Ada County Sheriff's Office
Forensic Lab
Biology Screening Analytical Method
Version 2.0

Content Page

1 Scope

This document is part of the Ada County Sheriff's Office (ACSO) Forensic Laboratory's quality management system. It does not include all available methods, but rather is a reference for procedures utilized by the Biology discipline and approved by the ACSO Forensic Laboratory. The intent is to provide thorough and consistent examination, processing, and documentation of evidentiary materials. The methods include procedures for locating potential biological stains, for conducting presumptive and confirmatory testing for the presence of blood and semen, and for the collection and/or preservation of items for additional testing.

These procedures shall be adhered to while conducting examinations, performing tests, and creating items that are subject to testing. Conformation with these documents helps facilitate assurance in the competence of the laboratory and acceptance of results. All forensic scientists performing analysis for the Biology discipline are responsible for performing work within these policies and procedures. However, it is not practical to provide procedures for all evidence that may be received, thus analysts must rely on their education, training, and experience to determine the most appropriate procedure(s) when examining evidence.

2 References

Ada County Sheriff's Office Forensic Lab Quality Assurance Manual (current version)

Ada County Sheriff's Office Forensic Lab Health and Safety Manual (current version)

NIST OSAC Subcommittee: Biological Methods (online)

SWGDAM Guidelines (online)

College/University level chemistry and biology text books

Scientific journals

Equipment manuals and product inserts

3 Terms and Definitions

Absorb/Absorption (light) – A process by which light strikes a surface and causes the substance/ material to absorb energy and transition to a higher energy state; an absorbing surface prevents reflection or diffusion of the light.

Acid Phosphatase (AP) – An enzyme found at elevated levels in semen and lower levels in some other body fluids.

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.

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

Body fluids – Any fluid in the body including, but not limited to, blood, semen, urine, saliva, sputum, sweat, milk, or vaginal secretions.

Catalyst – A material that increases the rate of a chemical reaction without undergoing any change in its own chemical structure.

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

Clean– A process to remove/reduce biological and/or chemical contaminants from surfaces, tools, and/or equipment (e.g., using 10% bleach or a comparable disinfectant).

Confirmatory test – A test that is specific for a body fluid, stain, or residue of interest, and reduces or eliminates false positive results.

Contamination – The unintended introduction of exogenous material/substance onto an item.

Controls – Samples of known type that are used to demonstrate that a reagent or procedure is working correctly.

DNA – Deoxyribonucleic acid; a molecule that carries genetic information for living organisms.

Epithelial cells – Cells that form a thin lining on the outside of the body and lines organs and cavities within the body (e.g., skin and vaginal surfaces); typically contain a nucleus.

False negative – A negative test result despite the presence of the targeted body fluid, stain, or residue of interest; possibly attributed to test limitations or limits of detection.

False positive - A positive test result despite the absence of the targeted body fluid, stain, or residue of interest; possibly attributed to a non-specific reaction.

Fluorescence – The property of absorbing light of shorter wavelength and emitting light of longer wavelength, which falls in the visible range.

Heme – The red, iron-containing compound that constitutes the nonprotein component of hemoglobin and some other biological molecules.

Hemoglobin – Protein in red blood cells that carries oxygen throughout the body.

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.

Negative Control – Analytical control used to determine contamination of test reagents; test reagent(s) without sample are expected to provide no response.

Occult blood – Blood that is present in amounts too small to be seen.

Oxidation – The loss of electrons (e.g., chemical substance gains oxygen or loses hydrogen).

Oxidizing agent – Oxidizes/takes electrons from another substance and becomes reduced (i.e., gives oxygen to another substance).

p30 – A protein used to confirm the presence of semen; also known as prostate specific antigen (PSA).

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**.**

Phenolphthalein – A presumptive test for the presence of blood.

Positive Control – Analytical control used to determine if a test is performing properly; test reagent(s) and a known targeted sample/fluid are expected to provide a positive response.

Presumptive test – A preliminary test to ascertain the presence of a targeted biological fluid; not confirmatory because it will react with other substances.

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.

Reduced – Chemical substance gained electrons (e.g., substance loses oxygen or gains hydrogen).

Sample Selection – A practice of selecting items or portions of items to test, based on training and experience; there is no assumption about homogeneity.

Screen/Screening – The process of examining an item for the presence of evidence; allows the analyst to make determinations about how to proceed with the examination and/or testing.

Semen – A viscous whitish secretion produced by the male reproductive organs and ejaculated during orgasm; also known as seminal fluid. It contains secretions of the prostate, seminal vesicles, and various other glands, epithelial cells, minor constituents, and may contain sperm.

Sperm/Spermatozoa – Microscopic male reproductive cells manufactured in the testicles and carried in semen; a single sperm cell may be called a spermatozoon.

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

4 General requirements

4.1 Impartiality

An awareness of various types of bias (e.g., allowing relationships to influence analysis/results or seeing what is expected) doesn't eliminate its occurrence, but can help mitigate its effects. Protocols should be followed to reduce the risk of biases that may impact the testing process. Detailed notes may allow for the detection of bias (e.g., conclusion or protocol violation) and help ensure a complete examination. Understanding the potential for bias aids self-reflection to question actions and conclusions during both the testing and review process. It is important to remain objective and admit to mistakes.

Analysts shall be thoughtful, thorough, and objective in their analysis, reviews, and testimony. Although a potential source of bias, reports/narratives or communications with persons having knowledge of the case can help determine valuable information regarding the associated items (e.g., probative value and body fluid(s) sought). Customer requests shall be satisfied when possible; however, the focus shall be maintaining the integrity of the item(s) and obtaining the most informative result(s) while minimizing sample consumption.

4.2 Confidentiality See ACSO Forensic Lab Quality Assurance Manual

5 Structural requirements See ACSO Forensic Lab Quality Assurance Manual

6 Resource requirements

6.1 General

The Biology discipline shall have available the personnel, facilities, equipment, systems, and support services necessary to perform accredited laboratory activities.

6.2 Personnel See ACSO Forensic Lab Quality Assurance Manual

6.3 Facilities and environmental conditions

Each analyst is responsible for maintaining a safe work environment and preventing contamination. This includes measures to maintain clean workspaces and to properly handle and store evidence items.

6.3.1 Cleaning of laboratory work areas and equipment is necessary to minimize the potential for contamination. Cleaning should follow the guidance below and as needed.

- Benchtops/work surfaces (including hoods) and the examination room floor should be cleaned prior to processing casework. Document cleaning in the log. It is also recommended to clean work surfaces between items and after processing is complete. Continued processing of the same case over multiple days does not necessitate additional documented cleaning.
- Examination tools/utensils (e.g., forceps, scissors, and measuring devices) shall be cleaned prior to use and between samples. Single-use/disposable tools may be used direct from the unopened package but shall be cleaned prior to additional usage.

Note: Bleach solutions for cleaning should be prepared fresh each day. It is advised to follow with a water rinse to avoid corrosion of metal surfaces. Utensils should be dried with a clean disposable tissue. Serrated tools should not be used as they are more difficult to clean.

6.3.2 Generally, only one item or a set of similar items (e.g., comingled clothing or bedding) will be examined at a time in order to prevent sample mix-up and/or cross-contamination. Items may be separated from each other by time and/or space. Items shall be stored in a secure area and should be repackaged and sealed as soon as practicable following the completion of analysis. Limiting the number of individuals with access to the workspace and/or items also minimizes the potential for contamination.

6.4 Equipment

6.4.1 Only suitable and properly operating equipment shall be employed in the laboratory. Operating manuals shall be maintained on the network drive, in a product information file, or near the instrument (particularly those referred to for instructions). Records of maintenance, failure, repair, etc. shall be kept in an equipment log. Instrument failure will result in removal from service and a sign placed to indicate this status. The instrument shall not return to service until it is repaired, passes a performance check, and/or is recalibrated, as appropriate.

Equipment should be evaluated for appropriate function with each use. General maintenance shall be performed on an "as needed" basis. Refer to the appropriate equipment manual for specific maintenance instructions. Any problems should be documented, and the Technical Lead or Lab Manager notified.

6.4.1.1 Microscopes shall be serviced as necessary by a qualified external technician. It is recommended that this interval not be longer than an accreditation cycle.

Equipment utilized for locating potential items/areas to test (e.g., flashlight, ALS, hand-held lens, and stereomicroscope) or documentation (e.g., camera and accessories) does not require routine calibration or maintenance.

Although not considered test equipment, performance of the alternate light sources shall be evaluated annually by an internal analyst. This will include an assessment of the visibility of some commonly encountered body fluid stains, to include semen.

6.4.1.2 Except for reference materials and ABAcard® test kits, equipment does not need to be specified in case documentation.

6.4.2 To prevent contamination of equipment, personnel, and exhibits, appropriate protective equipment (PPE) should be utilized (e.g., coat, gloves, and eye protection) and changed or cleaned when necessary. Disposable gloves should be changed or cleaned between handling different items and prior to using a computer/keyboard, camera, telephone, etc. Equipment that is moved between the laboratory and scenes should be maintained in a way to preserve its function and prevent contamination both on-site and during transport.

6.4.3 Reference Materials

Known blood and semen used to verify the accuracy of detection tests, reagents, and techniques, shall be assigned a unique lot number. This lot number shall be documented on the appropriate form and marked on the outer sample container(s). The source/donor may be identified, anonymous, or unknown and a general designation may be used instead (e.g., lab staff or human blood). Reference materials can be used to prepare additional known samples (e.g., stained fabric and swabs), which may be used for positive controls, validation, intralaboratory comparisons, etc. These items shall be labeled with the same lot number as the

original, as appropriate. The reference lot number shall be included in the case notes when used as a positive control.

6.4.4 Reagents prepared in the laboratory shall be labeled with the identity of the reagent and the preparation date or the Lab assigned lot number. For a reagent made in-house, the lot number shall be the date and preparing analyst's initials (e.g., 070716HC). This shall be recorded in the reagent prep log book. The log shall also include the chemicals utilized and an indication that reliability testing was conducted. It is not necessary to fully label reagents that are used and discarded on the day of preparation. BLUESTAR® FORENSIC and luminol working solution preparation and reliability testing may be documented in the case notes.

Expiration dates need not be documented, as use is permitted beyond labeled date(s) if reagents yield the appropriate response with controls as defined by the procedure.

If no safety data sheet (SDS) or comparable information is available for a mixture/reagent prepared in-house, it need not include hazard labels. Hazard labels are also not necessary for reagent containers that are to be discarded shortly after use (generally same day). Despite a lack of hazard labels, analysts are expected to treat all chemicals as potentially hazardous and to handle, use, and store chemicals in a safe and compliant manner.

6.4.4.1 Reagent Preparation

Reagents shall be prepared according to written protocols and stored appropriately.

Acid Phosphatase (AP) Test Reagents (Modified Brentamine)

AP Stock Buffer

Sodium acetate	6g
Glacial acetic acid	2ml
Deionized water	500ml

Mix the above ingredients and store refrigerated.

Note: Quality check new stock solution when the first working solutions are made from it.

AP Working Solution A

α-Naphthyl phosphate sodium salt	10mg
AP Buffer	5ml

Mix the above ingredients. Prepare fresh, daily at a minimum.

AP Working Solution B

o-Dianisidine, tetrazotized (Fast Blue B salt)	10mg
AP buffer	5ml

Mix the above ingredients. Prepare fresh, daily at a minimum.

Notes:

- Wear gloves. o-Dianisidine is a carcinogenic hazard.
- Working solutions are prepared and quality checked at the time of use.
- To optimize efficacy, store the working solution in a refrigerator/cooler when not in use and limit air and light exposure.

Hydrogen Peroxide, 3%

Generally, a commercial purchase. Diluted with water if preparing from a more concentrated form.

Notes:

- More hazardous as concentration increases.
- Begins to break down once exposed to air and the activity decreases over time. Extend efficacy by storing in a dark container and a cool location.

o-Tolidine Reagent

o-Tolidine	0.6 g
Glacial acetic acid	100 mL
Ethanol	100 mL

Dissolve o-tolidine in the acetic acid/ethanol mixture. Store refrigerated.

Notes:

- Wear gloves. o-Tolidine is a carcinogen.
- o-Tolidine is light sensitive and should be stored in a dark container.

Phenolphthalein (Kastle-Meyer) Test Reagents

May be a commercial purchase.

Phenolphthalein Stock Solution

Phenolphthalein	2 g
Potassium hydroxide	20 g
Deionized water	100 ml
Zinc, granular (~20 mesh)	20 g

Reflux the above ingredients until the solution becomes colorless (produces colorless phenolphthalin in ~2-3 hours). Store refrigerated in a dark/amber bottle with additional zinc to keep the solution in its reduced form.

Phenolphthalein Working Solution

Phenolphthalein stock solution	1 part
Ethanol	4 parts

Mix and store refrigerated in a dark/amber dropper bottle with additional mossy/granulated zinc.

6.4.5 All measurements are approximate. Therefore, measurement equipment (e.g., rulers, scales, balances, and volumetric plasticware or glassware) does not require routine calibration or maintenance, and it need not be documented and/or tracked. Precise measurements are not necessary for reagent preparation as those utilized for testing are quality checked prior to use.

6.5 Metrological traceability
Does not apply for biology screening.

6.6 Externally provided products and services
The equipment and supplies below have been determined to have a significant effect on the accuracy of test results.

6.6.1 Abacus Diagnostics ABAcard® Test Kits
The following procedures shall be employed to verify that the kits received function properly prior to use in casework. Purchasing documentation shall be retained and the kits shall be stored according to manufacturer guidelines. The date received should be marked visibly on the kit as well as the date of the acceptable quality check (QC). Additional QCs are not required for kits with the same lot number, but they shall still be marked as previously specified. Kits may be used beyond the expiration date if the controls perform as expected.

- ABAcard® HemaTrace® Test
  Perform the test as usual with an approximately 1mm^2 cutting or $^1/_{16}$ swab from a known bloodstain and a reagent blank. Record the extraction time and the results, including the time it took for the positive reaction to be visible. The samples must react appropriately within 10 minutes to pass. If the positive does not pass, attempt another test with the same sample after the extended extraction period or take a new, larger sample of approximately 2mm^2 or $^1/_8$ swab. If the negative does not pass perform a second test with new extraction buffer and/or a new test card. If the second test fails, contact the Technical Lead regarding how to proceed. Document the check on the Biology Test Kit QC Form.

- ABAcard® p30 Test
  Perform the test as usual with an approximately 1mm^2 cutting or $^1/_{16}$ swab from a known semen stain and a reagent blank. Record the extraction time and the results, including the time it took for the positive reaction to be visible. The samples must react appropriately within 10 minutes to pass. If the positive does not pass, attempt another test with the same sample after the extended extraction period or take a new, larger sample of approximately 2mm^2 or $^1/_8$ swab. If the negative does not pass perform a second test with new extraction buffer and/or a new test card. If the second test fails, contact the Technical Lead regarding how to proceed. Document the check on the Biology Test Kit QC Form.

6.6.2 Reagents
Non-water components utilized to prepare in-house testing reagents shall be tracked on the reagent preparation form or in case notes, as appropriate. Test reagents shall undergo a documented quality check prior to casework use to verify performance. Where distilled water is specified in reagents, deionized water may be used as well. Reagents shall be stored according to manufacturer guidelines, as available.

Passing criteria for the quality check is detailed on the reagent preparation form and/or in the associated procedure and generally consists of expected performance with controls. Reagents may be used beyond the expiration date if controls yield the appropriate response.

7 Process requirements

7.1 Review of requests, tenders, and contracts

7.1.1 The analyst ultimately decides the items to examine and the tests to be performed. An attempt shall be made to communicate with the customer(s), as needed, to clarify requests or to inform them of substantial changes.

7.1.2 An effort shall be made to save about half of each sample for independent testing. If testing would consume all or most of the sample, consider the need for alternate or additional testing (e.g., DNA) and notify an appropriate agency or case representative. Documented confirmation that the risk of consumption is understood must be received prior to testing.

7.2 Selection, verification, and validation of methods

Equipment and methods shall be shown to function as expected in the laboratory prior to their use for casework. Deviations from established procedures shall be approved prior to use with casework samples. Concerns regarding equipment/supplies, methods, analysis, or interpretation shall be brought to the attention of the Technical Lead.

7.3 Sampling

Not to be confused with sample selection, sampling is not utilized for biology screening. Sample selection makes no inference about the entire population. Selection of items and samples is left to the analyst's discretion and should be based on training and experience, while also considering the case circumstances.

7.4 Handling of test or calibration items

Analysts shall take precautions to ensure that evidence is not lost, contaminated, or otherwise compromised during collection, packaging, analysis, and storage.

Safety

Good practices in sample handling, including the proper use of personal protective equipment (PPE), prevents evidence from being contaminated by the examiner or other items and shields the examiner from potential hazards. All biological samples should be treated as potentially infectious. Analysts shall be aware of and utilize the various engineering and work practice controls, as available and appropriate. PPE should be used and changed when necessary and appropriate. Disposable gloves shall be worn when directly handling evidence and be mindful of contact with personal items. Replace gloves between items or as needed, especially if soiled (visibly or potentially) or defective. Eye protection should be worn whenever a hazard to the eyes exists (e.g., utilizing aerosolized chemicals or alternate light sources) and is recommended when making or using reagents that present a potential splash hazard.

Contamination Prevention

DNA contamination can be prevented by following minimal evidence handling guidelines. These guidelines should be followed while processing items to maintain their integrity. Clean the examination surface with a disinfecting product, remove/examine one item at a time, avoid contact of the evidence or examination surface to external packaging, and use new/clean examination paper(s) and clean/sterile utensils and supplies (e.g., scalpel, water, and swabs). To prevent contaminating items themselves, analysts should avoid talking during examinations, wear appropriate PPE (e.g., gloves, mask, and coat), and be mindful of leaning over and directly contacting items. When collecting a portion of an item for testing or preservation, avoid touching the cutting utensil, swab head(s), or testing portion of filter paper to anything other than the area

being sampled, cleaning or moistening fluids, test reagents, and/or the holding vessel or packaging, as appropriate.

Evidence should be processed in a logical manner to further avoid cross-transfer between examined items. Evidence shall be handled at a different time or in a different space from standards/known samples. It is generally advised to process questioned items prior to known samples, and to examine items from the victim prior to those from the suspect. Processing of samples from the scene depends largely on case circumstance. If items are received in a comingled state (i.e., multiple items packaged together) there is no need to separate them for analysis.

Packaging/Storage

Proper packaging and storage can help to prevent loss and degradation of biological material including DNA. The following are factors that may influence degradation: time, temperature (i.e., heat or freeze/thaw cycles), humidity, ultraviolet (UV) radiation exposure (e.g., sunlight), biological contaminants (e.g., bacteria, fungi, and enzymes), and chemical exposure (e.g., formalin or bleach). To avoid moisture, biological evidence should not typically be placed in airtight containers. Most samples should be packaged in breathable material (i.e., paper) and are preferably stored in a temperature-controlled environment. If biological stains cannot be fully dried, then frozen storage is often preferred. Liquid blood should be refrigerated, not frozen.

Items are not typically retained in the Laboratory for long periods of time. Non-optimal short-term storage is not likely to affect the overall condition of most evidence items. If an item is packaged in a manner that is likely to cause deleterious change or loss, it shall be repackaged appropriately. If it is not possible to properly package and/or store an item, the circumstance(s) shall be documented in the case notes.

Analysis Approach

Forensic biology testing, including eventual identity testing, may be utilized to associate an individual (e.g., suspect or victim) to a location, an item, or another person. The process typically begins with examination for the presence of a body fluid or other potential sources of DNA. Based on case information and/or visual examination items may be tested for blood and/or semen. Presumptive tests, sensitive but not specific, are a fast and inexpensive way to indicate the need for further testing on an item/area of interest. Confirmatory tests are more specific to a biological substance and reduce or eliminate false positives. Ultimately the source may be determined through DNA testing.

There are numerous techniques at an analyst's disposal that can be used to examine physical evidence for biological material. The method(s) chosen may depend on several factors including case considerations (i.e., probative value) and the evidence itself (e.g., substrate, condition, and targeted biological material). Focus on maintaining the integrity of the item and on minimizing sample consumption when considering appropriate methods.

Conservation of material for future testing is a priority and discretion shall be used with limited or poor condition samples. The analyst shall consider the information that may be gained from each test and additional or alternate testing that may be needed (e.g., DNA or BPA). Focus on maximizing the potential to obtain the most informative result(s) and avoid multiple attempts at testing. Techniques that minimize sample consumption (e.g., filter paper transfer, smaller cutting, and reduced volumes) may be selected or testing may be abandoned entirely, especially if DNA

testing is needed. If necessary, contact the customer or case representative to determine their needs and discuss options for how to proceed.

It is important that the analyst be able to recognize and evaluate the potential significance of the presence or absence of biological stains in relation to the evidence and to the entire case, especially when determining samples/stains to select for analysis. It may help to review the incident narrative(s) or contact the customer or case representative (e.g., submitting individual, detective, or attorney) to assist, as necessary and appropriate.

Whether requested or encountered, it is important to also be aware of other types of evidence. All recognized evidence shall be documented, conserved, preserved, and/or collected, as appropriate. Consider the ideal approach when an item requires multi-discipline analyses.

- Limit exposure to unnecessary individuals prior to collection of biology samples to prevent contamination.
- Unless circumstances dictate otherwise, biological examination should follow collection, preservation, and/or analysis of trace, firearms, and bloodstain pattern evidence.
- Unless circumstances dictate otherwise, biological testing and collection should be performed prior to latent print processing.
  - Cyanoacrylate or powdering of non-porous items is acceptable, but wet chemistry processing should be avoided.
  - Sterile/single-use brush and powder are optimal to minimize contamination.
- Consult with an analyst from the relevant discipline, as necessary and appropriate, to advise on areas to test/collect in order to avoid damaging other potential evidence. Document any relevant conversation/discussion.

<u>Controls</u>

Positive controls are known substances that have an expected result. Negative controls should not generally yield a response and are used to determine if reagents are contaminant-free. Appropriate controls are specified in each method and demonstrate that the reagents and tests perform as expected. Control performance may be documented to indicate individual results (e.g., positive and negative) or alternative wording may be used to express the acceptance or rejection of control results (e.g., pass, OK, or fail). Both the positive and negative control must perform as expected to be acceptable. Additional detail/explanation shall be provided for failure of any control.

If controls fail to perform as expected, they may be retested with newly prepared reagent or other components, as appropriate. If the problem persists, consult the Technical Lead for additional direction.

Substrate controls (i.e., unstained evidence substrate; often an area adjacent to the questioned stain) are not typically collected and/or tested. Where appropriate and available, the analyst may decide to test the substrate. If this occurs, the location tested, and the result shall be documented in the case notes. However, caution is advised as fluids may be more widely distributed on an item than can be visually observed. It is also likely for DNA to be detected on unstained areas, but the utility may be limited due to various unknown factors (e.g., time of deposition, persistence, and homogeneity).

Evidence/Stain Designators

Numbering shall be unique and follow a logical order (e.g., 1, 2, 3, … or A, B, C, …). Different formats are acceptable, and it is at the discretion of the analyst how to best designate items, subitems, stains, etc. Below are some examples.

- A single item (e.g., Item SG1, shirt with suspected blood stains) with:
  - one area/stain collected and packaged = SG1.1
  - additional areas/stains collected and packaged= …, SG1.2, SG1.3, etc.
- One item with multiple sub-items (e.g., Item SG2, purse with contents)
  - purse = Item SG2
  - contents = SG2.1, SG2.2, etc.
- Multiple items packaged together (e.g., Item SG3, bag of clothes)
  - shirt = SG3.1, pants = SG3.2, shoes = SG3.3

If multiple stains are designated on an item (e.g., carpet (SG4) with cuttings taken from three stains for ABAcard® HemaTrace® test, designated SG4.1, SG4.2, and SG4.3) but only one sample is ultimately removed, that sample shall retain its original designation (e.g., SG4.2) when packaged and labeled. It is not generally necessary to designate every tested stain with a number or letter.

7.4.1 General Schemes/Guidelines

The following guidelines and methods are meant to standardize biological processing. However, analysts may select the most appropriate procedure(s) for each item. Above all, the examination and analysis shall be relevant and conducted in a manner to preserve the integrity of the item.

7.4.1.1 Evidence Handling and Screening

Initial Examination

- Document the packaging and condition, as received (e.g., tape-sealed manila envelope).
- Clean the work surface and lay out clean paper (e.g., butcher paper or paper towel).
- Open the packaging without destroying existing seals, if possible.
- Label each item with the case number, item number, and analyst's initials, at a minimum.
  - Items may be marked on directly or labeled with a tag, tape, etc.
  - If an item cannot be/is not marked, document the reason and label the proximal packaging.
- Visually examine and describe the item(s)/contents.
  - Note the overall condition including visible stains and other potential evidence.
  - Examine all surfaces of the evidence, as available.
  - Photograph and/or sketch, as needed.
- Document the presence and/or absence of potential biological stains.
  - Not all stains may be visible to the unaided eye. Various lighting conditions and magnification may enhance the appearance of minute, faint, or low-contrast stains.
  - Certain body fluids, particularly semen, may cause a stiffening of the fabric. Tactile changes may indicate the presence of a stain in the absence of any visual characteristics.
  - Note the general appearance and location of stains. Details may be noted, as appropriate (e.g., color, texture, quantity, size, shape, directionality, and sequence of staining).

Testing

Utilize the appropriate tools and tests to evaluate potential stains (See general examination schemes for blood and semen).

- Confirm and document the proper performance of controls.
  - Document the lot number of the positive control.
  - Controls should be tested in the same manner as the evidence.
  - May be tested prior to casework or, if appropriate, concurrent with the test.
- Typically start with presumptive testing.
  - Reagents should not be applied directly to evidence, if possible. Transfer stains via swab or filter paper to perform testing.
  - Positive results only indicate the possible presence of the suspected fluid because reactions are also known to occur with other substances.
- Confirmatory tests may follow to eliminate or reduce presumptive test false positives.
  - May also follow an inconclusive or negative presumptive result at the analyst's discretion, which should be based on training and experience and dependent upon the item type and case circumstances.
- Test stains and document the results.
  - Record the number of stains/areas tested.
  - The approximate location(s) should also be documented, which may be accomplished using words, diagrams, photographs, or a combination thereof.
    - Prioritize depiction of positive and inconclusive results over negative results.
    - Note/clearly express when results are not depicted (e.g., negative stains not shown in sketch).
    - Document any cuttings taken; may include the approximate size.
  - Results may be marked directly on the item, if possible and appropriate.
    - More thorough documentation is necessary when results are not marked on the item in a manner that is likely to persist (i.e., cannot verify location/result by reexamining the item).
- Swabs/cuttings taken for performing tests are not considered evidence and are discarded after results are obtained and documented.
  - If collected but not utilized for testing (e.g., request to stop testing) the swab/cutting should be properly packaged and returned to the original item or repackaged separately.

Collecting and Packaging Items

Items that do/may require additional testing (e.g., DNA) shall be properly documented, collected, packaged, and preserved. The best practice is not necessarily the same for each item, especially for different substrates.

- Document the location sampled and the number of swabs used or approximate cutting size.
- Collect a portion of the item, if it is not possible or practical to collect/forward as a whole.
  - Porous items are generally cut or swabbed
  - Non-porous items are generally swabbed.
  - Avoid scraping dried stains.
- Swab collection
  - Material is typically transferred with a minimal amount of sterile water, if needed.
  - Transfers are typically concentrated at the swab tip; however, it may be appropriate to maximize the transfer over the surface area.
  - Consider noting how/where material is transferred when staining is not visible.
  - Should dry thoroughly prior to packaging and sealing.

- Very small evidence (e.g., hairs/fibers, flaked blood, and glass pieces) which may escape the packaging or be difficult to relocate should be placed in a smaller container (e.g., bindle, small envelope, folded sticky note) prior to placement in the outer packaging.
- Package separately or place inside the original packaging.
  - May depend on the case circumstances and/or availability of testing.
  - Seal the proximal packaging to prevent contamination, damage, or loss.
  - If separated from the parent item, minimally label with the case number, exhibit number, a brief description of the evidence, and begin a new chain of custody.
  - If repackaging with the original item, minimally label the proximal packaging with the case number, exhibit number (may be same as original), and initials of the analyst, and a brief evidence description.

7.4.1.2 Examination of Evidence for Blood

Visual examination, presumptive screening, and confirmatory testing for blood and indication of human origin.

Visual examination

- In most cases, bright light (e.g., flash light) is adequate to assist in locating stains that are visibly consistent with blood.
- Although blood does not fluoresce, an alternate light source may enhance contrast. When infrared (IR) lighting is selected, blood may appear as a dark stain on a light background, which may be helpful for dark substrates. (See Alternate Light Source)
- Magnification (e.g., stereomicroscope) may be used to enhance the observation of very small bloodstains.
- Continue with a presumptive test after potential stains are located.

Presumptive Tests:

- Sensitive, but not specific to blood and cannot distinguish human from animal.
- Chemiluminescence Tests (See BLUESTAR® FORENSIC and Luminol)
  - Although not commonly performed in the lab, these tests may be utilized when it is suspected that bloodstains have been cleaned/washed away or are otherwise not visible.
  - Peroxide-mediated oxidative tests catalyzed by the iron in hemoglobin result in excited molecules that release energy by temporarily emitting light.
  - Necessitates a dark environment (i.e., eliminate or reduce light sources).
  - Utilize standard low light photographic settings and techniques (e.g., ISO 400, aperture f/6.7, and 3 to 30 second exposure).
  - Positive reactions are known with substances other than blood (e.g., some acrylic paints, varnishes, and chlorine products).
  - Considered and reported as a presumptive test for blood; however, it is recommended to supplement with a color test and/or confirmatory test, if possible.
- Color Tests (See Phenolphthalein and o-Tolidine)
  - Chemical tests based on the peroxide-mediated oxidation of an organic compound, catalyzed by the peroxidase activity of hemoglobin. The compound is colorless in solution until oxidized to a colored solution.
  - Positive reactions are known with substances other than blood (e.g., plant peroxidases).
  - Multi-step testing helps minimize false positives due to chemical oxidants (e.g., rust).

- As appropriate, a positive presumptive test may be followed by a confirmatory test.
  - Often not necessary, especially for multiple samples.
  - An inconclusive result should be followed by an alternate presumptive test and/or a confirmatory test, especially if it represents the only potential blood stain for the case.

Confirmatory Test (See ABACard® HemaTrace®)
- To confirm the presence of blood and indicate human origin.
- Antigen-antibody reaction:
  - If target antigens (Ag) are present, they react with dye-labeled mobile antibody (Ab).
  - The formed Ab-Ag complex migrates toward the test and is captured by stationary Ab, forming an Ab-Ag-Ab sandwich which allows dye accumulation/pink line formation.
  - As an internal positive control, Ab-dye conjugates cannot bind to the test area and instead are captured at a control line to indicate that the test worked properly.
- Known to react with blood from higher order primates and ferret.
- Discretion should be used in testing small or poor condition samples if additional testing (e.g., DNA) may be needed.

7.4.1.3 Examination of Evidence for Semen

Visual/Alternate Light Source (ALS) examination, presumptive screening, and confirmatory testing for semen.

Visual/ALS examination (See Alternate Light Source)
- Normal room lighting and/or an ALS are often utilized to locate potential semen stains.
- Substrates and stains, including other body fluids (e.g., saliva and urine), may fluoresce
- Tactile examination
  - Semen is often stiff or starchy when dry.
  - Alternate or additional test when fluorescence is likely to be quenched (e.g., dark substrate) or otherwise not apparent.
  - Performed by lightly touching the fabric or moving it between gloved fingers to detect differences in the fabric's texture.
- Continue to a presumptive test after locating potential stains.
- If circumstances dictate and/or at the discretion of the analyst, presumptive testing may be performed in the absence of a visual cue (e.g., pants/panty crotch when worn after incident).

Presumptive Color Test (See Acid Phosphatase)
- Tests for an enzyme found in high levels in semen.
- Known to have positive reactions with other substances (e.g., vaginal secretions, fecal stains, and plant matter) so confirmatory testing is necessary.
- An inconclusive result should be followed by a confirmatory test, especially if it represents the only potential semen stain for the case.

Confirmatory Test (See ABACard® p30)
- The protein marker p30, or Prostate-Specific Antigen (PSA), is used to indicate semen and does not depend on the presence of sperm.
- Antigen-antibody reaction:
  - If target antigens (Ag) are present, they react with dye-labeled mobile antibody (Ab).

  - The formed Ab-Ag complex migrates toward the test and is captured by stationary Ab, forming an Ab-Ag-Ab sandwich which allows dye accumulation/pink line formation.
  - As an internal positive control, Ab-dye conjugates cannot bind to the test area and instead are captured at a control line to indicate that the test worked properly.
- Discretion should be used in testing small or poor condition samples if additional testing (e.g., DNA) may be needed.

7.4.1.4 Advisory for Other Biological Evidence

Some items may be sampled for DNA testing without first determining that a body fluid is present. This often involves targeting areas where DNA is suspected due to the way an item is likely to have been used, handled, worn, etc.

General collection

Collection is typically accomplished via cutting or swabbing and may depend on several factors, including, but not limited to, the substrate and the suspected substance (i.e., body fluid or touch DNA). Depending on the item and the case circumstances, it may be appropriate to employ an ALS.

One swab is recommended when small amounts of DNA are anticipated. Two swabs are generally recommended for items with a suspected fluid (e.g., saliva), wearer DNA, or more forceful contact. Additional swabs may be used depending on the amount of staining transferred to the swab, size of the item, etc.

Suspected fluid

Presumptive tests do exist for saliva, urine, and fecal matter; however, they are not currently utilized in the Laboratory. Saliva may be present on bottles, cans, utensils, tape with bite marks, etc. Target the portion(s) of the item likely to have been in contact with the mouth to collect a sample for DNA testing. Urine and feces may be present on an item, but the utility is typically limited, and DNA testing is often not performed on these samples.

Other body fluids (e.g., vaginal fluid/material and nasal mucus) may be forensically relevant but have no routine test. This does not prevent the collection of a sample for DNA testing. The area targeted and probative value are largely dependent on case circumstances.

Wearer DNA

Wearer DNA is deposited on an item as a result of the item being worn by an individual; however, some items may be shared. This can include items such as gloves, hats, bandanas, glasses, clothing, and shoes. Target the portion(s) of the item likely to have sweat transfer and/or direct contact with the skin.

Handler/Touch DNA

Touch DNA commonly refers to DNA from skin cells left on an object after it has been casually handled or touched. Target the portion(s) of the items likely to have been in contact with skin.

Firearms may be swabbed in their entirety in the absence of a latent print request and if there is no blood present. Otherwise, avoid areas that appear to be stained with blood. Focus on the following areas:

- Revolver: trigger, hammer, cylinder release, ridged surfaces on cylinder, and grip
- Semiautomatic handgun: trigger, slide serrations, hammer, and grip
- Magazine: bottom and feeding area
- Rifle or shotgun: trigger, stock, and forestock
- Other textured surfaces: magazine release, safety, and other buttons

Documentation

Indicate how and where the item is sampled in the case notes. Note if areas are avoided and why. If swabs are utilized, document the number used and note the color of the staining transferred, if present.

7.4.1.5 Advisory for Non-Biological Evidence

Non-biological evidence (e.g., trace, firearms, or latent prints) may be encountered while processing an item for biological evidence. If these types of evidence are observed, they should be documented and collected or otherwise preserved. The following may be used as a guide when such evidence is encountered, whether requested or not.

Refer to the ACSO Crime Scene Analytical Method and/or consult with a qualified discipline analyst for additional guidance.

Bloodstain Pattern Analysis (BPA)

BPA involves the study of the size, shape, and location of bloodstains in order to determine the physical events which gave rise to their origin. If a potential bloodstain pattern is observed, at a minimum, it should be documented photographically with a scale. Consultation with a qualified BPA analyst is preferred, as the item may need to be preserved and examined prior to using potentially destructive testing methods (e.g., swabbing or cutting).
The significance of patterns or recognition that BPA is relevant may not be realized until after processing has been completed. Thus, complete and accurate documentation of bloodstain patterns becomes imperative.

Documentation of bloodstain patterns should be accomplished in such a way that the orientation, location, size, and position can readily be determined. This can be accomplished through notes, photographs, sketches, or a combination thereof. The analyst should also record the following physical characteristics of the stain or pattern, as appropriate: color; apparent concentration, size, number, and distribution of stains; saturation of porous substrates (i.e., sits atop the surface or penetrates the material); clarity, sharpness, and symmetry of stain edges; and any other observable characteristics.

If biological testing must proceed without first consulting a BPA analyst, utilize methods that preserve the overall pattern and individual stain shapes as much as possible (e.g., sample from the stain interior). Selection of a larger stain will make it easier to avoid altering the outer perimeter.

Trace Evidence
Evidence (e.g., murder weapon or material found on a decedent) may be examined for transferred material such as hairs, fibers, glass, etc. Due to the often-transient nature of trace evidence and because there are currently no local trace analysts, it may not be the most ideal evidence. However, trace evidence can be valuable and may be all that is available. Consider the probative value, as trace evidence is not typically sought when an individual or item is known to be in contact with a person, location, etc.

Oblique lighting or an alternate light source may help reveal trace evidence. The collection method utilized may depend upon the evidence type, the substrate, and the need to determine exact location of the evidence, among other variables. Document the type of evidence, amount, and location it was collected from, if possible. Due to its typically small size, it is important to use a packaging that will not result in loss of the evidence. Evidence may be placed on the adhesive of a sticky note, in a bindle, or another appropriate container. As trace is often not able to be marked on directly, the proximal container shall be labeled.

Hair
A sufficient root is necessary to perform nuclear DNA testing on hair. Hair may be examined microscopically to determine if root material is present and, if possible, to indicate the growth phase. Roots resulting from an active or transitional growth phase are more likely to be successful with nuclear DNA testing. Depending on training and experience, an analyst may note relevant microscopic observations. The presence or absence of observed root material may be reported. Suitability of the hair for DNA analysis, based on the growth phase, shall not be reported.

Firearms Evidence
If apparent firearms evidence (i.e., gunpowder) is observed, document the location in the case notes. Avoid disturbing the area during processing to preserve the evidence for possible future analysis.

Latent Print/Impression Evidence
If apparent fingerprint or impression evidence is observed or there is a processing request and the anticipated biology processing may alter or destroy this evidence, the analyst should consult with a qualified discipline analyst prior to proceeding. The discipline analyst may advise on areas to avoid in order to protect potential evidence. Document relevant conversations, location(s) of apparent evidence, areas avoided, etc.

7.4.2 Alternate Light Source (ALS)
A specialized light that combines powerful illumination with the ability to select discrete wavelengths of light. This tool may help view or enhance physical evidence/stains when they are not readily observed with normal room or bright lighting. Some body fluids naturally fluoresce under certain wavelengths (e.g., semen, saliva, vaginal secretions, sweat, and urine) and others may absorb or reflect (e.g., blood). An ALS can be especially valuable when searching for potential evidence on large items such as bedding, clothing, furniture, and floors. In addition to body fluids the ALS may also aid in visualization of fibers, bone/tooth fragments, fingernails, glass, gunshot residue, etc.

Materials
Alternate Light Source
One color goggles/filters

Method
1. Examination should be conducted in a darkened room or space.

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

3. Select an appropriate wavelength and goggle/filter (red, orange, yellow, clear/UV protection) combination. This may depend on the ALS used, its available outputs, and the substrate/background being viewed. Visualization of biological fluids is typically optimal near 450 nm; however, it is recommended to test other wavelengths to determine the optimal contrast.

4. Lay out the item to be examined and systematically scan it for areas of fluorescence, absorption, or other contrast, as appropriate.

5. Circle or otherwise mark areas of fluorescence/absorption/contrast so they may be located under normal light conditions.

6. Record observations including the number of stains, appearance, general location, etc.

7. Proceed to additional screening procedures, as appropriate.

Result/Interpretation
Observation of fluorescence, absorption, or other contrast may indicate the presence of a biological fluid; however, it only indicates the location and does not specify the type of stain. Therefore, it is necessary to perform testing for the suspected biological fluid in order to determine its presence. Likewise, the absence of fluorescence/absorption does not confirm the absence of a biological fluid.

Comments
- Permanent eye damage can occur from direct illumination or reflected or refracted light. Proper eye protection shall be utilized by all individuals in the proximity of usage.
- Avoid direct or prolonged skin exposure as burns/damage may occur, especially direct from the unit (i.e., without a light guide).
- Intensity of the fluorescence/absorption/contrast may be affected by the substrate, stain concentration, and the light source.
- Several other materials may fluoresce, such as food, drink, cosmetics, detergent, and/or the substrate itself.
- Hair does not typically fluoresce, but may due to some treatment methods (e.g., dye or bleach)
- Some fabrics may absorb light (e.g., dark) and quench fluorescence. Tactile and/or presumptive tests may be conducted in the absence of visual cues.

7.4.3 Chemiluminescence Tests (BLUESTAR® FORENSIC and Luminol)

Both BLUESTAR® FORENSIC and luminol are used to locate non-visible blood or enhance bloodstain patterns and impressions. They are especially useful for dark-colored porous items or non-porous substrates when blood is suspected but little or no staining is visible (e.g., small traces in a large area, cleaned/diluted, or hidden/covered). BLUESTAR® FORENSIC is generally favored compared to luminol as it is convenient to prepare, is reported to be more sensitive, is longer lasting, can be applied multiple times, and can be visualized without complete darkness. All other analysis and search options should be exhausted prior to utilizing these tests and additional testing (i.e., alternate presumptive and/or confirmatory tests) is encouraged, as appropriate.

Luminol and BLUESTAR® FORENSIC testing shall be performed in accordance with the ACSO Forensic Lab Crime Scene Analytical Method manual.

7.4.4 Phenolphthalein Test

This oxidative test for the presumptive identification of blood is also known as the Kastle-Meyer test. The chemical indicator phenolphthalein is used to detect the possible presence of hemoglobin. The phenolphthalein test is extremely sensitive and can be used to detect visible or occult blood (e.g., diluted or cleaned). This test is generally more specific, but less sensitive than the o-tolidine test.

Materials

Phenolphthalein working solution (or commercial reagent)
Ethanol
3% Hydrogen peroxide
Water
Swabs, filter paper, or similar transfer material

Method

1. Positive (known bloodstain) and negative (swab or filter paper, may be moistened) controls are processed using the procedure below, prior to testing evidence samples (same day) to ensure the reagents function properly.

2. Sample the targeted stain with a swab or filter paper, which may be lightly moistened. Direct testing of a small cutting may also be performed, if necessary.

3. If a commercial reagent is utilized that does not contain ethanol, apply 1 to 2 drops of ethanol.

4. Place 1 to 2 drops of phenolphthalein reagent on the sample. Allow a few seconds for the reagent to soak in and for detection of a potential false positive.

5. Add 1 to 2 drops of 3% hydrogen peroxide to the sample and observe for the presence or absence of a pink color development.

6. Document results (controls and questioned samples) in the case notes.

Result/Interpretation

- Positive: Rapid development of a pink color within 10 seconds after the addition of the hydrogen peroxide indicates the possible presence of blood.
- Negative: No color change within 10 seconds indicates blood is not present or is too limited in quantity to be detected.
- Inconclusive: A positive reaction that occurs prior to the addition of the hydrogen peroxide, development of a different color, or other circumstances that causes the forensic scientist to be cautious about a positive or negative interpretation. Document the observation(s) that led to an inconclusive determination.

Comments

- In the absence of blood, the two reagents will react with each other and eventually develop a pink color over time due to the nature of oxidation reactions, thus it is important to adhere to the allotted reaction times.
- The color of the evidence (i.e., red/pink stains or substrates) may leach color and make results difficult to interpret.
- Negative results after latent print processing should be interpreted with caution, as certain processing techniques may interfere with presumptive blood testing. A disclaimer regarding the possible interference of latent print processing techniques may be reported.
- The working solution and hydrogen peroxide should be stored refrigerated with limited light and air exposure to optimize the efficacy of the chemicals.
- Ethanol increases sensitivity by cleaning around the hemoglobin to better expose heme.

7.4.5 o-Tolidine Test

The o-tolidine test is an extremely sensitive oxidative test for the presumptive identification of blood. It can be used to detect visible or occult blood (e.g., diluted or cleaned). The chemical indicator o-tolidine is used to detect the possible presence of hemoglobin. This test is generally more sensitive, but less specific than the phenolphthalein test.

Materials

o-Tolidine working solution
3% Hydrogen peroxide
Water
Swabs, filter paper, or similar transfer material

Method

1. Positive (known bloodstain) and negative (swab or filter paper, may be moistened) controls are processed using the procedure below, prior to testing evidence samples (same day) to ensure the reagents function properly.

2. Sample the targeted stain with a swab or filter paper, which may be lightly moistened. Direct testing of a small cutting may also be performed, if necessary.

3. Place 1 to 2 drops of o-tolidine working solution on the sample. Allow a few seconds for the reagent to soak in and for detection of a potential false positive.

4. Add 1 to 2 drops of 3% hydrogen peroxide to the sample and observe for the presence or absence of a blue/green color development.

5. Document results (controls and questioned samples) in the case notes.

Result/Interpretation

- Positive: Rapid development of a blue/green color within 10 seconds after the addition of hydrogen peroxide indicates the possible presence of blood.
- Negative: No color change within 10 seconds indicates blood is not present or is too limited in quantity to be detected.
- Inconclusive: A positive reaction that occurs prior to the addition of hydrogen peroxide, development of a different color, or other circumstance that lead the forensic scientist to be cautious about a positive or negative interpretation. Document the observation(s) that led to an inconclusive determination.

Comments

- In the absence of blood, the two reagents will react with each other and eventually develop a blue-green color over time due to the nature of oxidation reactions, thus it is important to adhere to the allotted reaction times.
- 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. A disclaimer regarding the possible interference of latent print processing techniques may be reported.
- The working solution and hydrogen peroxide should be stored refrigerated with limited light and air exposure to optimize the efficacy of the chemicals.

7.4.6 ABACard® HemaTrace® Test

The source of blood from items submitted for casework is often unknown and it may be useful to determine if it is of human origin. The immunochromatographic test uses mobile monoclonal antihuman hemoglobin antibodies to provide a means of detecting human hemoglobin to confirm the presence of blood. Blood from higher primates and ferrets has produced positive results; however, the likelihood of either being involved in a case is minimal. Case details may assist in this determination.

Materials

ABAcard® HemaTrace® test pouch (cards and dropper)
Extraction buffer (part of test kit)
Cutting utensil(s) (e.g., razor blade, scalpel, or scissors)

Method

1. Document the lot number and expiration date of the test kit used in the case notes.

2. Allow sample(s) to reach room temperature prior to running.

1. Testing of controls, positive (known human blood) and negative (buffer alone), is optional as each new lot is tested prior to release for casework. Controls may be run concurrent with the evidence sample(s), unless the lot is expired. Expired lots may only be used if acceptable results are obtained for controls prior to testing the evidence.

3. Add a portion of the stain/sample, generally ~2 to 3mm^2 cutting or ~$^1/_{16}$ to $^1/_4$ swab, into a minimum of 200 µl of extraction buffer for at least 1 to 5 minutes and up to 2 hours.

4. Open a test pouch and label the ABAcard® HemaTrace® card for each sample, including controls.

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

6. Allow the test to run for 10 minutes and observe for the development of a pink line in the "T" and/or "C" areas. Positive results may be determined prior to 10 minutes. Document results in the case notes.

Result/Interpretation

- Positive: The formation of two pink lines, one in the test "T" and one in the control "C" region, within 10 minutes indicates the presence of human hemoglobin. Faint lines are generally considered a positive result.
- Negative: Formation of one pink line in the control region "C" and no formation of a pink line in the test "T" region after 10 minutes, indicates no human hemoglobin was detected or a high level may have resulted in "High Dose Hook Effect" (see Comments). Confirmation of either may be achieved by concentration or dilution of the sample then repeating the test with a new card.
- Inconclusive: No formation of a pink line in the control "C" region or other circumstances that lead the forensic scientist to be cautious about a positive or negative interpretation. If no "C" line forms the test is invalid and should be repeated with a new card. This may occur if the sample is too viscous and does not migrate along the membrane; diluting the sample may correct the problem. Document the observation(s) that led to an inconclusive determination.

Comments

- If the control(s) fail, the same or a new sample may be run with a new test card. The Technical Lead shall be consulted if the failure recurs.
- Cutting size, extraction time, and/or extraction buffer volume may be adjusted depending on the amount and apparent quality of the stain.
- Degraded proteins may lose their ability to bind to the antibodies.
- A false negative (i.e., "High Dose Hook Effect") may occur with concentrated samples. Excess hemoglobin binds to the stationary anti-human hemoglobin antibody, which prevents the Ab-Ag complex from binding. This does not allow the dye to accumulate and causes a false negative result. If this is suspected, the sample may be retested using a dilution.
- Cross-reactivity is reported for other higher primates and ferret. Therefore, the report may declare human blood is indicated, rather than detected. If it is determined that the presence of a higher primate or ferret blood is possible, a disclaimer regarding the cross-reactivity may be reported.
- Due to sensitivity of the test, trace levels of hemoglobin may be detected in body fluid samples other than blood. Due to this, the HemaTrace® test should not be used if no staining consistent with blood is observed and/or a negative presumptive test result is obtained.

7.4.7 Acid Phosphatase (AP) Test

The AP enzyme is found in elevated levels in semen independent of the presence of spermatozoa. Acid phosphatase is primarily secreted by the prostate gland; however, the enzyme can be present in other substances, including other male and female biological materials (e.g., urine, vaginal secretions, and fecal material). The concentration is often lower in these other sources, which may be indicated by a weak and/or slower reaction.

Though not specific to semen, AP is a good indicator of its presence. The test can aid in locating and presumptively indicating the presence of semen stains on items submitted in allegations of sexual assault. When the enzyme is present, a phosphate is cleaved from sodium α-naphthyl phosphate to release α-naphthol. α-Naphthol coupled with o-Dianisidine (Fast Blue B) yields a purple compound.

Materials

AP working solutions A and B
Transfer pipettes
Water
Swabs, filter paper, or similar transfer material

Method

1. Prepare AP working solutions A and B fresh daily.

2. Positive (known semen) and negative controls (lightly moistened swab or filter paper) are processed each day of use with the procedure below to ensure the reagents function properly. This shall occur prior to testing evidence samples.

3. Sample the targeted stain with a lightly moistened swab or filter paper. Direct testing of a small cutting may also be performed, if necessary.

4. Place 1 to 2 drops of working solution A on the swab/paper and allow a few seconds for the reagent to soak into the substrate.

5. Add 1 to 2 drops of working solution B and observe for the presence or absence of a pink-purple color development.

6. Interpret and document the results in the case notes. It may be appropriate to note variations in the reaction color, strength, etc., as compared to the controls.

<u>Mapping</u> (for larger areas or to detect strongest reacting location)
In addition to or in place of the above testing for individual stains. The steps below are intended to follow Step 2 and precede Step 6 in the method above.

1. Place mapping/filter paper over the questioned stain/area and mark the paper(s) to indicate orientation relative to the item.

2. Moisten filter paper with water, place over the area of interest, and firmly press for several seconds.

3. Add working reagents A and B, stepwise as done above (Steps 4 and 5). Reagents may be applied with a dropper or sprayed. Observe for the presence or absence of a pink-purple color change.

Result/Interpretation

- Positive: Development of a pink/purple color within 60 seconds after the addition of working solution B indicates the possible presence of semen.
- Negative: No color change within 2 minutes indicates semen is not present or is too limited in quantity to be detected.
- Inconclusive: A pink/purple color occurring between 1 to 2 minutes or prior to the addition of working solution B, development of a different color, or other circumstance that lead the forensic scientist to be cautious about a positive or negative interpretation. Document the observation(s) that led to an inconclusive determination.

Comments

- 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.
- Do not place mapping paper back onto the original item after application of the working reagents.
- AP occurs in other body fluids, bacteria, fungi, and plants. It is not uncommon to obtain false positive results from vaginal secretions and fecal stains.
- AP degrades faster than sperm cells. At the discretion of the analyst, additional testing may be attempted for negative results. Document justification in the notes.

7.4.8 ABACard® p30 Semen Test

An immunochromatographic test for the qualitative detection of p30 protein, which is present in semen independent of spermatozoa. This test can be especially helpful when a semen from a vasectomized or azoospermic male is present. The protein, also known as Prostate-Specific Antigen (PSA), is not restricted to semen and may be found in other male or female body fluids and tissues (e.g., breast tissue, periurethral glands, breast milk, amniotic fluid, female urine, and endometrium), although at much lower concentrations. The Laboratory considers this to be a confirmatory test for semen in instances where a positive AP result was obtained.

Materials

Abacus Diagnostics OneStep ABAcard® p30 Test pouch (card and dropper)
Extraction buffer (in test kit)
Plastic microcentrifuge tube
Transfer pipette
Cutting utensil(s) (e.g., razor blade, scalpel, or scissors)

Method

2. Allow the sample/extract to warm to room temperature if it has been refrigerated or frozen.

3. Document the lot number and expiration date of the test kit used in the case notes.

4. Testing of controls, positive (known human semen) and negative (buffer alone), is optional as each new lot is tested prior to release for casework. Controls may be run concurrent with the evidence sample(s), unless the lot is expired. Expired lots may only be used if acceptable results are obtained for controls prior to testing the evidence.

5. Open a test pouch and label the ABAcard® p30 device card for each sample, including controls.

6. Add a portion of the stain/sample, generally ~2 to 3mm^2 cutting or ~$^1/_{16}$ to $^1/_4$ swab, into a tube. Add 200 µl of ABAcard® p30 extraction buffer for at least 1 to 5 minutes and up to 2 hours.

7. Mix thoroughly and transfer approximately 200 µl (4 drops) of the extract in the sample "S" well of the ABAcard® p30 test card.

8. Allow the test to run for 10 minutes and observe for the development of a pink line in the "T" and/or "C" areas. Positive results may be determined prior to the 10 minutes. Document results in the case notes.

Result/Interpretation

- Positive: The formation of two pink lines, one in the test region "T" and one in the control region "C", within 10 minutes indicates the presence of semen. Faint lines are generally considered a positive result.
- Negative: Formation of one pink line in the control region "C" and no formation of a pink line in the test "T" region, at 10 minutes indicates no semen was detected or a high level may have resulted in "High Dose Hook Effect" (see Comments). Confirmation of either may be achieved by concentration or dilution of the sample then repeating the test with a new card.
- Inconclusive: No formation of a pink line in the control "C" region or other circumstances that lead the forensic scientist to be cautious about a positive or negative interpretation. If no "C" line forms the test is invalid and should be repeated with a new card. This may occur if the sample is too viscous and does not migrate along the membrane; diluting the sample may correct the problem. Document the observation(s) that led to an inconclusive determination.

Comments

- If a control fails, the same or a new sample may be run with a new test card, as appropriate. Consult the Technical Lead if the failure recurs.
- Cutting size, extraction time, and/or extraction buffer volume may be adjusted depending on the amount and apparent quality of the stain.
- A false negative (i.e., "High Dose Hook Effect") may occur with concentrated samples. Excess p30 binds to the stationary anti-human p30 antibody which prevents the Ab-Ag complex from binding. This does not allow the dye to accumulate and causes a false negative result. If this is suspected, the sample may be retested using a dilution.
- Degraded proteins may lose their ability to bind to the antibodies.
- p30 has been reported in other body fluids. Therefore, the analyst may choose to report that semen is indicated, rather than detected.
  - Use caution interpreting results when the positive reaction develops closer to 10 minutes and there are circumstances that make a false positive more probable (e.g., weak/inconclusive AP results, reported medical condition, or other fluid(s) likely present).

- Some health conditions may cause elevated levels, but this is a relatively rare occurrence. If it is determined that another body fluid likely to contain p30 may be present, a disclaimer regarding the cross-reactivity may be reported or, with explanation, the analyst may choose to report the result as "inconclusive".

7.5 Technical records

Documentation is extremely important to provide a detailed record of events, aid in report writing, assist with testimony, and allow for independent review. Detailed notes help support conclusions, opinions, and interpretations and may also assist the analyst with information recall, especially after a time has passed. Documentation shall be enough to support reported conclusions and to allow another competent analyst to determine what was done and interpret the results.

Technical records include records of observations (e.g., notes, images, and sketches) that document the basis for findings resulting from analysis and test results. The date the case is started, and analysis date(s) are recorded in the notes. Examination records (e.g., data and observations) shall be recorded as they are made. Original documentation shall be retained unless an accurate electronic copy is captured.

Rejected observations, data, or results shall be explained and documented with the date and analyst initials. This includes justification for repeating tests and explanation for the final interpretation, as appropriate.

Item descriptions may be simplified for the report. When possible, descriptions should be discernable from other case items. The description in the case notes should include the following, as appropriate and necessary:

- Type of item and indication of quantity (e.g., two ankle socks).
- General description (i.e., color, size, brand, design, measurement, serial number).
- General condition (e.g., clean, damaged, worn, and missing portions).
- Location of/reported collection site.
- 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/packaged.
- Potential evidence located on the item.
  - The approximate location, number, and/or appearance of stains observed.
  - The number and/or approximate area(s) sampled for testing and/or collection.
  - The approximate location of any other apparent evidence observed and/or collected.
- Photography and sketches may aid evidence descriptions.

<u>Packaging</u>

Evidence packaging shall be documented for all items and include the condition of the package and/or seal, as appropriate. Document any differences in the evidence description between the request, ILIMS, external packaging, and/or what is contained within.

<u>Digital Images</u>

Photographic documentation of physical items or test results is not required. Images generated during the examination are for documentation purposes and no particular file format is required. These images are not considered evidence, but rather a supplement to the bench notes. Thus, it is

not necessary to track images, include images in the notes, nor is it required to retain all images captured.

Inclusion of a scale/ruler is encouraged for at least one image per item, but this may not be practical in some situations (e.g., close-ups or alternate lighting) and is not necessary for images of packaging or test results. Notations may be added to an image to specify stain areas, test results, etc. Enhancements need not be tracked. Images selected for inclusion in the case notes are attached via the LIMS. The case notes should indicate the item number and date, at a minimum, for at least one image/overall view of each item represented. Indicate in the case notes if images are uploaded to the records management system.

If capture of examination quality images is necessary, it would most likely pertain to documentation of non-biology screening evidence. The analyst should consult an appropriate ACSO discipline analyst and/or analytical method regarding guidance, to include any image resolution and file format requirements.

Sketches/diagrams
Drawn images shall minimally include a title or indication of what is depicted. All drawings are presumed to not be to scale; however, a scale or not to scale disclaimer may be added for clarity. Following approval of the case report/request, the version attached in the LIMS shall be considered the "original" and the hard copy may be destroyed.

Abbreviations
Abbreviations specific to ACSO are listed in the electronic quality records**.** The meaning for non-listed abbreviations shall be included in the case notes. Commonly accepted abbreviations, acronyms, and/or symbols do not need to be listed (e.g., element symbols).

7.6 Evaluation of measurement uncertainty
Does not apply for biology screening.

7.7 Ensuring the validity of results
Procedures, controls, and standards for monitoring tests are included in each method. Results are recorded in the case notes and/or quality records (e.g., reagent log), as appropriate.

See the ACSO Quality Manual for review criteria. Additionally, a reporting and review guide may be utilized to ensure appropriate notes and report content.

7.8 Reporting of results
Reports shall contain the elements delineated in the simplified reporting agreement. They should be as clear as possible to facilitate understanding.

The report shall include all items received. The degree of detail included in evidence descriptions is largely left to the analyst's discretion; however, it should be enough to distinguish between items listed in the same report and/or case, if possible. Multiple objects (e.g., a pair of shoes, oral swabs from the same source, or waste container contents) may be considered as a single item, as appropriate. Additional information regarding the stains/areas tested or sampled may also be included (e.g., location, size, or quantity).

Clearly convey and accurately represent results/conclusions, which are dictated by analysis findings. For tests that are presumptive in nature, ensure that a positive result provides an indication, but not confirmation, of the presence of the targeted body fluid. Absence of staining and/or negative test results don't necessarily indicate that the targeted fluid is not present, but rather that it was not detected.

The report shall make it clear that the results relate only to the samples or items tested. When a positive result is obtained, it is not typically necessary to also report inconclusive and/or negative results obtained from the same test on the same item. Likewise, it is not necessary to report negative results when an inconclusive result is reported. If multiple results/interpretations are reported for a single item, they should be in the following order: positive, inconclusive, negative, and not examined.

Reported "inconclusive" findings must state a reason in the report (e.g., invalid color test result or questionable sperm morphology). Supporting details/explanation shall be included in the notes.

The report wording guidelines may aid the analyst in generating the report. The tables below provide examples of case circumstances and corresponding statements. The statements are not all-inclusive; portions of statements may be used, modified, or combined, and there may be circumstances in which none are optimal.

General Examination

Circumstance	Statement
Item received, but not examined.	Not examined.
Item examined, but not tested or testing stopped due to <insufficient sample, poor quality, request to discontinue, etc.>.	Not tested at this time. No further analysis due to <limited sample, quality concerns, agency request, etc.>.
Reference another case/report or items previously examined.	See <Title/Discipline> Report <Case #> dated <MM/DD/YYYY>. This item was <re-analyzed, re-examined, previously examined>, see <Title/Discipline> Report <Case #> dated <MM/DD/YYYY>.

Blood Examination/Testing

Circumstance	Statement
• Positive presumptive test (phenolphthalein, o-tolidine, BLUESTAR® FORENSIC, and/or luminol) -and- • Positive ABAcard® HemaTrace® test	Test results detected/indicated the presence of human blood. Note: May add disclaimer regarding possible cross-reactivity.
• Positive presumptive test (phenolphthalein, o-tolidine, BLUESTAR® FORENSIC, and/or luminol) -and- • Negative ABAcard® HemaTrace® test	No human blood was detected.

• Positive presumptive test (phenolphthalein, o-tolidine, BLUESTAR® FORENSIC, and/or luminol) -and- • No [or inconclusive] confirmatory test	The result of a presumptive test indicated the possible presence of blood; however, a test to confirm blood and indicate human origin was not performed (or was inconclusive due to <reason>.).
• Positive presumptive chemiluminescent test (BLUESTAR® FORENSIC or luminol) -and- • Negative presumptive color test (phenolphthalein or o-tolidine)	The result of an initial presumptive test indicated the possible presence of blood; however, the result of a more specific presumptive test for blood was negative.
Inconclusive presumptive test (phenolphthalein, o-tolidine, BLUESTAR® FORENSIC, and/or luminol)	The result of a presumptive test for blood was inconclusive due to <reason> (e.g., color development prior to the addition of all test reagents).
Negative presumptive test (phenolphthalein, o-tolidine, BLUESTAR® FORENSIC, and/or luminol)	The result of a presumptive test for blood was negative. Note: May qualify results that follow latent print processing (e.g., However, certain latent print processing techniques may interfere with presumptive blood testing).
No blood stains observed (alternate light source, high intensity light source, visual and/or stereomicroscopic examination).	No stains consistent with blood were observed.

Semen Examination/Testing

Circumstance	Statement
• Positive AP test -and- • Positive ABAcard® p30 test	Test results detected/indicated the presence of semen.
• Positive AP test -and- • Negative ABAcard® p30 test	No semen was detected.
• Positive AP test -and- • No [or inconclusive] confirmatory test	The result of a presumptive test indicated the possible presence of semen; however, a confirmatory test was not performed [or was inconclusive due to <reason> (e.g., a failed control)].
Inconclusive AP test	The result of a presumptive test for semen was inconclusive due to <reason> (e.g., the timing of the color development).
Negative AP test	The result of a presumptive test for semen was negative.
No stains consistent with semen observed (alternate light source, high intensity light source, tactile, and/or visual examination).	No stains consistent with semen were observed.

Other Evidence Examination

Circumstance	Statement
Negative visual (e.g., unaided eye, room light, and bright light) or ALS examination	No apparent <stains/fibers/hair/trace, etc.> was/were observed.
Microscopic examination of hair for the presence of root material.	<Item> was examined microscopically and found to contain a hair with a root, which may be sufficient for nuclear DNA analysis. -or- <Item> was examined microscopically and found to contain an apparent hair with no root, which is insufficient for nuclear DNA analysis.

Collection/Disposition/Recommendation

Circumstance	Statement
Evidence/portion collected for possible additional testing.	<Type of evidence/a portion of/a cutting> from <Item #> was collected for <preservation, possible DNA testing, etc.>
Disposition	<A portion of/Item #> was: • returned to the agency from which it was received. • returned to ACSO Property and Evidence pending <additional/discipline specific> analysis. • forwarded for <additional/discipline specific> testing. • consumed during analysis.
Potential item(s) for DNA testing.	<Item #/This item> may be forwarded for DNA testing.
Recommend collection of reference sample(s). Note: In most cases, this statement should only be needed in one location.	Attempt to obtain any necessary reference samples. -or- A known reference sample from <name(s)> will be needed for DNA testing.

7.9 Complaints See ACSO Forensic Lab Quality Assurance Manual

7.10 Nonconforming work See ACSO Forensic Lab Quality Assurance Manual

7.11 Control of data and information management See ACSO Forensic Lab Quality Assurance Manual

8 Management system requirements See ACSO Forensic Lab Quality Assurance Manual

Document History

SECTION & COMMENTS	DATE ADOPTED	AUTHOR	REVIEWER(S)
Original version	10/04/2017	Campbell	
Re-write of manual formatted to ISO/IEC 17025:2017 standards and ANAB 3125 accreditation requirements and numbering format; added analysis schemes; modified options for transfer of stains for testing; removed reagent specifications and hazard labels; removed bulk of trace and chemiluminescence test methods and referenced CSI manual; added optional step to phenolphthalein method; modified report statements; and additional modifications throughout.	01/12/2021	Guess	Campbell Herink Kidwell Wheatley