

**WASHINGTON STATE PATROL**

**BIOCHEMICAL ANALYSIS PROCEDURES**

**CRIME LABORATORY DIVISION**

**January 2025**

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**1.0 INTRODUCTION**

This manual contains the technical procedures used for evidence examination and biological fluid testing used by forensic scientists working in the DNA functional area. Additional quality assurance policies and procedures are found in the Washington State Patrol Crime Laboratory Division *Quality Operations Manual* (QOM) and *DNA Quality Assurance (QA) Manual*.

## 2.0 GENERAL EXAM PROCEDURES

### 2.1 GENERAL PRINCIPLES

Examinations of clothing (and similar exhibits) shall be conducted with the following standards:

- The examination shall be relevant.
- The examination shall be conducted in a manner consistent with current safety requirements.
- The examination shall be conducted in a manner which will preserve the integrity of the evidentiary value of the exhibit.
- The examination shall meet peer standards of thoroughness and documentation.

### 2.2 GENERAL KNOWLEDGE REQUIRED

In order to meet the goals listed above, the examiner shall be aware of:

- The potential significance of the presence or absence of various biological stains and of any or all of the following: directionality/sequence-of-deposit of staining; trace evidence (hairs, fibers, paint, glass, soil, etc.); cuts, tears, punctures; heat damage. Any such significance may vary from case to case; therefore, a thorough understanding of the alleged facts of the case will contribute to assuring the examination is relevant (sources of such alleged facts include police and physician reports, investigating officers, prosecutors, newspaper accounts, etc.). Consulting technical publications and/or other examiners may be necessary in order to evaluate the potential significance of some attributes of an exhibit.
- Current laboratory requirements and recommendations regarding the handling and disposal of biological samples.
- Current peer standards regarding case management and documentation of examinations. Common ways in which evidence may be contaminated or lost during the examination of an exhibit (and ways to prevent or minimize such contamination or loss).

### 2.3 GENERAL INSTRUCTIONS REGARDING RELEVANCE OF EXAMINATIONS

Relevance of examinations can be assured only if the management and documentation of a case is responsive to the premise that each case is or may be of a non-routine nature.

A fundamental aspect of case management is to identify and document the unique characteristics of the case and of any evidence that is associated with the case. The examiner shall evaluate which examinations, if any, are appropriate for each exhibit submitted to the laboratory/examiner. Equally important, the examiner shall evaluate whether additional exhibits should be submitted in order to ensure that, to the extent practically possible, all pertinent forensic evidence is developed.

The Request for Laboratory Examination shall be carefully reviewed. Other available relevant documents, such as officer or medical reports or related earlier crime laboratory reports shall also be reviewed.

As needed and appropriate, the case facts and/or exhibits shall be discussed with knowledgeable individuals such as officers, prosecutor, and/or other examiners.

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## 2.4 GENERAL INSTRUCTIONS REGARDING SAFETY OF EXAMINATIONS

Biochemical and related examinations shall be carried out only by individuals having the equipment, supplies, and training needed to ensure the safety of the examiner and his/her co-workers. All evidence shall be considered potentially hazardous and shall be handled accordingly.

Before any examination is begun, an examination area of an appropriate size and location shall be available for use, safety equipment and supplies shall be readily accessible, and the examiner and the examination area shall be appropriately protected from contamination or other harm.

Required disposal containers, spill cleanup supplies, and personal protective equipment (PPE) shall be available and utilized when appropriate.

## 2.5 GENERAL INSTRUCTIONS REGARDING PRESERVING THE INTEGRITY OF EVIDENCE

Where there are alternative methods for storing, examining, and/or testing a particular exhibit, those methods which best ensure the integrity of the evidentiary value of the exhibit shall be utilized. Any exception shall be justified. Any actual or potential contamination, deterioration, or loss shall be documented.

The examination area shall be large enough to minimize the possibility of contamination of the evidence by other workers or other evidence.

Prior to the conducting an examination, the workbench shall be cleaned and covered with clean paper or other disposable material which is larger overall than the item. If the item is larger than the available examination surface, the item shall be examined in smaller sections. The workbench must be cleaned, and new paper/other material used between each individually wrapped exhibit. Exhibits packaged together in a single (innermost) wrapping may be examined on the same sheet of paper.

Evidence shall be handled as minimally as possible without compromising the thoroughness of the examination.

Collected samples shall be promptly documented, packaged, and labeled.

## 2.6 GENERAL INSTRUCTIONS REGARDING THOROUGHNESS OF EXAMINATIONS

The primary purpose of the examination is to locate, document, and collect (as appropriate) biochemical stains of possible significance. The secondary purpose of the examination is to locate, document, and collect (when appropriate) any additional materials/characteristics having potentially significant evidentiary value. The examiner shall document and be prepared to justify any significant deviation from current peer standards. It is acknowledged that two examiners are unlikely to approach a case in identical manners and that current peer standards, in order to be practical, must be expressed and interpreted in an appropriately flexible manner.

## 2.7 GENERAL INSTRUCTIONS REGARDING DOCUMENTATION OF EXAMINATIONS

The recording of case notes shall be carried out in a manner which will contribute to an efficient and proper resumption of examinations after an interruption of any time length, facilitate case review, and permit the examiner to appropriately prepare for a conference or testimony. Evidence and evidence packaging shall be marked according to the regulations in the current versions of the QOM and DNA QA manual.

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Case notes shall include descriptions of evidence items. When appropriate, diagrams, sketches and/or digital images may also be utilized.

Case notes shall be recorded according to the requirements of the QOM and DNA QA manual and the following guidelines:

- Each page of notes shall include the case number, analyst's initials, page number, and the date(s) of analysis.
- Evidence packaging shall be described.
- Evidence items shall be described as to type and identifying characteristics.
- Details of examination procedures used and resulting observations, including the location of stains and location and size of samples collected shall be documented.
- All tests and their results conducted shall be recorded. Lot numbers for reagents utilized in testing shall be recorded.

## 2.8 EXAMINATION PROTOCOL

As the examination for biological stains is carried out, other potential evidence such as hair which is observed shall be documented and/or collected. After properly packaging any such collected evidence, it may be placed inside the package holding the item from which it was collected, or it may be packaged separately, given a unique identifier, and entered into evidence. Consult with the appropriate functional area section for procedural advice concerning other evidence observed. It may be appropriate for the DNA Forensic Scientist to evaluate hairs to determine suitability for DNA analysis (see EVALUATION OF APPARENT HAIR).

An appropriately thorough examination may require inspecting the front, back, outside, and inside of the garment (pockets, cuffs, etc.) using the unaided eye, oblique lighting, backlighting, an ultraviolet light, an alternate light source, a handheld lens, and/or a stereomicroscope. The testing and sampling of an item shall be based upon the training and experience of the analyst, taking into account the nature and quantity of available evidence, the request for analysis and the known facts of the case. However, the analyst's decisions must pass the scrutiny of the peer review process.

Not all contents of a sexual assault kit may require testing (e.g. oral swabs, body swabs, underwear). The analyst will determine which contents require initial screening based on information provided at the time of submission.

After the examination is completed, items (whenever possible) shall be returned to the original packaging. The packaging shall then be sealed according to the QOM.

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### 3.0 ALTERNATE LIGHT SOURCE

#### 3.1 PURPOSE:

To screen an item of evidence for the presence of potential biological fluids when no stains are observed under normal lighting.

#### 3.2 INTRODUCTION:

An Alternate Light Source (ALS) is a specialized light that combines powerful illumination with specific wavelengths. Physical evidence, such as fibers and biological fluids, may fluoresce or absorb when exposed to this type of light.

#### 3.3 EXAMINATION PROCEDURE:

- An examination using an ALS shall be conducted in a darkened room.
- Follow the manufacturer's recommendations for proper operation of the ALS.
- Select an appropriate wavelength and filter/goggles for the type of search being conducted.
- Spread out the item to be examined on clean paper and systematically scan the item for areas of fluorescence/absorption as appropriate.
- Mark and/or note any areas for further testing.

#### 3.4 INTERPRETATIONS:

Fluorescence/absorption shows the location of a possible biological fluid for additional testing. Further analysis (acid phosphatase test, sperm search, amylase, or phenolphthalein test) must be performed before the presence of a biological fluid is indicated. The absence of a fluorescence/absorption result does not confirm the absence of a biological fluid.

## 4.0 BLOOD: PHENOLPHTHALEIN TEST

### 4.1 PURPOSE:

To test for the possible presence of blood.

### 4.2 INTRODUCTION:

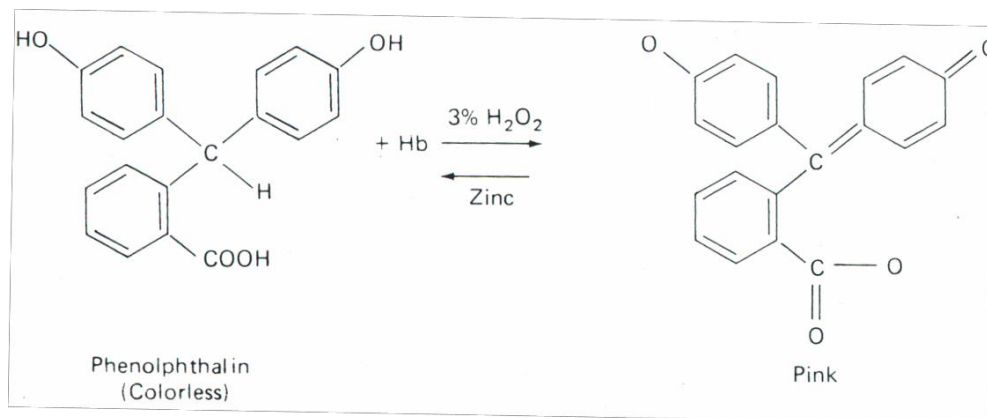
All catalytic blood tests depend upon an oxidation reaction in which an oxidant, hydrogen peroxide for example, oxidizes a colorless material to a colored one (e.g., phenolphthalin to phenolphthalein). Ionic iron forms cyclic chelate structures with many organic compounds, and often such iron-chelates possess catalytic activity in oxidation reactions. An example of such a biological catalyst is peroxidase, wherein a chelated metal decomposes hydrogen peroxide to form hydroxide ion and an oxy radical, which are ultimately converted to water and a metal-hydroxide ion complex:



Hemoglobin contains an iron-heme chelate complex which has demonstrated this particular behavior.

### 4.3 THE PHENOLPHTHALEIN TEST:

Phenolphthalin is an organic molecule which is oxidized by a compound with a reactive metal-oxygen bond commonly found in peroxidases. Prior to its use in this test, the phenolphthalin must be reduced to phenolphthalin. The reduced form is then used to test for the presence of the Fe(IV)=O species. In a positive test, formation of a pink color indicates the oxidation of phenolphthalin back to phenolphthalein (under basic conditions).



The phenolphthalein test has a maximum reported sensitivity for liquid blood of a 1:10,000,000 dilution and a maximum reported sensitivity on a dried blood stain of 1:10,000. While this test is highly sensitive to a minute trace of hemoglobin and its derivatives, it suffers from interference by other iron-heme containing materials, such as catalase, peroxidase, and cytochromes. Biological structures containing copper are also a noted strong interferent. In addition, strong oxidizing agents and metallic salts can also produce spurious reactions.

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#### 4.4 TESTING PROCEDURES:

Before use on casework samples, working reagents shall be tested with a positive blood control each day of use. A negative control shall also be tested. The results of these tests shall be documented in case notes.

Note: All efforts shall be made to avoid performing DNA extraction on a swab used for phenolphthalein testing. Phenolphthalein reagent has been shown to negatively impact the ability to extract DNA from samples. It is recommended organic extraction be used versus EZ1/2 if a phenolphthalein-tested swab must be extracted for DNA.

The test is performed by one of the following methods:

##### IF THE STAIN IS VISIBLE:

The stain may be rubbed with a clean cotton swab (or filter paper) moistened with sterile dH<sub>2</sub>O. Phenolphthalein reagent is then added to the cotton swab or the filter paper. After visual inspection, a similar amount of 3% hydrogen peroxide is applied to the test swab or paper.

A portion of the stain may be cut out or scraped off an object and placed into a spot well or suitable vessel. Phenolphthalein reagent is then added to the sample. After visual inspection, a similar amount of 3% hydrogen peroxide is added to the sample.

##### IF THE STAIN IS NOT VISIBLE:

A general swab or mapping of the area may be performed. Phenolphthalein reagent is then added to the cotton swab or the filter paper. After visual inspection, a similar amount of 3% hydrogen peroxide is applied to the test swab or paper.

#### 4.5 INTERPRETATIONS:

A color change to pink (within 5 seconds after adding 3% H<sub>2</sub>O<sub>2</sub>) indicates a positive reaction.

The phenolphthalein test is a test for the possible presence of blood. Careful consideration shall be given to positive reactions on/in certain substrates or substances (i.e. leather and feces). When a positive result is obtained, it is necessary to consider carefully whether the test result could have been given by something other than heme from blood. The specificity of various catalytic reagents has been studied extensively. A spurious reaction may be obtained from substances other than bloodstains. These substances may be conveniently divided into two groups:

##### CHEMICAL OXIDANTS AND CATALYSTS:

Copper and nickel salts most frequently show reactions.

Rust, formalin, potassium permanganate, potassium dichromate, some bleaches, hypochlorite, iodine, and lead oxides.

Oxidizing compounds such as copper, potassium ferricyanide, and nickel and cobalt nitrates, and some sulfocyanates.

The behavior of chemical oxidants is quite different from that of blood. Chemical oxidants will give a discoloration before the addition of the hydrogen peroxide. Therefore, a reaction for blood is distinguished by use of the two-solution test procedure outlined above.

The color of the stain tested must be considered and the evidence of usual observation added to the intelligent use of this test. A stain on cloth of these salts strong enough to give a positive reaction will (a) discolor the cloth (unless the cloth is of identical color) and (b) show small crystals in the cloth where the salt has dried. The stain color will not be that of blood. Invisible

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traces of blood can be detected. Invisible traces of these salts are not detected using the method described.

#### PLANT SOURCES:

Vegetable peroxidases are the most important class of substances which show false positive reaction with chemical color tests. The following plant tissues may react with the phenolphthalin reagent and be mistaken for blood: apple, apricot, bean, blackberry, Jerusalem artichoke, horseradish, potato, turnip, cabbage, onion, and dandelion root.

The color of the stain must be observed before any tests are done, and in most cases, this is not the color of blood staining. Green and white are the most common colors to find associated with plant materials. The plant peroxidases appear to reside in the particulate contents of the cells of the plant tissue, the juice of the plant giving a negative or only a faint positive reaction. A strong positive reaction could be associated with plant tissue or tissue fragments.

As an alternative, heating the sample extract to 100° C for five minutes will differentiate the plant peroxidases from blood sources, since heme is stable at this temperature while plant peroxidases are rapidly deactivated.

#### 4.6 CONCLUSIONS:

If HemaTrace was also performed on the sample, see section 5.5 for conclusions.

#### NEGATIVE REACTION:

Blood was not detected using the PHT test.

#### POSITIVE REACTION:

Blood was indicated using the PHT test.

#### INCONCLUSIVE REACTION:

The PHT test did not provide any information regarding whether blood is or is not present due to *[reason, e.g., insufficient sample/test malfunction]*.

#### 4.7 REFERENCES:

Culliford BJ. (Phenolphthalein). In: The Examination and Typing of Bloodstains in the Crime Laboratory. U.S. Department of Justice, Law Enforcement Assistance Administration; 1971; p. 2-93 to 2-101.

Gaensslen RE. (Phenolphthalein). In: Sourcebook in Forensic Serology, Immunology and Biochemistry. Washington, D.C.: United States Department of Justice; 1983; p. 101-16.

Higaki RS, Philp WM. A Study of the Sensitivity, Stability and Specificity of Phenolphthalein as an Indicator Test for Blood. Canadian Society of Forensic Science Journal 1976; 9 (3):97-102.

Lee HC. Identification and Grouping of Bloodstains In: Saferstein R, editor. Forensic Science Handbook.

Englewood Cliffs: Prentice Hall; 1982; p. 272-6.

Spalding RP, Cronin WF. Technical and Legal Aspects of Forensic Serology: A Laboratory Manual. Washington D.C.: U.S. Department of Justice, Federal Bureau of Investigation; 1984.

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## 5.0 BLOOD: HEMATRACE TEST

### 5.1 PURPOSE:

To test for the possible presence of human hemoglobin, a component of blood. This test shall only be conducted after a positive phenolphthalein test. There may be special exceptions where HemaTrace is used as the sole independent test however the rationale to explain the testing approach used needs to be well documented in the notes and the conclusion and or remarks would need to be modified as appropriate to reflect the limitations of the testing performed. Prior approval (date and initials) of a Forensic Scientist 4, Forensic Scientist 5, or the DNA Technical Leader shall be documented for these special exceptions. Due to possible reactivity with semen, this test should be used with caution on stains suspected of containing semen.

### 5.2 TESTING PROCEDURES:

1. Place a sample into a sterile microcentrifuge tube.

Note 1: For old stains, add approximately 15  $\mu$ l of 1% ammonia solution to the sample in the tube. Incubate at room temperature for about 15 minutes, vortexing occasionally. Proceed to step two and then skip step three. Follow the procedure from step four.

Note 2: If the 37°C incubation described for sperm search is used to prepare an extract, skip to step 5.

2. Add approximately 200  $\mu$ l of sterile dH<sub>2</sub>O (or PBS) to the sample in the tube and vortex.
3. Incubate at room temperature for approximately 15 minutes, vortexing occasionally.
4. Place cutting in a spin basket and spin at maximum speed for  $\approx$ 3 minutes.
5. Remove a portion of the supernatant. Do not disturb the pellet.
6. Dilute the removed supernatant until it appears light yellow-brown (straw) colored and it is a minimum of 150  $\mu$ l in volume.
7. The microcentrifuge tube with the pellet (and cutting) may be used for any extraction procedure.
8. Place 80 or 150  $\mu$ l of liquid (depending on manufacturer instructions) on the sample well, well "S" of the HemaTrace cassette.
9. The result shall be read at 10 minutes. A positive result may be seen earlier.

Note: The result shall not be read after 10 minutes since nonspecific reactions may result in a false positive.

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## 5.3 INTERPRETATIONS:

POSITIVE HEMATRACE RESULT:

The formation of a visible pink line in the test “T” and control “C” regions.

NEGATIVE HEMATRACE RESULT:

No formation of a visible pink line in the test “T” region, and a visible pink line in the control “C” region.

INCONCLUSIVE HEMATRACE RESULT:

An incomplete or questionable visible pink line in the “T” region, and a visible pink line in the control “C” region.

INCONCLUSIVE TEST (FAILED HEMATRACE CASSETTE):

No visible pink line in the control “C” region regardless of whether or not the test “T” region exhibits a visible pink line. The test sample should be rerun, if appropriate and possible.

Note: Interpretation of the result of the test shall be documented in the case file.

## 5.4 INTERPRETATION CAUTIONS:

Product literature reports that the lower limit of sensitivity for human hemoglobin is approximately 1 to 1,000,000 dilution of whole human blood.

A “High Dose Hook Effect” may be seen for extremely concentrated human hemoglobin samples (such as whole human blood) which appears as a negative result (no pink line in the test “T” region, pink line visible in the control “C” region). If such a sample is suspected, the supernatant should be retested using a 10-to-10,000-fold dilution.

It has been reported that ferret blood and upper primate blood can produce false positives with this product. A 1 to 10 dilution of blood from an orangutan tested negative during internal validation work.

An approximately 1 to 100 dilution of neat semen and a neat semen stain were both positive during internal validation work (a dilution of 1:1000 neat semen was negative). Therefore, this test should be used in conjunction with the phenolphthalein test to decrease the possibility of false positives with semen stains. The product literature suggests that the sensitivity of the product can detect trace levels of hemoglobin that might be present in other body fluid samples (e.g. urine, semen, feces, saliva, vaginal fluid, perspiration).

The reported sensitivity for the phenolphthalein test is approximately 10 times greater than the sensitivity of the HemaTrace cassettes. Therefore, a weak positive phenolphthalein reaction may occur (or may be obtained) with a negative test using the HemaTrace cassette with human blood.

An internal study indicated that materials treated with phenolphthalein reagents, when added to the HemaTrace assay, may cause a false negative result. Therefore, the HemaTrace test should not be run on samples which have been exposed to phenolphthalein reagents.

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## 5.5 CONCLUSIONS:

### PHT AND HEMATRACE POSITIVE:

Human blood was indicated using the PHT and HemaTrace tests.

### PHT POSITIVE AND HEMATRACE NEGATIVE:

Blood was indicated using the PHT test; however, human blood was not detected using the HemaTrace test.

### HEMATRACE POSITIVE:

Human blood was indicated using the HemaTrace test.

### HEMATRACE NEGATIVE:

Human blood was not detected using the HemaTrace test.

### INCONCLUSIVE HEMATRACE RESULT:

The HemaTrace test did not provide any information regarding whether blood is or is not present due to [reason, e.g., insufficient sample/test malfunction].

## 5.6 REFERENCES:

Abacus Hematrace Technical Information Sheet, ABACard HemaTrace For The Forensic Identification of Human Blood. Abacus Diagnostics Inc; 2022

Hochmeister MN. Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. Journal of Forensic Sciences 1999; 44 (6):597-602.

Johnston S. Validation study of the Abacus Diagnostics ABACard HemaTrace membrane test for forensic identification of human blood. Canadian Society of Forensic Science Journal 2003; 36 (3):173-83.

Rowley B. Commentary on Hochmeister, MN. "Validation studies of an immunochromatographic 1-step test for the forensic identification of blood." Journal of Forensic Sciences 1999; 44 (6):1323-4.

## 6.0 SEMEN: ACID PHOSPHATASE TEST

### 6.1 PURPOSE:

To screen for the possible presence of semen in forensic samples by testing for acid phosphatase activity.

### 6.2 INTRODUCTION:

Acid phosphatase (AP) is an enzyme found at elevated levels in semen. When the Brentamine reagent is applied to the test sample, a purple color forms in the presence of AP. The AP enzyme cleaves phosphate from sodium  $\alpha$ -naphthyl phosphate, freeing naphthol. The freed naphthol then couples with buffered Fast Blue B resulting in a purple azo dye.



### 6.3 TESTING PROCEDURES:

Before use on casework samples, working reagents shall be tested with at least a 1:100 diluted positive semen control each day of use. A negative control shall also be tested. The results of these tests shall be documented in the case notes.

Note: It is recommended to prepare the diluted positive semen control with PBS (instead of water) to increase its long-term reactivity if stored frozen and used after a long period of time.

The test itself may be performed by one of the following methods:

#### SPOT TEST (INDIVIDUAL STAINS):

1. Moisten a piece of filter paper or swab with dH<sub>2</sub>O.
2. Press or gently rub the filter paper or swab on the suspected semen stain.
3. Apply Brentamine reagent to the paper or swab and allow 2-3 minutes for color development.
4. Test result and approximate location(s) of stain(s) shall be documented in the case file.

#### MAPPING (WHOLE GARMENTS, BED SHEETS, UNDERWEAR CROTCH PANELS, ETC.):

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

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

3. After pressing, hang the filter paper in a chemical fume hood and spray with Brentamine reagent and allow 2-3 minutes for color development.

Note: Brentamine reagent may also be applied drop-wise to the area to avoid aerosolizing the

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reagent. The drop-wise application does not require a chemical fume hood.

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

#### 6.4 INTERPRETATIONS:

Semen stains should give a fast (immediate to within three minutes) purple color reaction depending on the amount of AP present. Slower developing color reaction may be due to a weak semen stain or from AP in other body fluids.

#### 6.5 INTERPRETATION CAUTIONS:

1. This test assists in locating possible semen stains. Positive reactions may be followed by a p30 test and/or a microscopic sperm search. Slow or weak reactions and different color reactions may not be seminal fluid.
2. The enzyme acid phosphatase occurs in other body fluids, bacteria, fungi, and many plants. Common weak reactions may be seen in:
  - Vaginal secretions (especially during pregnancy or with bacterial infection). Generally, the reaction is slow, and the resulting color is faint. However, some females may have higher endogenous levels of AP activity that may provide stronger results.
  - Fecal stains. The reaction is slow, and the resulting color is faint pink.
  - Certain plants and fungi.

#### 6.6 CONCLUSIONS:

See section 8.6 for conclusions.

#### 6.7 REFERENCES:

Baechtel F. The Identification and Individualization of Semen Stains. In: Saferstein R, editor, Forensic Science Handbook Vol. 2. Englewood Cliffs Prentice Hall; 1988; p. 347-55.

Davies A, Wilson E. The Persistence of Seminal Constituents in the Human Vagina. Forensic Science 1974; 3 (1):45-55.

Gaensslen RE. (AP). In: Sourcebook in Forensic Serology, Immunology and Biochemistry. Washington, D.C.: U.S. Department of Justice 1983; p. 155-69.

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Serological Research Institute. "The Brentamine Reaction" procedure. In: Serological Research Institute Methods Manual. Vol. Document No. MM I-B, Revision No. 2 2002; p. 1-3.

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## 7.0 SEMEN: SPERM SEARCH / CHRISTMAS TREE STAIN

### 7.1 PURPOSE:

To isolate and search for spermatozoa and other cellular components (if present) through microscopy and staining techniques.

### 7.2 INTRODUCTION:

This staining procedure, coupled with the microscopy techniques, aids in distinguishing sperm cells from other cellular material/components. The nuclear material of the cell is stained red, while the cellular membranes are stained green. Sperm heads are usually well differentiated with the acrosomal cap unstained or lightly stained and the posterior (nuclear) portion more darkly stained. This color staining is augmented by phase contrast microscopy in which the staining appears reversed, with the acrosomal cap appearing darker than the posterior (nuclear) region.

### 7.3 TESTING PROCEDURES:

1. Remove a portion of the stain, stained material, or vaginal aspirate and place into an appropriate microcentrifuge tube.

#### Notes:

- If an unstained smear slide was received as a case sample, proceed to step 9.
  - If an appropriately stained smear slide without a cover slip was received as a case sample, proceed to step 13.
2. Add 100–1000  $\mu\text{L}$  of sterile PBS to the microcentrifuge tube and incubate at 37 °C for a minimum of 30 minutes.
  3. Following incubation, pulse spin the sample(s). Transfer the substrate material into a spin basket insert and place the basket into the tube containing the stain extract.
  4. Centrifuge for 5 minutes (at top speed or as appropriate).
  5. Remove the basket insert with the substrate material from the extract tube and set aside.
  6. While being careful not to disturb the pellet in the microcentrifuge tube, remove most of the supernatant fluid (leaving approximately 20–50  $\mu\text{L}$ ) and transfer the supernatant into a separate labeled sterile microcentrifuge tube.

Note: The transferred supernatant may be used for PBS extraction compatible serological tests which includes p30 and amylase. If the sample supernatant is not used immediately, it may be frozen for later use.

7. Resuspend the cell pellet in the remaining fluid in the microcentrifuge tube and place 3  $\mu\text{L}$  of the suspended material on a microscope slide. (In some circumstances it may not be necessary to resuspend the pellet.)

Note: The extract and substrate may be taken forward for further DNA extraction procedures. The extracts shall be stored under appropriate conditions until further testing is performed.

8. Heat fix the sample to the microscope slide (a Bunsen burner, small torch, hot plate, or oven may be utilized).

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9. Apply Christmas Tree Stain A (red, nuclear fast red) to the heat-fixed cells on the microscope slide and allow stain to remain on the sample for 10–15 minutes.
10. Gently rinse the slide with dH<sub>2</sub>O.
11. Apply Christmas Tree Stain B (green, picro indigo carmine) to the sample on the microscope slide and allow the stain to remain on the sample for 5–10 seconds.
12. Gently rinse the slide with ethanol and allow the slide to dry.
13. Using permount (or another mounting medium), apply a coverslip to the slide.
14. Conduct a systematic search of the slide using a microscope which has phase contrast capabilities between 200X to 400X magnification or retain the slide for possible future examination.

**Notes:**

If a slide is examined and no spermatozoa are observed, proceed with testing for p30. If few spermatozoa are observed and/or weak p30 results are obtained, additional sample may be removed and combined with the original substrate, and a differential slide may be made (see *Casework STR Analysis Procedures*, Lysis: Differential, step 8). For oral swabs and genital/anal swabs collected for sexual assaults, the analyst may choose to delay the microscopic exam for sperm as described in step 8 of the *Casework STR Casework Procedures*, Lysis: Differential procedure until after the washes in step 21. A second exam after step 21 is optional.

**7.4 INTERPRETATIONS:**

Case notes shall document whether spermatozoa were observed or not, the relative number of spermatozoa observed, any unusual characteristics the spermatozoa exhibit (pyriform heads, tail defects, etc.), other cellular and/or non-cellular materials observed to include identity (or general characteristic) and relative number. As appropriate, the relative location of spermatozoa on the slide shall be documented in the case notes.

**7.5 CONCLUSIONS:**

See section 8.6 for conclusions.

**7.6 REFERENCES:**

Allard JE. The Collection of data from finding in cases of Sexual Assault and the Significance of Spermatozoa on Vaginal, Anal, and Oral Swabs. *Science and Justice* 1997; 37 (2):99-108.

Baechtel F. The Identification and Individualization of Semen Stains. In: Saferstein R, editor, *Forensic Science Handbook Vol II*. Englewood Cliffs: Prentice Hall 1988 p. 364-8.

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Davies A, Wilson E. The Persistence of Seminal Constituents in the Human Vagina. *Forensic Science* 1974; 3 (1):45-55.

Enos WF, Beyer JC. Spermatozoa in the Anal Canal and Rectum and in the Oral Cavity of Female Rape Victims. *Journal of Forensic Sciences* 1978; 23 (1):231-3.

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Serological Research Institute. "A Gram Modified Christmas Tree Stain" procedure. In: Serological Research Institute Methods Manual Vol. Document No. MM II-C, Revision No. 2; 2002 p. 1-2.

Willott GM, Allard JE. Spermatozoa – Their Persistence After Sexual Intercourse. Forensic Science International 1982; 19 (2):135-54.

## 8.0 SEMEN: p30 TEST

### 8.1 PURPOSE:

To test for the possible presence of semen by detecting p30 in forensic samples.

### 8.2 INTRODUCTION:

The p30 test is an immunoassay used to detect p30, a protein found in high levels in semen.

### 8.3 TESTING PROCEDURES:

1. Apply 80 or 200  $\mu$ L (depending on manufacturer instructions) of room temperature supernatant from the test sample to the sample well "S" on the ABACard for p30. Maintain at room temperature.

Note: The supernatant used is from the PBS extraction step of the Sperm Search section of this manual. A dilution of the supernatant may be needed to yield 200  $\mu$ L of liquid.

2. The result shall be read at 10 minutes. A positive result may be seen earlier.

Note: The result shall not be read after 10 minutes since nonspecific reactions may occur and may result in a false positive.

### 8.4 INTERPRETATIONS:

#### POSITIVE P30 RESULT:

The formation of a visible pink line in the test "T" and control "C" regions.

#### NEGATIVE P30 RESULT:

No formation of a visible pink line in the test "T" region, and a visible pink line in the control "C" region.

#### INCONCLUSIVE P30 RESULT:

An incomplete or questionable visible pink line in the "T" region and a visible pink line in the control "C" region.

#### INCONCLUSIVE TEST (FAILED CASSETTE):

No visible pink line in the control "C" region regardless of whether or not the test "T" region exhibits a visible pink line. The test sample should be rerun, if appropriate and possible.

Note: Interpretation of the result of the test shall be documented in the case file.

### 8.5 INTERPRETATION CAUTIONS:

Product literature reports that the lower limit of sensitivity for p30 detection is 4 ng/ml. A few studies have shown that a very small minority of women may have endogenous levels of p30 close to the minimum detectable level of the ABACard assay. Therefore, caution should be used in interpreting lines that develop close to the 10-minute time limit of this test. The strength of the acid phosphatase result could also be a factor considered.

A "High Dose Hook Effect" may be seen for extremely concentrated p30 samples (such as neat semen on an item) which appears as a negative result (no pink line in the test "T" region, pink

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line visible in the control “C” region). If such a sample is suspected, the supernatant should be retested using a 10-to-10,000-fold dilution.

Internal validation work on this procedure reveals that a PBS (rather than dH<sub>2</sub>O) extract is more stable over time. In addition, extracted samples should be run immediately or stored frozen and run within approximately 72 hours.

Concentrated male urine (approximating neat urine) may give a positive result.

The use of a substrate sample to aid in interpretation may be appropriate for some types of evidence.

False positive test results have been reported from recently deceased and decomposed post-mortem anal swabs.

8.6 CONCLUSIONS:

INCONCLUSIVE  
The [microscopic examination or AP/p30 test] did not provide any information regarding whether semen is or is not present due to [*reason, e.g., insufficient sample/test malfunction*].

OTHER RESULTS  
Select the applicable conclusion from the table below according to the semen tests performed (AP, p30, and/or microscopy) and their results. Test results are designated as positive (+), negative (–), or not tested (NT). This table is not intended to suggest case approach and the presence of a result set in the table does not imply those tests are appropriate for any given case or sample.

AP	p30	Micro	Conclusion
–	–	–	Semen was not detected using the AP and p30 tests, nor were sperm cells observed microscopically.
–	–	+	Semen was indicated through microscopic observation of sperm cells; however, neither AP nor p30 were detected.
–	–	NT	Semen was not detected using the AP or p30 tests.
–	+	–	Semen was indicated using the p30 test; however, AP was not detected, nor were sperm cells observed microscopically.
–	+	+	Semen was indicated through microscopic observation of sperm cells and the p30 test; however, AP was not detected.
–	+	NT	Semen was indicated using the p30 test; however, AP was not detected.
–	NT	–	Semen was not detected using the AP test, nor were sperm cells observed microscopically.
–	NT	+	Semen was indicated through microscopic observation of sperm cells; however, AP was not detected.
–	NT	NT	Semen was not detected using the AP test.
+	–	–	Semen was indicated using the AP test; however, p30 was not detected, nor were sperm cells observed microscopically.
+	–	+	Semen was indicated through microscopic observation of sperm cells and the AP test; however, p30 was not detected.
+	–	NT	Semen was indicated using the AP test; however, p30 was not detected.
+	+	–	Semen was indicated using the AP and p30 tests; however, sperm cells were not observed microscopically.
+	+	+	Semen was indicated using the AP and p30 tests and the microscopic observation of sperm cells.
+	+	NT	Semen was indicated using the AP and p30 tests.
+	NT	–	Semen was indicated using the AP test; however, sperm cells were not observed microscopically.
+	NT	+	Semen was indicated using the AP test and the microscopic observation of sperm cells.
+	NT	NT	Semen was indicated using the AP test.
NT	–	–	Semen was not detected using the p30 test, nor were sperm cells observed microscopically.
NT	–	+	Semen was indicated through microscopic observation of sperm cells; however, p30 was not detected.
NT	–	NT	Semen was not detected using the p30 test.
NT	+	–	Semen was indicated using the p30 test; however, sperm cells were not observed microscopically.
NT	+	+	Semen was indicated through microscopic observation of sperm cells and the p30 test.
NT	+	NT	Semen was indicated using the p30 test.
NT	NT	–	Semen was not detected, as sperm cells were not observed microscopically.
NT	NT	+	Semen was indicated through microscopic observation of sperm cells.

## 8.7 REFERENCES:

Abacus p30 Test Technical Information Sheet ABACard p30 Test For The Forensic Identification of Semen. Abacus Diagnostics Inc; 2022.

Baechtel F. The Identification and Individualization of Semen Stains. In: Saferstein R, editor,

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Stubbings NA, Newell PJ. An Evaluation of Gamma-Glutamyl Transpeptidase (GGT) and p30 Determinations for the Identification of Semen on Postcoital Vaginal Swabs. Journal of Forensic Sciences 1985; 30 (3):604-14.

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## 9.0 SALIVA: PHADEBAS PAPER AMYLASE DIFFUSION

### 9.1 PURPOSE:

The Phadebas paper amylase diffusion method can be useful in indicating the possible presence of  $\alpha$ -amylase, a protein found in saliva, in a variety of body fluid stains and swabs.

### 9.2 INTRODUCTION:

Phadebas paper has a water-insoluble starch polymer that carries a blue dye. If  $\alpha$ -amylase is present,  $\alpha$ -amylase hydrolyzes the starch polymer to form water-soluble blue fragments. This method can be useful in indicating the presence of  $\alpha$ -amylase, a protein found in saliva. The paper may be used to "map" the location of possible saliva stains on items of evidence.

### 9.3 TESTING PROCEDURES:

1. Spray sterile distilled water liberally onto the back (non-blue side) of the Phadebas paper. Be sure the paper is wet enough so it will not dry out during the testing process but is not too wet.
2. Dampen item test area with sterile distilled\* water using a spray bottle. Alternatively, the non-blue side (back) of the paper can be dampened again after placement on the test area.
3. Place the dampened piece of paper, blue side down on the desired test area.
4. Place 2–3 layers of butcher paper over the paper and apply light weight to ensure continual contact.
5. Allow contact to occur at room temperature for a maximum of 40 minutes or until a positive reaction is observed.

After recording the results, the paper may be tested for acid phosphatase activity (AP) by applying Brentamine reagent to the paper and allow 2–3 minutes for color development.

Note: If the paper is further tested for AP, a positive 1:100 semen control shall be run successfully in conjunction with, or on each day of testing.

\*Note – using deionized water instead of distilled water can result in reduced sensitivity for the AP test.

### 9.4 INTERPRETATIONS:

Areas of a uniform blue color on the non-reagent side of the paper are indicative of the presence of  $\alpha$ -amylase.

Note: The dye cleaved by amylase activity diffuses to the back white-side and the blue side becomes faded.

#### INTERPRETATION CAUTION:

$\alpha$ -Amylase activity is also reportedly found in semen, sweat, vaginal fluid, urine and fecal stains.

### 9.5 CONCLUSIONS:

If RSID-Saliva testing was also performed on the sample, see section 10.6 for conclusions.

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UNIFORM BLUE COLOR ON NON-REAGENT SIDE:

Saliva was indicated using the Phadebas test.

NO BLUE COLOR ON NON-REAGENT SIDE:

Saliva was not detected using the Phadebas test.

9.6 REFERENCES:

Willott GM. An Improved Test for the Detection of Salivary Amylase in Stains. Journal - Forensic Science Society 1974; 14 (4):341-4.

Willott GM, Griffiths M. A new method for locating saliva stains – spotty paper for spotting spit. Forensic Science International 1980; 15 (1):79-83.

<http://www.phadebas.com/>

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## 10.0 SALIVA: RSID SALIVA TEST

### 10.1 PURPOSE:

To screen for the possible presence of saliva by detecting human alpha-amylase in forensic samples.

### 10.2 INTRODUCTION:

The RSID Saliva cards can be used to determine the possible presence of amylase in vaginal swabs, semen stains, and other body fluid stains that could indicate the presence of saliva.

### 10.3 TESTING PROCEDURES:

1. Extract the sample in PBS buffer or dH<sub>2</sub>O as described in sections 5.2 or 7.3.
2. Combine 20 µL of the room temperature supernatant from the test sample with 80 µL of room temperature TBS+ Running Buffer or Universal Buffer provided in the RSID Saliva kit.
3. Apply the 100 µL total volume prepared in Step 1 to the sample well "S" on the RSID Saliva test card. Maintain at room temperature.
4. The result shall be read at 10 minutes. A positive result may be seen earlier.

### 10.4 INTERPRETATIONS:

#### POSITIVE AMYLASE RESULT:

The formation of a visible pink line in the test "T" and control "C" regions.

#### NEGATIVE AMYLASE RESULT:

No formation of a visible pink line in the test "T" region, and a visible pink line in the control "C" region.

#### INCONCLUSIVE AMYLASE RESULT:

An incomplete or questionable visible pink line in the "T" region, and a visible pink line in the control "C" region.

#### INCONCLUSIVE TEST (FAILED CASSETTE):

No visible line in the control "C" region regardless of whether or not the test "T" region exhibits a visible pink line. The test sample should be rerun, if appropriate and possible.

Notes: Interpretation of the result of the test shall be documented in the case file.

### 10.5 INTERPRETATION CAUTION:

Concentrated neat urine, feces, and breast milk may give a positive result close to the 10-minute time limit of this test.

Internal validation work on this procedure reveals that the test is more sensitive when a PBS (rather than dH<sub>2</sub>O) extract is used.

### 10.6 CONCLUSIONS:

#### POSITIVE AMYLASE RESULT:

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Human saliva was indicated using the RSID test.

NEGATIVE AMYLASE RESULT:

Human saliva was not detected using the RSID test.

INCONCLUSIVE AMYLASE RESULT OR TEST:

The RSID test did not provide any information regarding whether saliva is or is not present due to [reason, e.g., insufficient sample/test malfunction].

PHADEBAS AND RSID SALIVA POSITIVE:

Human saliva was indicated using the Phadebas and RSID tests.

PHADEBAS POSTIVE AND RSID SALIVA NEGATIVE:

Saliva was indicated using the Phadebas test; however, human saliva was not detected using the RSID test.

## 11.0 EVALUATION OF APPARENT HAIRS

### 11.1 PURPOSE

To evaluate the suitability of apparent human hairs for DNA analysis based on macroscopic and microscopic characteristics.

### 11.2 INTRODUCTION

Macroscopic and microscopic observations of apparent hairs may be used to determine whether the sample is appropriate for DNA analysis.

### 11.3 PROCEDURE

1. When apparent hairs and/or fibers will not be subjected to DNA analysis, documentation that they are present is sufficient (i.e. using terms such as possible hair, apparent hair, or hair-like)
2. To evaluate the suitability of apparent hairs and/or fibers for DNA analysis, examine the sample using a stereomicroscope
  - a. Observe the entire sample, noting any adhering cellular material and the physical appearance of any root

### 11.4 INTERPRETATION

1. Samples with adhering cellular material and/or a root may be suitable for DNA analysis
2. Samples without either adhering cellular material or a root may be unsuitable for DNA analysis. Other testing approaches, such as examination by a Materials Analysis hair analyst or alternative DNA testing technologies should be considered and discussed with the investigating officer as appropriate.

### 11.5 CONCLUSIONS

#### POSSIBLE ROOT MATERIAL WAS OBSERVED

Possible root material was observed by microscopic examination.

#### NO ROOT MATERIAL WAS OBSERVED

No root material was observed by microscopic examination.

### 11.6 REFERENCES

Chewning, D.D., Deaver, K.L., and Christensen A.M., Persistence of Fibers on Ski Masks During Transit and Processing, Forensic Science Communications, 2008: 10(3).

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Linch, C.A., The Ultrastructure of Tissue Attached to Telogen Hair Roots, J Forensic Sci, 2008: 53(6), pp. 1363-1366.

Moore, T.D., Identification of Dorsal Guard Hairs of Some Mammals of Wyoming, Wyoming Game and Fish Department, 1974.

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## 12.0 WHOLE BLOOD PROCESSING

### 12.1 PURPOSE:

To prevent contamination of whole blood samples, and to prepare the whole blood for testing.

### 12.2 PROCEDURE:

THE ANALYST SHALL NEVER HAVE BLOOD TUBES FROM MORE THAN ONE SOURCE OPEN SIMULTANEOUSLY. Barrier tips shall be used to decrease the chance for contamination while making DNA stains.

THE STAINS FOR DNA SHALL BE ISOLATED FROM THE PREPARATION AREA BEFORE BLOOD TUBES FROM ANOTHER SOURCE OF BLOOD ARE OPENED. This isolation can be done by moving the prepared stains to another area, by covering the prepared stains, or by placing a barrier between the prepared stains and the blood processing area.

### 12.3 STAINS FOR DNA

If enough blood is present, deposit on the paper DNA cards several aliquots of whole blood. The cards should be labeled with case number, name of the blood source, date the stains are made, and the initials of the individual making the stain. Additionally, if the blood is from a tube other than a lavender-top tube, a notation indicating the deviation (i.e. "gray-top") may also be written on the card. If using some bodily substance other than blood (i.e. liver tissue), the body substance may be noted on the card.

If the blood (or other bodily substance) is not in a liquid form, such as coagulated blood in a red-top tube, then some of the solid material should be placed on and worked into the card using an applicator stick, stirring rod, or other clean utensil.

Prepared reference cards shall be dried. The reference card may be used in a DNA extraction procedure. When the testing has been completed on the case, the card shall be returned to the submitting agency.

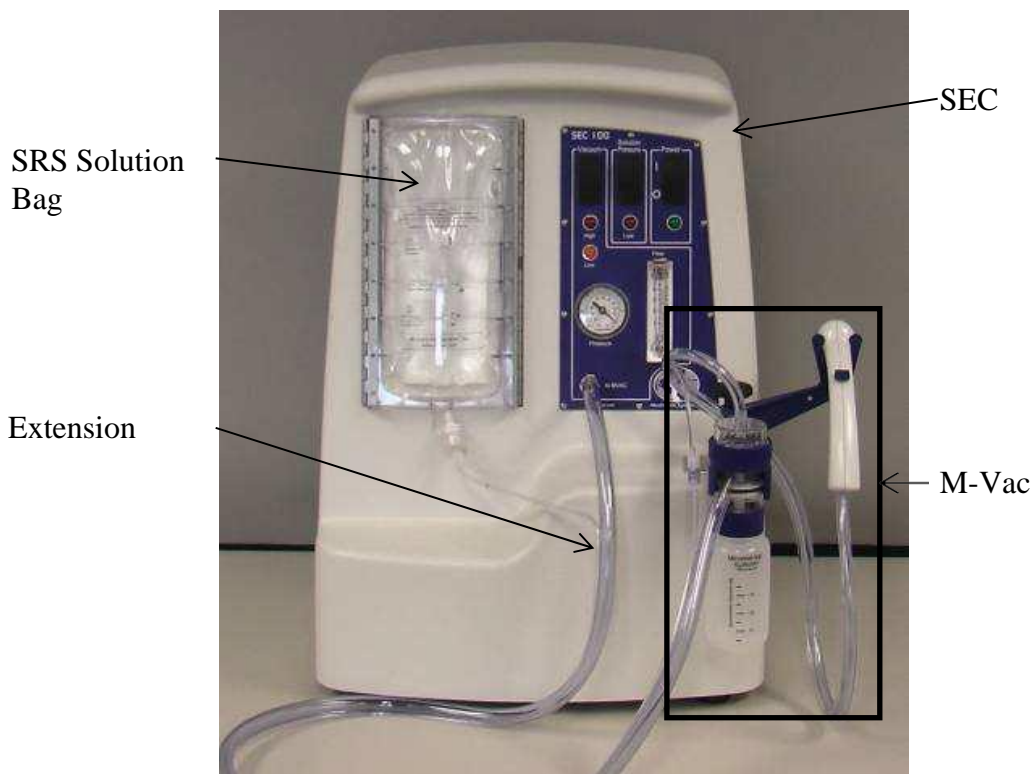
Alternatively, a small aliquot (~3–5 µL) of liquid blood may be placed directly into a tube and extracted for DNA content; however, a reference card should still be made for long-term storage.

### 13.0 SAMPLE COLLECTION USING THE M-VAC SYSTEM

The M-Vac System is for the wet-vacuum collection of cellular material from forensic samples where limited amounts of DNA may be present and where other collection techniques may not be successful.

The M-Vac System consists of a Support Equipment Case (SEC, which provides the power and vacuum), extension tubing to the M-Vac, a sterile surface rinse solution (SRS) bag, and the M-Vac (see below).

Each sample collection requires a new M-Vac (see System Set-Up, Sample Collection, & Removing/Replacing M-Vac) where the cellular sample is suspended in a collection bottle included with the M-Vac (see Removing/Replacing Sample Collection Bottle). The SRS bag provides the buffer enabling the suspension of cellular material and is required for sample collection; the SRS bag can remain on the SEC for multiple collection events and should be replaced as needed for sample collection (see Replacing SRS Bag). For some collection events, multiple collection bottles or SRS bags may be needed. The cellular sample in the collection bottle must be filtered prior to DNA extraction (see Filtering a Sample).



Note: Prior to using the MVAC, DNA Scientists shall consult with the local Materials Analysis section supervisor or designee to determine whether trace evidence analysis should be performed prior to DNA analysis or whether other analytical considerations are appropriate to preserve potential trace evidence.

### 13.1 SYSTEM SET-UP:

1. Turn ON the Support Equipment Case (SEC) power switch.
2. Remove over wrap from the Surface Rinse Solution (SRS) bag and hang the bag on the solution door.
3. Prepare the M-Vac for use
  - a. Remove the M-Vac and tighten the lid (tighten, release, tighten)
  - b. Turn OFF the solution switch on sampling head by pulling back
  - c. Place separation unit and sampling head in holder on SEC
4. Open extension tubing. Attach the solution line fitting to the M-Vac fitting on the M-Vac. Lightly attach the vacuum tubing.
5. Aseptically break tip off the SRS bag. Connect spiked fitting of the tubing to the bag port. To connect, push and twist the fittings together until fully seated.
6. Close the door until it is locked shut by the hinged latch. Turn Solution Pressurization switch ON. (It is pressurized when the low pressurization indicator light turns OFF.)
7. Connect vacuum side of tubing to SEC by slipping the quick-connect fitting into the vacuum port on SEC labeled "to MVAC."

### 13.2 SAMPLE COLLECTION:

1. Turn the Vacuum switch of the SEC to ON.
2. With the vacuum pump ON, retighten the lid on the Separation Unit.
3. Place the sampling head against the surface to be sampled. (Try to keep all the flexible feet in light contact with the surface while sampling.)
4. Turn ON and OFF the solution by toggling the switch on the sampling head as needed.
5. When the sample has been taken, or if additional bottles are needed to complete the sample, turn the Vacuum switch on the SEC to OFF. Remove the sample collection bottle.

### 13.3 REMOVING/REPLACING M-VAC:

1. Turn OFF the vacuum.
2. Turn OFF the Solution Pressurization (optional).
3. Pull vacuum tubing off hose barb on the side of the Separation Unit.
4. Disconnect the solution line at the Separation Unit by unthreading the M-Vac fitting from the check valve.
5. Remove the Separation Unit and Sampling Head from their holders and discard.
6. Open and connect a new M-Vac (optional)

### 13.4 REPLACING SRS BAG:

1. Depressurize the Solution Pressurization chamber by turning the Solution Pressurization switch to OFF.
2. Open Solution Pressurization Chamber and remove used SRS bag from hook.
3. Disconnect spiked fitting on solution line from threaded SRS bag port fitting. Discard old SRS bag.
4. Connect a new SRS bag (see "System Set-up")

### 13.5 REMOVING/REPLACING SAMPLE COLLECTION BOTTLE:

1. Turn Vacuum switch to OFF.

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2. Unscrew the bottle from the Separation Unit.
3. Place a lid on the removed bottle and tighten.
4. If the M-Vac is going to be used to continue sampling an area or to take another sample, screw a new bottle on the Separation Unit.

### 13.6 FILTERING A SAMPLE:

1. Attach the M-Vac vacuum tubing to the vacuum filter.
2. Support the vacuum filter in the upright position during processing.
3. Turn ON the vacuum on the SEC.
4. Swirl the sample in the bottle and then slowly pour into the vacuum filter funnel.
  - a. Optional: use of the pre-filter system should be used for samples with significant debris content. Continue vacuuming until all the solution has passed through the pre-filter and proceed to step 4. If the M-Vac vacuum is used for the pre-filter process (as opposed to in-lab vacuum system), use a pre-filter shield to avoid aspirating liquid into the M-Vac system.
5. Continue vacuuming until all the solution has passed through the filter.
6. If desired, turn OFF the vacuum, remove the collection bottle on the bottom of the filter, pour the filtered water back into the sample bottle, swish and re-filtrate after the vacuum filter is reassembled and the vacuum is turned ON.
7. If desired, the vacuum filter funnel can be rinsed with DI water at the end of filtering to rinse the sides of the funnel.
8. Discard the filtered solution.
9. Handle the filter/filter funnel apparatus with standard evidence handling practices (if desired, the filter can be stored and dried while in the housing for later processing).
10. Using a sterile scalpel remove and process the entire filter for DNA using an appropriate extraction method.

### 13.7 M-VAC SYSTEM MAINTENANCE:

1. Day of use:
  - a. Ensure the switches are OFF on the Vacuum, Solution Pressure, and Power.
  - b. If the vacuum tubing or solution will be used again, leave an M-Vac (sampling head and separation unit) attached and in the OFF position.
  - c. In the event of contamination, wipe down SEC with a suitable disinfectant.
2. Periodic (schedule may vary based on usage):
  - a. Check the liquid trap and empty if needed.
  - b. Inspect the ventilation filter, and if dirty, clean with a mild soap or replace if needed.
  - c. Replace the Vacuum Exhaust HEPA Filter anytime there is reason to believe contamination is present in the vacuum exhaust.



## 14.0 REPORTING BIOCHEMICAL EXAMINATION AND TEST RESULTS

- 14.1 General report requirements may be found in the QOM and the DNA QA Manual.
- 14.2 The conclusions specified in test procedures of this manual shall be used in the Results and Conclusions section of laboratory reports to express results of each method performed.
- 14.2.1 If a standard conclusion statement does not accurately reflect the testing or results obtained, or for situations not addressed by standard conclusions, consult a technical lead scientist or supervisor. A technical lead scientist, supervisor, or the technical leader must approve significant deviations from standard wording.
- 14.3 The following method statements will be included in the methods section of laboratory reports when applicable.

Method used	Method statement in report
Phenolphthalein	Blood testing was conducted using the phenolphthalein test.
HemaTrace	Human blood testing was conducted using the ABACard HemaTrace test.
Phadebas	Saliva testing was conducted using the Phadebas amylase test.
RSID Saliva	Human saliva testing was conducted using the RSID Saliva test.
Acid phosphatase	Semen testing was conducted using the AP test.
p30	Semen testing was conducted using the ABACard p30 test.
Microscopy for sperm	Semen testing was conducted using microscopic examination for sperm cells.
M-Vac	The M-Vac collection system was used for sample collection.
Microscopy for possible hair root material	Microscopic examination for possible hair root material was conducted.

- 14.4 Applicable report remarks listed in the *Casework STR Analysis Procedures* and in the table below will be included in the remarks section of all laboratory reports.

Criteria	Remark in report
Serology testing performed	A positive body fluid test indicates the possible presence of the body fluid of interest; however other biological materials may also produce a positive result. A negative body fluid test indicates the body fluid of interest was not detected but does not confirm its complete absence.

- 14.5 The following definitions will be included in the glossary section of all laboratory reports containing serology test results when applicable.

Method used	Definition in report
Phenolphthalein	Phenolphthalein test (PHT): a chemical test for the presence of hemoglobin, a protein found in blood.
HemaTrace	ABACard HemaTrace test: a test for the presence of human hemoglobin, a protein found in blood.
Phadebas	Phadebas amylase test: a chemical test for the presence of amylase, a protein found in saliva.
RSID-Saliva	RSID Saliva test: a test for the presence of $\alpha$ -amylase, a protein found in human saliva.

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Acid phosphatase	Acid phosphatase (AP) test: a chemical test for the presence of AP, a protein found in semen.
p30	ABAcad p30 test: a test for the presence of p30, a protein found in semen.
Microscopy for sperm	Sperm cells (spermatozoa): the male reproductive cell found in semen.
M-Vac	M-Vac: a wet vacuum collection device.

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## 15.0 SAFETY

During daily serological routines, the analyst is likely to come into contact with hazardous and potentially hazardous chemicals. Skin contact with any chemical shall be avoided. Use personal protection equipment, including gloves, lab coat, mask, and safety glasses as required. Adequate ventilation shall be used when handling chemicals, especially liquids. Safety Data Sheets (SDSs) shall be readily available to reference. Questions not answered by the SDS regarding the safe use of any chemical shall be directed to the laboratory safety officer.

Below is a list of commonly used chemicals in serology, which should be handled carefully.

Chronic exposure to any chemical may increase the potential for Carcinogenic, Teratogenic, Mutagenic, and Reproductive hazards.

### 15.1 ACETIC ACID:

Combustible.

Causes severe burns, harmful in contact with skin.

### 15.2 ACETONE:

Flammable.

Irritating to eyes and skin.

Inhalation may cause drowsiness and dizziness.

### 15.3 BROMOTHYMOL BLUE:

Contact and inhalation hazard.

### 15.4 DIANISIDINE TETRAZOTIZED (FAST BLUE B):

Toxic.

May cause cancer.

Harmful if swallowed.

### 15.5 INDIGO CARMINE:

Harmful if swallowed

### 15.6 NUCLEAR FAST RED:

Irritant to eyes, respiratory system, and skin.

### 15.7 PHENOLPHTHALEIN:

Possible carcinogen.

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15.8 PHOSPHORIC ACID:

Corrosive.

Causes burns.

Harmful if swallowed.

15.9 POTASSIUM HYDROXIDE:

Corrosive.

Causes severe burns.

Harmful if swallowed.

15.10 SODIUM HYDROXIDE:

Corrosive.

Causes severe burns.

Exothermic in contact with skin.

15.11 SODIUM  $\alpha$ -NAPHTHYL PHOSPHATE:

Irritating to eyes, respiratory system, and skin.

15.12 SODIUM PHOSPHATE, MONOBASIC:

May cause skin irritation.

15.13 SODIUM PHOSPHATE, DIBASIC:

May cause skin irritation.

15.14 ZINC CHLORIDE:

Corrosive.

Causes burns.

Harmful if swallowed.

Dangerous to the environment.

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## 16.0 REAGENT PREPARATION

Use reagent grade chemicals unless otherwise noted. Commercially prepared, reagent grade chemicals can be substituted for many of the recipes below. Prepare all solutions using deionized water (dH<sub>2</sub>O). Wear appropriate personal protection equipment such as gloves and follow safety recommendations provided by manufacturer for handling chemicals. Comply with any and all laws, regulations, or orders with respect to the disposal of any hazardous or toxic chemical, material, substance or waste. Store all reagents at room temperature unless otherwise noted.

The chemicals which require special handling are indicated with a designation of "HAZMAT".

### 16.1 PHOSPHATE BUFFERED SALINE (PBS)

PBS shall be purchased from a commercial supplier and shall have a formulation of approximately pH 7.4, 137 mM salt and at minimum 10 mM PO<sub>4</sub>. PBS can be purchased in a 1X or 10X solution.

#### HAZMAT

PBS is a skin, eye, and respiratory irritant.

### 16.2 PHENOLPHTHALEIN

### 16.3 PHENOLPHTHALIN STOCK SOLUTION

2 g Phenolphthalein CAS No. 77-09-8 (HAZMAT)

20 g Potassium hydroxide (HAZMAT)

100 ml dH<sub>2</sub>O

The mixture is refluxed with 20 grams of powdered zinc (approximately two hours) until the solution becomes colorless. The stock solution shall be stored in an appropriate container in a cold dark place (i.e. refrigerator) with some zinc added to keep it in the reduced form.

#### **Phenolphthalin Stock Solution (alternate method)**

2g Phenolphthalin CAS No. 81-90-3 (HAZMAT)

20 g Potassium hydroxide (HAZMAT)

100 ml dH<sub>2</sub>O

Place 20g of potassium hydroxide pellets into a 500ml amber reagent bottle with zinc turnings and 2g of phenolphthalin powder. Add 100ml dH<sub>2</sub>O to the contents of the bottle and swirl until dissolved. The stock solution shall be stored in an appropriate container in a cold dark place (i.e. refrigerator) with some zinc added to keep it in the reduced form

This reagent expires 3 years after its preparation. A lot number, which will be the six-digit expiration date, shall be assigned and recorded on the bottle and in the Reagent Log.

#### HAZMAT

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Phenolphthalein is a possible carcinogen.

Potassium hydroxide is corrosive and will cause severe burns when contacting skin, eyes, and mucous membranes. Harmful if swallowed.

#### DISPOSAL

Zinc may be stored until it is picked up by a chemical waste removal company. Zinc shall not be disposed of in a waste paper basket! All waste shall be disposed of according to local regulations.

### 16.4 PHENOLPHTHALIN WORKING SOLUTION

10 ml Phenolphthalin stock solution

40 ml EtOH

Combine 10 ml of phenolphthalin stock solution and 40 ml ethanol. The prepared working solution shall be stored in an appropriate container with some zinc to keep it in the reduced form and may be stored in the refrigerator or at room temperature.

The lot number, which is the lot number of the stock solution followed by a unique identifier (i.e. - A, -B, -1, -2), shall be recorded on the bottle. Before use on casework samples, working reagent shall be QC checked and recorded in the Reagent Log. Working reagent will also be tested with a positive blood control and negative control each day of use. (The results of these tests shall be recorded in the case notes).

#### DISPOSAL

All waste shall be disposed of according to local regulations.

Zinc may be stored until it is picked up by a chemical waste removal company. Zinc shall not be disposed of in a waste paper basket.

### 16.5 ACID PHOSPHATASE

#### 16.5.1 ACID PHOSPHATASE STOCK SOLUTION (A)

1 g Fast Blue B (HAZMAT)

20 g Sodium acetate, trihydrate

10 ml Glacial acetic acid (HAZMAT)

100 ml dH<sub>2</sub>O

Combine and store in a cool dark place (i.e. refrigerated).

This reagent expires 6 months after its preparation. Each lot must be recorded in the reagent log. A lot number, comprised of the six-digit expiration date, shall be recorded on the bottle.

#### HAZMAT

Fast Blue B is a suspected carcinogen. Avoid ingestion and inhalation.

Glacial acetic acid is combustible and can cause severe burns to the skin, eyes, and mucous membranes. Avoid inhalation.

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DISPOSAL

All waste shall be disposed of according to local regulations.

## 16.5.2 ACID PHOSPHATASE STOCK SOLUTION (B)

0.4 g Sodium  $\alpha$ -naphthyl acid phosphate (HAZMAT)

5 ml dH<sub>2</sub>O

Combine and store in a cool dark place (i.e. refrigerated).

This reagent expires 6 months after its preparation. Each lot must be recorded in the reagent log. A lot number, comprised of the six-digit expiration date shall be recorded on the bottle.

HAZMAT

Sodium  $\alpha$ -naphthyl acid phosphate is an irritant. Avoid inhalation and contact with skin and eyes.

DISPOSAL

All waste shall be disposed of according to local regulations.

## 16.6 ACID PHOSPHATASE WORKING SOLUTION

Brentamine reagent, also known as Acid Phosphatase Working Solution

10 ml Solution A

1 ml Solution B

89 ml dH<sub>2</sub>O

Combine and store in a cool dark place (i.e. refrigerated).

The expiration date (2 weeks after preparation) shall be recorded on the bottle. Before use on casework samples, working reagent shall be QC checked and recorded in the Reagent Log. Working reagent will also be tested with a documented semen control each day of use. A negative control shall also be tested. The results of these tests shall be recorded in case notes.

Alternative preparation method:

AP Spot Test (SERI) may be prepared following the manufacturer's directions.

Per manufacturer's instructions, the AP Spot reagent will be made fresh each day of use.

Before use on casework samples, working reagent shall be QC checked. Working reagent will also be tested with a documented semen control each day of use. A negative control shall also be tested. The results of these tests shall be recorded in case notes.

DISPOSAL

All waste shall be disposed of according to local regulations.

## 16.7 SPERM SEARCH

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It is preferable to obtain Stains A and B of the Christmas Tree Stain procedure commercially.

Mounting media (i.e. Permount or Cytoseal) can be used beyond its commercially designated expiration date as long as its functional properties remain intact.

#### 16.8 PHADEBAS

Phadebas Paper (purchased commercially)

Distilled H<sub>2</sub>O (do not use de-ionized water instead of distilled)

Note. Distilled water will be given a six digit lot number depicting a three-year expiration date from receipt if an expiration date is not already supplied by the vendor and will be recorded in the chemical inventory log.

Store Phadebas paper at room temperature. A lot number, comprised of the six-digit expiration date supplied by the vendor, shall be recorded on the packaging. Prior to use in casework, each lot of paper must be QC checked using a positive neat saliva control swab and recorded in the Reagent Log.

All waste shall be disposed of according to local regulations.

#### 16.9 0.1 N SODIUM HYDROXIDE

0.4g NaOH (HAZMAT)

100 ml dH<sub>2</sub>O

10% Sodium Hydroxide

10g NaOH (HAZMAT)

100ml dH<sub>2</sub>O

Combine and mix. This reagent expires 3 years after its preparation. A lot number, comprised of the six-digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log.

Alternatively, a more concentrated stock solution can be made with a lot number assigned, and preparation or expiry date recorded on the bottle. As required an appropriate dilution can be prepared with the preparation date recorded on the bottle.

##### HAZMAT

NaOH is poisonous by ingestion and intraperitoneal routes. It is a skin and eye irritant. It has marked corrosive action on all body tissue. It can react violently with acids.

##### DISPOSAL

All waste shall be disposed of according to local regulations.

#### 18.0 BROMOTHYMOL BLUE (BTB)

150mg BTB (HAZMAT)

10.0ml dH<sub>2</sub>O

50 µL 1:10 Phosphoric acid (HAZMAT)

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Dissolve BTB in dH<sub>2</sub>O with the addition of 1:10 phosphoric acid.

This reagent expires when the bromothymol blue stock expires (or three years after preparation if no expiry date is provided by the manufacturer). Each lot must be recorded in the reagent log. A lot number, comprised of the six-digit expiration date, shall be recorded on the bottle.

#### HAZMAT

Bromothymol blue may be harmful by inhalation, ingestion, or skin absorption. It may cause eye irritation and skin irritation.

Phosphoric acid is corrosive and may cause burns to the skin, eyes, and mucous membranes. Harmful if swallowed.

#### DISPOSAL

All waste shall be disposed of according to local regulations.

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## 17.0 REVISIONS

Biochemistry Procedures Manual version 08-2006

Extensive update and re-organization of manual and addition of Phadebas Paper amylase testing method

Table of Contents version 03-2007

Addition of Revisions section

Reagent Preparation version 03-2007

Acid Phosphatase working solution wording of lot number and preparation or expiration date changed to just preparation date recorded on bottle. Allowance added for using a more concentrated NaOH to make dilutions of needed working solutions.

RSID Saliva Cards version 06-2007

New method added.

Biochemistry Procedures Manual version 09-2008

Converted to CLD ISO format, p30 procedure update, reagent prep update, HemaTrace update, Introduction update.