Ada County Sheriff's Office

Forensic Lab

Biology Screening Analytical Method

Version 1.0

1.0 Scope

1.1. Provide uniform processing of evidentiary material for the presence of human blood and semen.

1.2. Examine items of evidence for the presence and identification of human blood and semen using visual examination, presumptive screening and confirmatory testing.

2.0 References

2.1. ACSO Policy Manual located at https://aces.ada.net.gov/sites/ADAPortal/departments/sheriff/policy/Documents/ACSO%20Policy%20Manual%20112414.pdf

2.2. Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

2.3. Ada County Sheriff's Office Forensic Lab Health and Safety Manual

2.4. SWGDAM located at https://www.swgdam.org/

2.5. NIST OSAC Subcommittee: Biological Methods at https://www.nist.gov/topics/forensic-science/osac-biological-methods-subcommittee

2.6. College/University level Chemistry and Biology text books

2.7. Operation and maintenance manuals for each instrument

3.0 Terms and Definitions

3.1. Acid Phosphatase (AP) – an enzyme found at elevated levels in semen and lower levels in some other body fluids (to include vaginal fluid and saliva).

3.2. Alternate Light Source (ALS) – an instrument which 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.

3.3. 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).

3.4. Blood – body fluid that circulates through the body delivering nutrients and and removing metabolic waste. It is composed of cells and plasma.

3.5. Body fluids – blood, semen, blood products, vaginal secretions, cerebrospinal fluid, synovial fluids, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, and concentrated HIV and HBV viruses. Care should also be taken with other biological materials such as body parts and tissues, saliva, urine, feces, and blood typing reagents.

3.6. Chemiluminescence – the emission of light during a chemical reaction that does not produce significant quantities of heat.

3.7. 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).

3.8. Confirmatory test – one or more procedures, the result(s) of which justifies a conclusion of identification.

3.9. Contamination – the unwanted transfer of material from another source to a piece of physical evidence.

3.10. Fluorescence – the property of absorbing light of shorter wavelength and emitting light of longer wavelength.

3.11. HemaTrace test® – a test for hemoglobin; confirmatory for blood and indicative of human origin.

3.12. Heme – iron-containing compound of the porphyrin class that forms the non-protein part of hemoglobin and some other biological molecules.

3.13. **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.

3.14. **Latent print –** a print impression not readily visible, made by contact of the hands or feet with a surface resulting in the transfer of materials from the skin to that surface.

3.15. **Occult blood –** blood that is present in amounts too small to be seen and can be detected only by chemical analysis or microscopic examination.

3.16. **o-tolidine –** a presumptive test for the presence of blood.

3.17. **Oxidative –** relating to the process or result of oxidizing or being oxidized.

3.18. **p30 –** a protein used to identify semen.

3.19. **p30 test –** a test for human p30.

3.20. **Personal protective equipment (PPE) –** articles such as disposable gloves, masks, and eye protection that are 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**.**

3.21. **Phenolphthalein –** a presumptive test for the presence of blood.

3.22. **Presumptive test –** a preliminary test to ascertain the presence of a biological substance.

3.23. **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.

3.24. **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.

3.25. **Semen –** seminal fluid and spermatozoa.

3.26. **Seminal fluid –** organic fluid secreted by the gonads.

3.27. **Spermatozoa –** male reproductive cells.

3.28. **Trace evidence –** physical evidence that results from the transfer of small quantities of materials (e.g., hair, textile fibers, paint chips, glass fragments, gunshot residue particles).

4.0 Management Requirements

4.1. Organization See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.2. Management system See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.3. Document control See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.4. Review of requests, tenders, contracts See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.5. Subcontracting tests See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.6. Purchasing services and supplies

4.6.1. Purchasing documents shall indicate that the defined specifications are met for the following supplies listed below.

4.6.1.1. ABAcard® HemaTrace® Test

4.6.1.2. ABAcard® p30 Test

4.7. Service to customer See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.8. Complaints See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.9. Nonconforming work See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.10. Improvement See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.11. Corrective action See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.12. Preventive action See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.13. Control of records

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.

4.13.1. Documentation includes a combination of notetaking, photography, and diagramming.

4.13.2. Documentation shall take place as the analysis is occurring.

4.13.3. All original documentation shall be retained.

4.13.4. The description of evidence in the case notes should include the following:

4.13.4.1. Type of item and quantity (i.e. two brown knee high dress socks).

4.13.4.2. A general description of the item (i.e. color, size, brand, and designs on clothing, bedding, towels, etc.).

4.13.4.3. Condition of the item (i.e. cleanliness, damage, holes, buttons missing, hooks bent, etc.).

4.13.4.4. The approximate location of any stains seen and their visual appearance.

4.13.5. Document the control results in case notes. Record positive (+) as indicated by the development of the correct color change, or negative (-) as indicated by the absence of the color change. This shall be done prior to casework.

4.13.6. The approved abbreviation list for the biology discipline can be found in the electronic lab documents**.** Commonly accepted abbreviations or abbreviations that are readily recognizable to a reviewer do not need to be listed, such as those for elements.

4.13.6.1. Should an analyst use an abbreviation that is not listed in the abbreviation list, then a reference shall be included in the case notes as to what the abbreviation means.

4.14. Internal audits See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

4.15. Management reviews See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

5.0 Technical Requirements

5.1. General

5.1.1. ABA cards shall have a quality control (QC) check performed prior to use in casework. This shall include a positive and negative control.

5.1.1.1. The results of these tests shall be documented in the case notes.

5.1.1.2. If the quality control passes, ABA cards may be used passed their expiration date.

5.1.2. Reagents prepared in the laboratory shall be labeled with the identity of the reagent, date of preparation and/or lot number, and identify of who made the reagent.

5.1.2.1. For a prepared reagent, the lot number shall be the date and the initials of the analyst who prepared it (i.e. 070716HC).

5.1.3. Reagents prepared in the laboratory shall be recorded in the reagent prep log book. This shall include the date the reagent was made, the initials of the preparer, the chemicals utilized, lot numbers of the chemicals utilized, and the reagent name.

5.1.4. A positive and negative control shall be run to verify the reagent preparation. This control shall occur before use or, if appropriate, concurrent with the test.

5.1.4.1. The results of the reagent quality control tests shall be documented in the case notes.

5.2. Personnel See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual

5.3. Accommodation and Environmental conditions

5.3.1. Standard laboratory safety protocols shall be followed. Refer to the ACSO Forensic Lab Health and Safety Manual.

5.3.2. To prevent contamination of personnel and the exhibit, the appropriate protective equipment should be utilized (e.g., gloves, eye protection, etc.) To minimize cross-contamination, personal protective equipment should be worn and changed when necessary.

5.3.3. Appropriate eye protection shall be worn whenever a hazard to the eyes exists. This would include chemical exposures and alternate light sources (UV, laser, etc.).

5.3.4. Examination utensils (e.g., forceps, scissors, measuring devices, etc.) used in processing shall be disinfected between items of evidence.

5.3.5. When using aerosol type chemicals, proper engineering controls, work practice controls, or PPE must be employed to avoid chemical exposure.

5.3.6. Prior to, during, and/or after evidence processing, sweep and mop the floor and wipe off examination work areas and tools with a freshly prepared 10% solution of bleach or a solution that will remove/degrade the DNA.

5.3.6.1. Document the date of the sweeping and mopping.

5.3.7. Work with only one item at a time to avoid sample mix-up and/or contamination.

5.3.8. Place each item of evidence on a new sheet of paper (i.e. Kimwipe, butcher, etc.)

5.3.9. Crime scene samples shall be handled at a different time or in a different space from standards/known samples.

5.4. Testing Methods

5.4.1. Phenolphthalein Test

This oxidative test for the presumptive identification of blood is based on the catalytic activity of the heme group within the hemoglobin molecule. The phenolphthalein test is extremely sensitive and can be used to detect visible blood or occult blood that has been diluted or washed away.

5.4.1.1. Reagents

Make stock solution below or purchase commercially made phenolphthalein.

Stock Solution

Phenolphthalein	2 g
Potassium hydroxide, ≥85%	20 g
Deionized water	100 ml
Zinc, granulated, particle size approximately 20 mesh	20 g

Reflux until the solution becomes colorless (2-3 hours). Store in refrigerator in amber bottle with additional zinc added.

Working Solution A

Stock solution	1 part
Ethanol, ≥99.5% (200 proof)	4 parts

Store in dropper bottle with mossy or granulated zinc.

Working Solution B

3% Hydrogen peroxide

5.4.1.2. Method

5.4.1.2.1. Take a small cutting or swabbing of a targeted stain.

5.4.1.2.2. Place 1 to 2 drops of phenolphthalein working solution A on the cutting or swab. Allow the reagent to soak into the sample.

5.4.1.2.3. Place 1 to 2 drops of working solution B on the sample.

5.4.1.2.4. Interpret and document the results in the case notes.

5.4.1.3. Interpretation

Positive: A positive reaction will show a pink color within 10 seconds after the addition of working solution B (hydrogen peroxide).

Negative: A negative reaction will not have a pink color change within 10 seconds.

Inconclusive: A color change that is suspected to be from substrate contamination, a positive reaction that occurs with the addition of working solution A only, or other circumstances that lead the forensic scientist to be cautious about a positive or negative interpretation.

Note: the forensic scientist shall document in their case notes the observation(s) that led them to make an inconclusive determination.

5.4.1.4. Caution/Safety

- Do not place reagents directly on the evidence. Sample the evidence with a cutting, swabbing or filter paper.
- 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.
- In order to optimize the efficacy of hydrogen peroxide, forensic scientists shall 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 and give a hot pink color with time.

- The phenolphthalein test is a presumptive test and substances other than blood may yield positive reactions.

- Negative results obtained with the phenolphthalein test after latent print processing should be interpreted with caution as certain latent print processing techniques may interfere with presumptive blood testing.

  NOTE: Presumptive blood testing should be performed prior to latent print processing, unless circumstances dictate otherwise.

5.4.1.5. Controls

Positive: A known blood stain.

Negative: Unstained swab, fabric, filter paper, empty spot well, or water.

Working solutions are checked at the time of use.

A new stock solution is quality checked at the time the first working solutions are made from it. The results are documented in the appropriate reagent log.

5.4.2 o-tolidine Test

This oxidative test for the presumptive identification of blood is based on the catalytic activity of the heme group within the hemoglobin molecule. The o-tolidine test is extremely sensitive and can be used to detect visible blood or occult blood that has been diluted or washed away.

5.4.2.1 Reagents

o-tolidine	0.6 g
Glacial acetic acid	100 mL
Ethanol	100 mL
Hydrogen Peroxide	1-2 drops

Dissolve o-tolidine in acetic acid/ethanol mixture.

5.4.2.2 Method

5.4.2.2.1 Take a small cutting or swabbing of a targeted stain.

5.4.2.2.2 Place 1 to 2 drops of o-tolidine reagent on the cutting or swab. Allow the reagent to soak into the sample.

5.4.2.2.3 Place 1 to 2 drops of hydrogen peroxide on the sample.

5.4.2.2.4 Interpret and document the results in the case notes.

5.4.2.3 Interpretation

Positive: A positive reaction will show a blue/green color within 10 seconds after the addition of hydrogen peroxide.

Negative: No color change within 10 seconds.

Inconclusive: A color change that is suspected to be from substrate contamination, a positive reaction that occurs with the addition of o-tolidine only, or other circumstances that lead the forensic scientist to be cautious about a positive or negative interpretation.

Note: the forensic scientist shall document in their case notes the observation(s) that led them to make an inconclusive determination.

5.4.2.4 Caution/Safety

- Do not place reagents directly on the evidence. Sample the evidence with a cutting or swabbing.
- 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.
- In order to optimize the efficacy of hydrogen peroxide, forensic scientists shall store the working solution in a refrigerator/cooler when not in use and limit the reagents' exposure to air and light.
- The o-tolidine test is a presumptive test and substances other than blood may yield positive reactions.
- o-tolidine is designated as a potential carcinogen and should be used with caution.
- Negative results obtained with the o-tolidine test after latent print processing should be interpreted with caution as certain latent print processing techniques may interfere with presumptive blood testing.

  NOTE: Presumptive blood testing should be performed prior to latent print processing, unless circumstances dictate otherwise.

5.4.2.5 Controls

Positive: A known blood stain.

Negative: Unstained swab, fabric, filter paper, empty spot well, or water.

Reagents are checked at the time of use.

5.4.3 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 blood tests, such as phenolphthalein or o-tolidine, may be subsequently used on Bluestar® positive areas/stains.

5.4.3.1 Supplies

BLUESTAR® FORENSIC

For the BLUESTAR®FORENSIC tablets you will need distilled water & a 125 ml spray bottle (mister) equipped with an adjustable spray nozzle.

5.4.3.2 Method

The working solution is prepared before use. For optimal results, this solution should be used within 3-4 hours after mixing. Each BLUESTAR® Forensic tablet reagent packet contains a beige tablet (containing Sodium Hydroxide) and a white tablet (containing Hydrogen Peroxide – Urea) which will make 125 ml of solution. 125 ml is generally sufficient for a 250 sq. ft. area.

5.4.3.2.1 Remove the two tablets from the reagent packet and add to 125 ml of distilled water.

5.4.3.2.2 Allow to completely dissolve (~1 to 2 minutes).

5.4.3.2.3 Gently stir with a circular motion. DO NOT shake the container upside down.

5.4.3.2.4 Test controls. Document control reactions.

5.4.3.2.5 Determine the area to be sprayed with BLUESTAR®. Application must occur in a dark environment to view any resulting chemiluminescence.

5.4.3.2.6 Have a camera on a tripod or stabilized ready to document any positive reaction.

5.4.3.2.7 Lightly mist the area and photograph immediately after application.

5.4.3.2.8 Additional application may be necessary. However, use caution to avoid saturation and dilution of the target.

5.4.3.2.9 Samples should be collected from the area(s) which displayed luminescence for additional testing.

5.4.3.3 Photography

Utilize standard low light photographic techniques for ISO, aperture setting, shutter speed, and subject lighting.

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.

5.4.3.4 Interpretation

Positive: Chemiluminescence is observed when sprayed with Bluestar®.

Negative: No chemiluminescence observed.

5.4.3.5 Identifying "false" reactions

False chemiluminescence reactions may occur in the presence of certain household detergents, chlorine, some paints and varnishes, copper, certain iron metabolizing plants and soils containing iron.

Such "false" reactions 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.

5.4.3.6 Cautions/Safety

- All other analysis and search options should be considered prior to BLUESTAR® application.
- Spraying of BLUESTAR® dilutes bloodstains.
- Because the BLUESTAR® reagent is primarily water it 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 in proceeding.
- BLUESTAR® works with blood stains and impressions. Consider testing with a more specific presumptive test for blood prior to application, when possible.
- Any samples collected after BLUESTAR® processing should be thoroughly dried prior to packaging. If possible, sample prior to use of BLUESTAR®.
- Photographic equipment should be set up and ready for use prior to BLUESTAR® application.
- BLUESTAR® is a relatively non-specific presumptive test for blood, so other substances such as chlorine, metallic ions like manganese, copper and iron, some paints and varnishes, some vegetable and fruits, etc. may yield a positive reaction.
- Use in a ventilated area when possible.
- Wear eye protection and masks when spraying any reagent.

5.4.3.7 Controls

Positive: Known blood stain on a substrate

Negative: Unstained substrate

Controls are checked prior to use.

5.4.4 Luminol Test

Luminol is used on suspected blood samples to locate non-visible blood or to enhance bloody impressions. Luminol is a clear, colorless reagent. When Luminol comes into contact with the hemoglobin, an oxidation reaction, catalyzed by the peroxidase-like activity of hemoglobin, occurs. A positive result is a temporary light blue chemiluminescence, visible best in a dark environment. The resulting chemiluminescence may increase the contrast of a blood impression on a dark substrate.

Luminol is especially useful for suspected blood deposited on dark colored, porous or non-porous surfaces. Results may be best on faint impressions or samples. Luminol may also be used to locate non-visible blood stains.

More specific presumptive blood tests, such as phenolphthalein or o-tolidine, may be subsequently used on Luminol positive areas/stains.

5.4.4.1 Reagent

The reagent is mixed just prior to use.

Sodium carbonate, anhydrous ≥99.0%	10 gm
Luminol > 97.0%	0.2 gm
Deionized water	200 ml
Sodium perborate monohydrate	1.4 gm

Dissolve the sodium carbonate, sodium perborate, and Luminol in the 200mL deionized water. Complete dissolution may take several minutes at room temperature. Place the reagent in a non-metal fine-mist sprayer for application.

Luminol is considered expired within 1-2 hours of preparation or when the controls don't yield the expected results. Controls will be checked every hour to ensure the reagent is working properly.

Note: reference articles on Luminol do not distinguish between the use of sodium perborate monohydrate (CAS 10332-33-9) and sodium perborate tetra- or 4-hydrate (CAS 10486-00-7) therefore, both are acceptable for use presuming expected results with controls are obtained.

5.4.4.2 Method

5.4.4.2.1 Determine area to be sprayed with Luminol. Application must occur in a dark environment to view any resulting chemiluminescence.

5.4.4.2.2 Have a camera on a tripod or stabilized ready to document any positive reaction.

5.4.4.2.3 Mix reagent and test controls. Document control reactions.

5.4.4.2.4 Lightly spray the area with Luminol and photograph any positive reactions.

5.4.4.2.5 Repeat spraying may be necessary. However, use caution since this will dilute the stain and may cause running.

5.4.4.2.6 Samples may be collected as needed from the positive area(s) for additional testing and/or preservation.

5.4.4.3 Photography

Utilize standard low light photographic techniques ISO, aperture setting, shutter speed, and subject lighting. Longer exposure times will typically be necessary to assure quality, so use of a tripod is essential.

Viewing and photographing Luminol reactions requires the need to reduce or eliminate available light. Indoor scenes should be darkened as much as possible. Aluminum foil or other light blocking material may be necessary to cover windows and doors. Outdoor scenes should be photographed at night with as few lights illuminated as possible.

5.4.4.4 Interpretations

Positive: A light blue chemiluminescence is observed almost immediately upon application. The strength of the chemiluminescence will fade, typically over 1 to 2 minutes.

Negative: No chemiluminescence observed.

5.4.4.5 Caution/Safety

- Other analysis and search options should be exhausted prior to Luminol application.
- Blood impressions may be dissolved by the Luminol reagent causing running or obliteration of fine detail. This is more likely with repeated spraying.
- Applying a chemical blood fixative (e.g. 2% 5-sulfosalicylic acid solution) prior to Luminol treatment will reduce the Luminol reaction and is not recommended.
- Any samples collected after Luminol processing should be thoroughly dried prior to packaging. If possible, sample prior to the use of Luminol.
- Photographic equipment should be set up and ready for use prior to Luminol application.

- Upon drying, Luminol contaminates the substrate with its component chemicals leaving a white, crusty residue.

- Consider testing with a more specific presumptive test for blood prior to application, when possible. Luminol is a relatively non-specific presumptive test for blood, so other substances such as chlorine, rust, iron-containing soil, metals, etc. may yield a positive result.

- Use in a ventilated area when possible.

- Wear eye protection and masks when spraying any reagent.

5.4.4.6 Controls

Positive: Known blood on a substrate

Negative: Unstained substrate

Controls are checked prior to use.

5.4.5 ABACard® HemaTrace® Test

The ABAcard® HemaTrace® test made by Abacus Diagnostics, Inc. is an immunochromatographic test for human hemoglobin (hHb). Hemoglobin from primates (anthropoideae) and ferrets have produced positive results with this test. Body fluids other than blood (e.g., urine, semen, and saliva) have also produced positive results with this test. The HemaTrace® test is a presumptive test that is not specific for human hemoglobin. The presence of human blood is indicated when a positive result from the HemaTrace® test is obtained in combination with a positive result from phenolphthalein, o-tolidine, Luminol, and/or BlueStar®.

5.4.5.1 Supplies

ABAcard® HemaTrace® test card(s) and tube(s) of extraction buffer. The extraction buffer tubes are provided with the HemaTrace® cards.

5.4.5.2 Method

5.4.5.2.1 Label an ABAcard® HemaTrace® test device for each sample, including controls.

5.4.5.2.2 Extract a portion of the stain or swab in a minimum of 200 µl of the ABAcard® HemaTrace® extraction buffer for at least 1-5 minutes and up to 2 hours.

5.4.5.2.3 Place approximately 200 µl (4 drops) of the extract in the sample well of the ABAcard® HemaTrace® test card, filling the sample well completely.

5.4.5.2.4 Read result at 10 minutes and document results in the case notes.

5.4.5.3 Interpretation

Positive: The formation of a pink line in the test "T" and control "C" regions of the membrane within 10 minutes.

Negative: No formation of a pink line in the test "T" region within 10 minutes and a visible pink line in the control "C" region. **See Caution/Safety**.

Invalid: No formation of a pink line in the control "C" region. 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.

5.4.5.4 Caution/Safety

5.4.5.4.1 A false negative may occur when very high levels of hHb are present in the tested sample, causing a so-called "high dose hook effect". Under these conditions, unbound hHb could reach the test area before the mobile antigen-antibody complex, potentially resulting in a false negative result. If you suspect that this may have occurred, you should dilute the remaining extract and re-test.

5.4.5.4.2 Because positive results can be obtained from body fluids other than blood (e.g., urine, semen, and saliva), the HemaTrace® test should not be used if no staining consistent with blood is observed and a negative result with phenolphthalein, o-tolidine, Luminol, and/or BlueStar® is obtained.

5.4.5.5 Documentation

5.4.5.5.1 The lot number and expiration date of the ABAcard® HemaTrace® test cards used shall be documented in the case notes.

5.4.5.5.2 The test card results, for both the casework and the positive and negative control, shall be documented in the case notes.

5.4.5.6 Controls

Positive: Known human blood

Negative: Extraction buffer

A positive and negative control will be run with each batch of tests run.

5.4.6 Acid Phosphatase (AP) Test

The purpose of the Acid Phosphatase (AP) test is to detect the enzyme acid phosphatase, which is found in elevated levels in seminal fluid. This presumptive test is used to indicate the presence of seminal fluid; it does not confirm the presence of seminal fluid since the enzyme can be found in lower concentrations in other body fluids such as vaginal secretions and fecal material.

5.4.6.1 Reagents

Modified Brentamine Test

AP Stock Buffer (Brentamine)

Sodium acetate	6g
Glacial acetic acid, ≥99.5%	2ml
Deionized water	500ml

Mix the above ingredients and pH to an approximate pH of 5.0.

Working Solution A

1-naphthyl phosphate sodium salt, ≥98% titration	~10mg
AP buffer	~5ml

Working Solution B

o-dianisidine, tetrazotized (zinc chloride complex)	~10mg
AP buffer	~5ml

Working solutions are prepared at the time of use.

5.4.6.2 Method

5.4.6.2.1 Take a small cutting or swabbing of a targeted stain.

5.4.6.2.2 Place 1-2 drops of working solution A on the sample to be tested and allow the reagent to soak into the substrate for at least 30 seconds.

5.4.6.2.3 Place 1-2 drops of working solution B on the sample.

5.4.6.2.4 Interpret and document the results in the case notes.

5.4.6.3 Interpretation

Positive: A purple color change within 60 seconds.

Negative: The absence of a purple color change within 2 minutes.

Inconclusive: A purple color change occurring in 1-2 minutes.

The timelines listed above are guidelines only. Items with negative or inconclusive results may be submitted to an approved laboratory that performs DNA analysis. The determination is left to the analyst's judgement and should

be based on training and experience, and bearing in mind the circumstances of the case.

5.4.6.4 Caution/Safety

- When interpreting AP results, make sure to compare the color of the reaction for the tested sample to the controls. 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.
- o-dianisidine (Fast Blue B) is a possible carcinogen and should be handled cautiously.
- In order to optimize efficacy, store the working solution in a refrigerator/cooler when not in use and limit the reagents exposure to air and light.

5.4.6.5 Controls

Positive: A known semen or seminal fluid stain.

Negative: Unstained swab, fabric, filter paper, or empty spot well.

Reagents are tested at the time of use and the results are documented in the case notes:

A new stock solution is quality checked at the time the first working solutions are made from it. The results are documented in the appropriate reagent log.

It is recommended that the prepared reagents be re-tested 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 case notes.

5.4.7 ABACard® p30 Semen Test

The ABAcard® p30 test made by Abacus Diagnostics, Inc. is an immunochromatographic test for the detection of p30. p30 is a seminal fluid specific protein. Its presence in semen is independent of the presence of spermatozoa. The ABACard® p30 Semen Test is considered a confirmatory test for the presence of human semen/seminal fluid in instances where a positive AP result was obtained.

5.4.7.1 Supplies

ABAcard® p30 test card(s) and tube(s) of extraction buffer. The extraction buffer is provided with the p30 cards.

5.4.7.2 Method

5.4.7.2.1 Label an ABAcard® p30 test device for each sample, including controls.

5.4.7.2.2 Extract a portion of the stain or swab in a minimum of 200 µl of the ABAcard® p30 extraction buffer for at least 1-5 minutes and up to 2 hours.

5.4.7.2.3 Place approximately 200 µl (4 drops) of the extract in the sample well of the ABAcard® p30 test card, filling the sample well completely.

5.4.7.2.4 Read result at 10 minutes and document results in the case notes.

5.4.7.3 Interpretation

Positive: The formation of a visible pink line in the test "T" and control "C" regions of the membrane within 10 minutes.

Negative: No formation of a pink line in the test "T" region within 10 minutes and a visible pink line in the control "C" region. **See Caution/Safety**.

Invalid: No formation of a pink line in the control "C" region. 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.

5.4.7.4 Caution/Safety

- A false negative may occur when very high levels of p30 are present in the tested sample, causing a so-called "high dose hook effect". Under these conditions, unbound p30 could reach the test area before the mobile antigen-antibody complex, potentially resulting in a false negative result. If you suspect that this may have occurred, you should dilute the remaining extract and re-test.

- Positive results have been obtained from post-ejaculate urine and male urine from adult men, when the urine samples were directly added to the test.

5.4.7.5 Documentation

5.4.7.5.1 The lot number and the expiration date of the ABAcard® p30® test cards used shall be documented in the case notes.

5.4.7.5.2 The test card results, for both the casework and the positive and negative control, shall be documented in the case notes.

5.4.7.6 Controls

Positive: Known human semen or seminal fluid

Negative: Extraction buffer

A positive and negative control will be run with each batch of tests run.

5.4.8 Physical Examination

There are a number of techniques at an analyst's disposal that can be used to thoroughly examine physical evidence for biological material. The method(s) chosen will depend on the evidence substrate, the condition of the evidence, the targeted biological material, and case specific considerations as to what is probative.

If examination of an item involves disciplines other than Biology Processing, the involved examiners should confer before any work is undertaken. Unless circumstances dictate otherwise, examinations for biological stains should be done after collection and/or preservation of trace evidence, firearms evidence, and advanced bloodstain pattern analysis, but before collection and/or preservation of latent print evidence.

5.4.8.1 Method

5.4.8.1.1 Lay out the evidence onto a clean piece of paper.

5.4.8.1.2 Describe the evidence in the case notes.

5.4.8.1.3 Examine evidence for stains that appear biological in nature, using visual and tactile techniques. Examine all surfaces of the evidence that are relevant.

5.4.8.1.3.1 Various lighting conditions, including magnification, may be used in general evidence examination to enhance the observation of blood or to help locate minute, faint, or low-contrast stains. A high intensity light source with appropriate lens filter may also aid in the location of stains on dark substrates.

5.4.8.1.3.2 Certain body fluid stains, particularly semen, may cause a stiffening of the fabric. Examine fabric evidence for tactile changes that might indicate the presence of a stain in the absence of any visual staining characteristics. Perform this by lightly moving the fabric between gloved fingers to detect differences in the fabric's texture.

5.4.8.1.4 Document the visual appearance of any stains found and their location in case notes.

5.4.8.1.4.1 This can be done with a diagram or a photograph.

5.4.8.1.5 Not all biological stains may be visible to the unaided eye. Use of an alternate light source shall be performed dependent upon the case specifics. **See 5.5.1**.

5.4.8.1.6 Swab, use pre-moistened filter paper, or take a small cutting (~2-3mm^2) from the targeted area of the evidence.

5.4.8.1.7 Test the sample with the reagent appropriate for the targeted biological material.

5.4.8.1.8 Record the area tested, the method used for sampling (swab, cutting, filter paper), and the results in the case notes.

5.4.8.1.9 Repeat until all locations requiring testing have been sampled and tested.

5.4.8.1.10 If the results indicate a presumptive positive result for blood, and it has been requested the laboratory determine if it is of human origin, an ABAcard® HemaTrace® test shall be performed and documented.

5.4.8.1.11 If the results indicate a presumptive positive result for semen/seminal fluid, an ABAcard® p30 test may be performed, dependent upon the circumstances of the case. Results shall be documented if the test is performed.

5.4.8.1.12 Label the evidence with the following information:

- Case number
- Item number
- Initials of analyst

Should the evidence item not lend itself to marking, the proximal container should be marked.

5.4.8.1.13 If trace evidence, firearms evidence, and/or latent print evidence is observed during examination of an item for biological processing, collection of such evidence should be done for preservation purposes. **See 5.4.9**

5.4.8.1.14 If interpretation of the method of blood deposit (bloodstain pattern analysis) is relevant, preserve the evidence photographically and/or refer the evidence to a qualified analyst to perform Bloodstain Pattern Interpretation and/or Reconstruction **prior** to using destructive methods such as swabbing for presumptive tests.

5.4.8.1.14.1 The significance of some patterns or the recognition that bloodstain pattern analysis is relevant may not be realized until after all the processing has been completed or all the information gathered. Thus, the accurate documentation of the bloodstain patterns through notes, diagrams, and photographs becomes imperative.

5.4.8.1.14.2 Documenting the bloodstain patterns must be accomplished in a way such that the orientation, location, size, and position can readily be determined.

5.4.8.1.14.3 The location of any bloodstains or patterns on an evidence item shall be recorded. This can be accomplished through notes, photographs, sketches, or a combination thereof.

5.4.8.1.14.4 When documenting bloodstain patterns, the analyst should 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.

5.4.8.1.15 Swabs received as evidence shall be sampled via cuttings, unless noted otherwise.

5.4.8.1.16 If a located 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, selecting a swabbing or filter paper technique instead, or omit presumptive testing entirely.

5.4.8.1.16.1 If an analyst opts to omit testing entirely, the item shall be submitted to an approved laboratory that performs DNA analysis.

5.4.8.2 Cautions/Safety

- 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.
- Use an enclosed space hood and/or wear a face mask as appropriate.
- Any lab surface that will be used to examine evidence shall be cleaned with a dilute bleach solution (or product containing bleach) prior to beginning evidence processing and upon completion of all analysis.
- Use clean paper under items of evidence and change paper as necessary.

- 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 of evidence.

- All laboratory utensils used to sample evidence (e.g., scissors, tweezers, etc.) shall be cleaned with the following procedure:
    - A dilute bleach solution (approximately 10%) dip immediately followed by an alcohol dip. Dilute bleach solutions for utensil decontamination will be prepared on the day of use.

    - Wiping the utensil with a clean disposable tissue is strongly recommended. Serrated tools should not be used for sampling biological evidence.

5.4.9 Screening Techniques – Trace Evidence, Firearms Evidence, and Latent Print Evidence

Trace evidence, firearms evidence, and latent print evidence may be encountered during the processing of biological evidence. The following techniques may be used when collection/analysis of trace evidence, firearms evidence, and/or latent print evidence was not requested by the submitting agency but such evidence is incidentally observed during examination for biological stains and/or should be collected for preservation purposes.

5.4.9.1 Trace Evidence

The analyst shall use the following collection techniques: particle picking and tape lifting. The choice of collection method will depend upon the evidence type, the substrate, and the need to determine exact location of the evidence, among other variables.

5.4.9.1.1 Particle picking

5.4.9.1.1.1 Particle picking is the recommended technique when visible apparent hair evidence is to be collected.

5.4.9.1.1.2 Particle picking may be performed without equipment, using only the analyst's gloved fingers, or with the aid of tweezers, forceps, or other tools. Serrated tools are not recommended as they are difficult to clean and keep contamination-free.

5.4.9.1.1.3 Dependent upon the trace evidence, you may place trace evidence on the adhesive of a post-it note, than fold the note over on itself, or place the evidence in an appropriate container.

5.4.9.1.1.3.1 If utilizing post-it notes, label the post-it note with the case number, exhibit number and initials.

5.4.9.1.1.3.2 Place the post-it note into another container and seal to prevent contamination, damage, or loss.

5.4.9.1.1.3.3 Label the exterior of the packaging with the case number, exhibit number, date and initials of analyst and a brief description of the evidence collected.

5.4.9.1.1.3.4 Place this sealed package inside the packaging with the original exhibit.

5.4.9.1.1.4 Document the evidence in the case notes to include (if possible): type of evidence (i.e. hair), amount, where the trace evidence was collected from.

5.4.9.1.2 Tape lifting

5.4.9.1.2.1 Tape lifting is useful when collecting trace evidence that is not visible or apparent to the unaided eye.

5.4.9.1.2.2 The technique collects trace evidence from an area, so it is impossible to determine the exact location of a specific piece of trace evidence.

5.4.9.1.2.3 This method is not recommended for substrates that will strongly adhere to the tape lift adhesive (i.e. paper products, cardboard, etc.)

5.4.9.1.2.4 This technique will be performed after samples have been collected for DNA analysis, since adhesive lifting techniques have the potential for removing DNA material.

5.4.9.1.2.5 Scotch tape, fingerprint lifting tape, mailing tape or commercially purchased adhesive lifters are all acceptable materials to be used.

5.4.9.1.2.6 Peel away the protective layer to expose the adhesive layer of the tape lift. Do this just prior to use to avoid contamination.

5.4.9.1.2.7 Repeatedly pat the tape lift over the evidence item in an area defined by the analyst. The size of the area is dependent upon how much the evidence itself sheds fiber/particles, how much dirt/debris/trace evidence is present, and the overall size of the tape lift used.

5.4.9.1.2.8 After collection, affix tape lifts to a clear, colorless plastic sheet (i.e. transparency film, sheet protectors, etc.) The plastic sheet must allow for the tape lift to be removed or peeled away from it and may be pre-printed with a grid.

5.4.9.1.2.8.1 Label sheet with the case number, exhibit number, date and initials of analyst, and location of the tape lift collection.

5.4.9.1.2.8.2 If multiple tape lifts are collected from the same piece of evidence, the total number used shall be documented in the case notes.

5.4.9.1.2.8.3 Do not separate the tape lift from the plastic sheet unless unavoidable. Each time it is separated, the possibility of contamination and/or loss is introduced.

5.4.9.1.2.9 Place the tape lift(s) into a clean container and seal to prevent contamination, damage, or loss.

5.4.9.1.2.9.1 Label the exterior of the packaging with the case number, exhibit number, date and initials of analyst and a brief description of the evidence collected.

5.4.9.1.2.9.2 Place this sealed package inside the packaging with the original exhibit.

5.4.9.1.2.10 Document the location(s) where the tape lift(s) were taken in the case notes.

5.4.9.2 Firearms Evidence

If apparent firearms evidence (i.e. gunpowder) is observed**,** document in the case notes the location where the apparent firearms evidence was observed. Use caution when sampling and attempt to preserve the apparent firearms evidence for future analysis.

5.4.9.3 Latent Print Evidence

If apparent latent print evidence (i.e. apparent friction ridge skin detail) is observed during examination of an item for biological stains and/or the expected biology processing examinations have the potential to alter or destroy latent print evidence, the analyst will immediately stop processing the evidence and consult with a Latent Print analyst. Collection of the latent print evidence should be performed by a Latent Print analyst after any further examination for biological stains. While processing an item for biological stains, care should be taken to avoid swabbing any areas that may be processed for latent print evidence (i.e. area where apparent friction ridge skin detail is observed).

5.4.9.3.1 Document the location where the apparent latent print evidence was observed in the case notes.

5.5 Equipment

5.5.1 Alternate light source (ALS)

The alternate light source is a tool that can be used to visualize possible semen stains, saliva stains, urine stains, and other body fluids on physical evidence. An

ALS is a specialized light that combines powerful illumination with the ability to select discrete wavelengths of light. Certain combinations of wavelength and colored goggles/glasses/filters may result in fluorescence of physical evidence such as body fluids. ALS should not be considered an alternative to chemical presumptive tests when possible semen stains or bloodstains are visible.

5.5.1.1 Method

5.5.1.1.1 An examination using an ALS shall be conducted in a darkened room.

5.5.1.1.2 Follow the manufacturer's recommendations for proper operation of the ALS.

5.5.1.1.3 Select the wavelength of light and goggle/filter appropriate for the biological material targeted and document in the case notes. A combination of 450nm with a yellow filter is a good starting point for many biological stains.

5.5.1.1.4 Spread out the item to be examined on clean paper and systematically scan the item for areas of fluorescence/absorption as appropriate.

5.5.1.1.5 Circle or otherwise mark areas of fluorescence/absorption so they may be located for follow-up testing and document the locations of those areas in the notes. The locations where areas of fluorescence were noted may be recorded using words, diagrams, photographs, or a combination thereof.

5.5.1.2 Interpretations

5.5.1.2.1 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 a fluorescence/absorption result does not confirm the absence of a biological fluid.

5.5.1.3 Caution/Safety

- Precautions should be used when operating any ALS.
- Proper eye protection shall be worn by anyone operating an intense light source.
- Permanent eye damage can occur from direct illumination to the eye or reflected or refractive light hitting the eye.
- Exposing the skin to the beam of light can cause burns and other skin damage.

- All persons in proximity of usage shall adhere to the above safety guidelines.
- A number of 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 necessarily fluoresce, it depends on the stain and other factors.
- Blood will appear dark and will likely not fluoresce when viewed with an ALS.
- Blood serum and semen stains mixed with blood may fluoresce when viewed with an ALS.

5.5.2 Only suitable and properly operating equipment shall be employed in the laboratory.

5.5.3 The manufacturer's operation manual and other relevant documentation for each piece of equipment shall be readily available.

5.5.3.1 All maintenance is recorded in a logbook.

5.5.3.2 General maintenance shall be performed on an "as needed" basis. Refer to the manufacturer's manuals for specific maintenance instructions.

5.6 Traceability

5.6.1 Known blood, semen and/or seminal fluid reference materials used to verify the accuracy of detection tests, reagents, and techniques shall be assigned a unique lot number.

5.6.2 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 (i.e. lab staff).

5.6.3 Reference materials are subjected to the tests, reagents, and techniques for which it is used as a positive control in casework and an accurate positive result must be obtained.

5.6.4 The lot number of the standard that is used as a positive control shall be noted in the case notes (i.e. "o-tol +/- controls OK, Std lot # 010203AB")

5.7 Sampling See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual.

5.8 Handling of test items See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual.

5.9 Assuring the quality See Ada County Sheriff's Office Forensic Lab Quality Assurance Manual.

5.10 Reporting the Results

5.10.1 The report shall contain the following for all evidence:

5.10.1.1 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.

5.10.1.2 Location of biological stains, areas sampled for biological evidence, and/or apparent trace/firearm/latent evidence found.

5.10.1.2.1 If it is not possible to determine the exterior and interior surfaces of an item (e.g., condom), then the results should be reported with respect to how the item was submitted.

5.10.1.3 The results/conclusions of each test or series of tests shall be reported for each item of examined evidence.

5.10.1.4 The report shall be clear that the results/conclusions are for only the samples or evidence tested. Multiple swabs that are collected from the same source at the same time (e.g., vaginal swabs, cervical swabs, etc.) can be considered a single sample.

5.10.1.5 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 the item shall be submitted to an approved laboratory that performs DNA analysis.

5.10.1.6 The following report wording guidelines 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.

Analysis Results	Report Wording
Positive phenolphthalein,o-tolidine, BlueStar® test, and/or Luminol test	A statement that the result is from a presumptive test for blood (i.e. a positive result with a presumptive test for blood was obtained or testing indicated the presence of blood).

Positive phenolphthalein,o-tolidine, BlueStar® test, and/or Luminol test and a positive ABAcard® HemaTrace® test	A statement that testing indicated the presence of human blood.
Positive phenolphthalein,o-tolidine, BlueStar® test, and/or Luminol test and a negative ABAcard® HemaTrace® test	A statement that testing indicated the presence of blood; however, no human blood was detected.
Inconclusive phenolphthalein,o-tolidine, BlueStar® test, and/or Luminol test	A statement that an inconclusive result with a presumptive test for blood was obtained. The reason(s) why the result was inconclusive must also be included in the report (i.e. slow reaction).
Negative phenolphthalein,o-tolidine, BlueStar® test, and/or Luminol test	A statement that no blood was detected. If a negative phenolphthalein result is obtained after latent print processing, the report must qualify the result (i.e. A negative result with a presumptive test for blood was obtained; however, this result must be interpreted with caution, as certain latent print processing techniques may interfere with presumptive blood testing).
Negative screening results for blood (alternate light source, high intensity light source, visual and/or stereomicroscopic examination)	A statement that no staining consistent with blood was observed.
Positive AP test	A statement that a positive result with a presumptive test for seminal fluid was obtained.
Negative AP test	A statement that a negative result with a presumptive test for seminal fluid was obtained.
Negative screening results for semen/seminal fluid (alternate light source, tactile, and/or visual examination)	A statement that no staining consistent with semen/seminal fluid was observed.
Positive AP test and positive ABAcard® p30 test	A statement that semen/seminal fluid was indicated.
Positive AP test and negative ABAcard® p30 test	A statement that no semen was detected. A notation may 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.

Inconclusive AP test	A statement that an inconclusive result with a presumptive test for seminal fluid was obtained. The reason(s) why the result was inconclusive must also be included in the report.
Collection of trace evidence	A statement that trace evidence was collected. **Example:** Trace evidence was collected for preservation purposes but was not examined at this time.

Biology Screening Analytical Method History

Issuing Authority: Quality Assurance Manger

SECTION & COMMENTS	DATE ADOPTED	AUTHOR	REVIEWER(S)