <u>human blood was indicated</u> .
Positive TMB and/or Bluestar® test and a negative RSID™ -Blood test	A statement that <u>presumptive testing indicated</u> the presence of <u>blood</u>; however, a <u>negative</u> result with a <u>confirmatory test</u> for <u>human blood</u> was obtained. May include an insufficient quantity and quality statement. May include a false positive statement.
Positive TMB and/or Bluestar® test and Inconclusive RSID™ -Blood test	A statement that <u>presumptive testing indicated</u> the presence of <u>blood</u>; however, an <u>inconclusive</u> result with a <u>confirmatory test</u> for <u>human blood</u> was obtained. The reason(s) why the result was inconclusive must also be included in the report (e.g., shadow band). May include an insufficient quantity and quality statement. May include a false positive statement.
Inconclusive TMB and/or Bluestar® test and a positive RSID™ – Blood test	A statement that <u>testing indicated</u> the presence of <u>human blood</u> .
Inconclusive TMB and/or Bluestar® test and a negative RSID™ – Blood test	A statement that <u>no human blood was indicated</u>. May include an insufficient quantity and quality statement.
Inconclusive TMB and/or Bluestar® test and Inconclusive RSID™ -Blood test	A statement that an <u>inconclusive</u> result with a <u>presumptive and confirmatory test</u> for <u>blood</u> was obtained. The reason(s) why the result was inconclusive must also be included in the report.

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	May include an insufficient quantity and quality statement.
Semen	
Positive AP test	A statement that <u>presumptive testing indicated</u> the presence of <u>semen</u>. Include presumptive test statement. May include a false positive statement.
Negative AP test	A statement that a <u>negative</u> result with a <u>presumptive test</u> for <u>semen</u> was obtained. Include presumptive test statement. May include an insufficient quantity and quality statement.
Inconclusive AP test	A statement that an <u>inconclusive</u> result with a <u>presumptive test</u> for <u>semen</u> was obtained. No determination can be made regarding the presence or absence of semen on the item. The reason(s) why the result was inconclusive must also be included in the report (e.g., A slow, faint pink color change occurred, which may indicate the presence of other body fluids (e.g., vaginal secretions, fecal material) and/or interfering substances, such as certain plants and fungi). Include presumptive test statement. May include an insufficient quantity and quality statement.
Positive AP test and positive RSID™-Semen test	A statement that <u>human semen</u> was <u>indicated</u> .
Positive AP test and negative RSID™-Semen test	A statement that <u>no semen</u> was <u>indicated</u>. A notation should also be included to inform the reader that presumptive testing indicated the presence of acid phosphatase, an enzyme present in high levels in semen but also in other body fluids. May include an insufficient quantity and quality statement. May include a false positive statement.

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Positive AP test and inconclusive RSID™ - Semen test	A statement that <u>presumptive testing indicated</u> the presence of <u>acid phosphatase</u>, <u>an enzyme present in high levels in semen but also in other body fluids</u>; however, an <u>inconclusive result with a confirmatory test for human semen</u> was obtained. The reason(s) why the result was inconclusive must also be included in the report. May include an insufficient quantity and quality statement. May include a false positive statement.
Inconclusive AP test and a positive RSID™ - Semen	A statement that <u>testing indicated</u> the presence of <u>human semen</u>.
Inconclusive AP test and negative RSID™ - Semen test	A statement that <u>no human semen</u> was <u>indicated</u>. May include an insufficient quantity and quality statement.
Inconclusive AP test and inconclusive RSID™ -Semen test	A statement that an <u>inconclusive</u> result with a <u>presumptive and confirmatory test for semen</u> was obtained. No determination can be made regarding the presence or absence of semen on the item. The reason(s) why the result was inconclusive must also be included in the report. Include presumptive test statement. May include an insufficient quantity and quality statement.
Saliva	
Positive RSID™ Saliva	A statement that <u>presumptive testing indicated</u> the presence of <u>saliva</u>. Include presumptive test statement. May include a false positive statement.
Negative RSID™ Saliva	A statement that a <u>negative</u> result with a <u>presumptive test for saliva</u> was obtained. Include presumptive test statement. May include an insufficient quantity and quality statement.

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Inconclusive RSID™ Saliva	A statement that an <u>inconclusive</u> result with a <u>presumptive test</u> for <u>saliva</u> was obtained. No determination can be made regarding the presence or absence of saliva on the item. The reason(s) why the result was inconclusive must also be included (e.g., Due to areas of a uniform blue color on the non-reagent side of the paper where fecal stains are present). Include presumptive test statement. May include an insufficient quantity and quality statement.
Confirmatory Test without a Presumptive Test	
No presumptive test and positive RSID™ Blood or Semen test	The report shall indicate that no presumptive test was performed. A statement that <u>testing indicated</u> the possible presence of <u>human [blood or semen]</u>.
No presumptive test and negative RSID™ Blood or Semen test	The report shall indicate that no presumptive test was performed. A statement that <u>no human [blood or semen]</u> was <u>indicated</u>. May include an insufficient quantity and quality statement.
No presumptive test and inconclusive RSID™ Blood or Semen test	The report shall indicate that no presumptive test was performed. A statement that an <u>inconclusive</u> result with a <u>confirmatory test</u> for <u>[blood or semen]</u> was obtained. No determination can be made regarding the presence or absence of blood or semen on the item. The reason(s) why the result was inconclusive must also be included in the report. May include an insufficient quantity and quality statement.
Other situations	
Analyst opts to omit testing entirely due to consumption issues	A statement shall be included in the report if testing was requested but not conducted due to consumption concerns.

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	If testing is not requested and stain(s) are observed but not tested due to consumption concerns, then the following statement may be included in the report. A statement including “Not analyzed further due to consumption issues” and a note that recommends the item be submitted to an approved laboratory that performs DNA analysis.
The item of evidence opened; however, no serological testing was performed	A statement that the item was opened; however, no further <examination/testing> at this time
An item of evidence was received, but not examined	A statement that the item was received, not examined
An item of evidence was requested, but not examined	A statement that the item was not examined
Referencing items for which analysis was halted during processing.	“The analysis of this item was halted at the written request of or per a conversation with <Title, Name, Ada#> received on <date>.”

22. Appendix B: Biology Screening Technical Manual History

ISSUING AUTHORITY: Chief Administrative Officer / Quality Assurance-Lab Manager / Supervisor / Technical Leader		
DATE	SECTION AND COMMENTS	AUTHOR/REVIEWER
8/20/2020	Current Manual 2020 (V1)	Delaney / Voorhees
8/19/2021	V2 2021 – See “Bio Screening Tech Manual Changes” for Version 1, dated 8/18/21	Voorhees / Delaney
9/7/2021	V3 2021 – See “Biology Screening Technical Manual changes to V2”	Voorhees / Delaney
11/16/2022	V4 2022 – See “BS Tech Manual V3 tracked changes for V4” document	Wenetzky / Delaney
11/4/2023	V5 2023 – See “BS Tech Manual V4 tracked changes for V5” document	Wenetzky / Leung and Delaney
11/7/2024	V6 2024 – See “BS Tech Manual V5 tracked changes for V6”	Wenetzky / Oldemeyer and Delaney

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12/8/2025	V7 2025 – See “BS Tech Manual V6 tracked changes for V7”	Wenetzky and Tuscher/ Oldemeyer and Delaney
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