

**TENNESSEE BUREAU OF INVESTIGATION**  
*Forensic Services Division*

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Forensic Biology Policy and Procedures Manual

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**Tennessee Bureau of Investigation**

**Forensic Services Division**

**Forensic Biology Policy and Procedures Manual**

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#### 1. Blood Case Policy

Analysis of bloodstains may be limited to the identification of blood and/or human blood unless known blood standards are submitted for comparison.

Analysis of bloodstains on a multi-item case will be limited to a minimum number of significant items. A particular item will be examined only if the Statement of Facts (Request for Examination form) clearly states the necessity for that examination.

Analysis of victim's clothing may be performed if information suggests blood other than the victim's might be on the garments.

If a blood sample is not available from the deceased, the victim's bloody clothing, buccal swab (saliva standard), toothbrush, etc. may be used as a standard.

Liquid blood samples received with no other physical evidence for comparison may not be typed. Bloodstain cards will be made and returned to the submitting agency. Liquid blood tubes will be destroyed by TBI Laboratory personnel (see below).

Missing persons – This policy applies to samples that are to be retained for identification purposes only. If an official case has been created, then the evidence is to be processed through evidence receiving.

Blood samples received into the lab from an unidentified body are processed through Evidence Receiving. Each sample is given a lab number and exhibit number. Blood samples from relatives of missing persons are not processed through Evidence Receiving (see Combined DNA Index System or CODIS Convicted Offender protocol). DNA (deoxyribonucleic acid) results obtained will be entered into the Missing Person database to be compared to other DNA samples.

**Note:** All policies are subject to review and individual cases will be considered on a case-by-case basis, if questions arise. Tennessee Bureau of Investigation (TBI) management will have the ultimate authority to approve casework for DNA analysis on a case-by-case basis.

DNA analysis may not be performed without prior request from the District Attorney General, a TBI Special Agent in Charge, a Court Order, or an Agreed Order.

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#### Handling Blood Stain Cards:

Analysts are to prepare a blood stain card from the liquid blood standard submitted as evidence in a case and then return the blood stain card, along with other evidence assigned with the case, to the submitting agency or other designated law enforcement agency.

The stain card is to be labeled with the laboratory/exhibit number, the victim/subject name, the analyst's initials and the date. The stain card is to be air-dried at least 16 hours then placed in a "Ziploc"-type bag. The package is to be properly sealed with TBI evidence tape across which the analyst will place his/her initials.

Swatch cards made from blood samples received from a Medical Examiner Office or a law enforcement agency will be returned to the local law enforcement agency handling the case or other designated law enforcement agency.

#### Retention and Disposal of Liquid Blood Samples:

All blood tubes from completed cases will be collected monthly and stored in plastic bags in a refrigerator. Blood tubes will be destroyed after six months.

Work sheets will note the following:

*"Liquid blood standard destroyed on the first working day of \_\_\_ (month) \_\_\_, \_\_\_ (year) \_\_\_\_."*

#### Disposition Statement for Liquid Bloods:

The following disposition statement has been added to the LIMS system to be used for exhibits corresponding to liquid blood samples:

*"A portion of this liquid blood sample has been dried onto a swatch and stored with the evidence. The liquid blood tube will be destroyed after 6 months."*



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## **2. Sexual Assault Case Policy**

Based on the statement of facts and/or medical history, testing should begin with the most intimate sample which is generally an internal swab (i.e. vaginal, anal, or oral swab).

1. For single subject cases, screening may stop once a positive result is obtained that is sufficient for obtaining a CODIS eligible profile. If no CODIS eligible profile is obtained from the initial DNA testing, additional testing should be completed on the minimum number of items necessary to obtain a CODIS eligible profile. Testing may also stop at the analyst's discretion if a probative profile of the victim is obtained e.g. victim's profile on subject's penis swabs.
2. For cases with multiple subjects, the minimum number of items should be tested based on the statement of facts. If the number of different CODIS eligible profiles obtained from the initial DNA testing is less than the number of subjects indicated, additional testing of items should be completed.
3. For cases involving an unconscious victim or a non-verbal victim, screening may stop once a positive result is obtained that is sufficient for obtaining a CODIS eligible profile; however, an analyst may proceed with further screening if the statement of facts indicates the assault may involve multiple subjects.
4. Cases requiring a known subject standard and/or an elimination standard should not have additional testing performed until submission of the appropriate standard(s). If a profile is determined to match the elimination standard, additional testing of items should be performed.
5. Additional testing should not be performed for cases in which a CODIS eligible profile has been obtained unless a request is made by a District Attorney General and appropriate standards are submitted if applicable. For single subject cases, requests for additional testing will generally be limited to internal swabs if a CODIS eligible profile has been obtained from the initial testing.
6. Cases involving digital, oral, or penile penetration of the vagina or anus may proceed to Y-STR testing when no saliva or semen is detected. Y-STR testing may only be performed after receipt of the appropriate standard(s)



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and a request from the District Attorney General. Y-STR testing should be limited to swabs from the site(s) of penetration. Y-STR testing should only be offered when no exhibits are applicable for STR testing or no probative STR result is obtained in the case.

#### **LIMS Reporting Statements**

Note: The following statements are examples and may be edited or amended as necessary.

1. Known subject case with no consensual partner:

Please submit a proper standard from the subject, \_\_\_\_\_, for comparison to the unknown profile.

2. Known subject case with consensual partner:

Please submit a proper standard from the subject, \_\_\_\_\_, and the consensual partner for comparison to the unknown profile. If the unknown profile is determined to match the consensual partner standard, additional testing will be performed if applicable.

3. Unknown subject case with a consensual partner:

Please submit a consensual partner standard for comparison to the unknown profile. If the unknown profile is determined to match the consensual partner standard, additional testing will be performed if applicable.

4. A match is made to the known subject(s):

Testing of additional items may be performed upon request by the District Attorney General.

5. Digital, oral, or penile penetration cases with no semen/saliva detected:

This case may be suitable for Y-STR testing. Y-STR testing may be performed upon receipt of a subject standard and a request from the District Attorney General. Contact the undersigned analyst for additional information.

**NOTE:** All policies are subject to review and individual cases will be considered on a case-by-case basis if questions arise. TBI Management will

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have the ultimate authority to approve casework for DNA analysis on a case-by-case basis. Requests for additional STR testing of items in single subject cases will generally be limited to internal swabs i.e. vaginal, anal, and oral swabs. Scientists should attempt to obtain an NDIS eligible profile for CODIS entry. If an SDIS only profile is obtained, a scientist should obtain approval from the supervisor prior to stoppage of testing when additional items remain.

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### 3. Verification of Tests and Reports

#### Verification of Tests:

Tests which require verification are:

1. All DNA Analyses. The case analyst and a second qualified DNA analyst (technical reviewer) will initial the allele call sheet(s).
2. Any slide which has only one (1) sperm head shall be verified by a qualified scientist.
3. Assuming controls work properly, all other tests can be performed alone without verification.
4. Verification of results requires the second analyst's initials and date.

EXAMPLE: "Verified sperm head, MLM 11-30-00".

#### Verification of Notes and Reports:

All case notes and data sheets will be technically reviewed by another qualified analyst, to assure that there are no discrepancies. The results are accurately reported on the data work sheets and to confirm the results are consistent with the report.

Acceptable report wording is provided in this document, but is not intended to cover all possible scenarios. Analysts may edit/change report wording in order to further clarify results when necessary. The term "indicated" or "presumptive" will be used for test results that are not confirmatory. The indication of blood, human blood, and saliva is based on physical characteristics that are inherent, but not unique, to the body fluid being tested. Except for microscopic identification of spermatozoa, the indication of semen is based on physical characteristics that are inherent, but not unique, to semen. Identification of spermatozoa by microscopic examination is confirmatory for the presence of spermatozoa/semen.

An analyst and/or supervisor will administratively review all final reports. The case analyst will apply a computer-generated signature to all final reports.

In the event the case analyst is unavailable (unable to sign the report), the supervisor may sign the final report for the case analyst.

Prior to issuing a report, the technical reviewer and the case analyst must agree on the interpretation of the data and the conclusions derived from that data. If there is a

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difference of opinion, the casework is to be referred to the Unit/Regional Supervisor and DNA Technical Leader (TL). In the event the disagreement is still unresolved, the matter must be referred to the Assistant Director who may confer with the Quality Assurance Manager (QAM).

Refer to the Division QA Manual for additional information on Administrative and Technical Reviews and Conflict Resolution.

Tests and Controls Routinely Run:

All controls used will be annotated in the case notes in order to confirm the validity of the results. If controls do not work properly, testing should be repeated. Case results will not be reported unless controls work properly.

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**4. Case Controls**

1. Tetramethylbenzidine (TMB) and Phenolphthalin (P)  
Positive control - known blood → TMB-blue/green, P-pink  
Negative reagent control → no color change  
Negative substrate control (if available) → no color change
2. Hematrace  
Positive control - known blood → pink line in “C” and “T”  
Negative reagent control → pink line in “C”, no color in “T”
3. Bluestar  
Positive control - known blood → blue chemiluminescence  
Negative reagent control → no chemiluminescence  
Negative substrate control (if available) → no chemiluminescence
4. Alternate Light Source  
Positive control - known semen swatch → fluorescence  
Negative substrate control → no fluorescence of unstained swatch
5. Acid Phosphatase  
Positive control - known semen → purple color  
Negative reagent control → no color  
Negative substrate control (if available) → no color
6. Prostate Specific Antigen (also known as p30)  
Positive control - known semen → pink line in “C” and “T”  
Negative reagent control → pink line in “C”, no color in “T”
7. Amylase  
Positive control - known saliva → pink line in “C” and “T”  
Negative reagent control → pink line in “C”, no color in “T”
8. STR DNA Analysis  
See STR Protocol

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**5. Case Assignment Policy**

Cases will be assigned on rotational basis by the regional/unit supervisor or designee.

Cases suspected of being serial assaults or linked by subject(s) will be assigned to a single examiner, when possible.

No cases will be assigned to an analyst that will be out of the lab for more than two weeks for medical or maternity/paternity reasons.

Any cases where the original examiner is no longer a member of the Forensic Biology unit, another analyst will be assigned on a rotational basis.

Vehicles submitted for processing should be assigned on a rotational basis with a list kept current by the regional/unit supervisor or designee.

RUSH case requests will only be approved on a case-by-case basis. They can only be approved by TBI management, the regional supervisor, or the supervisor of the unit performing the analysis. These requests should be documented in the case file. RUSH cases should be assigned on a rotational basis with a list kept current by the regional/unit supervisor or designee.

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## 6. Paternity/Familial Testing

For cases needing familial testing (i.e. identifying a stain back to biological relatives) or criminal paternity testing, the TBI Forensic Biology unit may perform DNA testing on the evidence at the discretion of TBI management when a request is made by the District Attorney General. However, the TBI Forensic Biology unit will not perform paternity inclusions or statistical calculations on the resulting profiles. The Official Forensic Biology Report should indicate that the profiles obtained by TBI were or will be forwarded to a private paternity lab for comparison and statistical calculations. Upon receipt of the official report from the vendor lab, the vendor lab report will be added to LIMS. An Official TBI Forensic Biology Report will be generated and will include instructions for the submitting agency on how to access the vendor lab report from i-Results.

Cases needing familial or criminal paternity testing may also be forward to a private paternity lab for both testing and comparison at the discretion of lab management. An aborted fetus or product of conception will be forwarded to a private paternity lab for both testing and comparison.

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### **7. Evidence Handling and Sample Collection**

#### **7.1. Contamination Prevention**

1. A laboratory coat, disposable gloves, and a face mask will be worn during evidence processing and screening.
2. Disposable gloves will be changed between handling different items of evidence and before using a telephone, keyboard, camera, etc.
3. Disposable gloves should be changed anytime the gloves come into contact with a surface that may contain a contaminant e.g. drawer handles, a person's skin/hair/eyes, clothing, etc.
4. Work with only one item of evidence at a time to avoid cross contamination.
5. Clean work area with 10% bleach in between each item of evidence.
6. Use new clean bench paper in between each new item of evidence.
7. Clean scissors, scalpels, and forceps with 10% bleach before collecting a sample. A fresh scalpel blade may also be used to cut each item of evidence.
8. General work areas and fume hoods should be cleaned with 10% bleach as needed.
9. Refer to the Forensic Biology STR Typing Manual for contamination prevention during DNA testing.

#### **7.2. Sample Collection**

1. Liquid blood samples will be handled as outlined in Chapter 1 of this manual.
2. Dry biological stains (e.g. blood, semen, saliva) may be collected by cutting or swabbing based on the surface of the item.



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3. A sterile cotton swab moistened with sterile or distilled water may be used to collect dried stains. The double swab technique of using a moist cotton swab followed by a dry cotton swab can be used for collection of limited samples.
4. Condoms should be swabbed using sterile cotton swabs. Each side of the condom should be swabbed and packaged separately.
5. Touch DNA evidence is evidence with no visible staining but would likely contain DNA resulting from transfer of epithelial cells from the skin to an object. Touch DNA can be collected using the double swab technique.
6. Touch evidence does not include items where saliva might occur or articles of clothing submitted to determine the wearer. Wearer DNA from clothing may be collected by swabbing or cutting the item. A clean scalpel may be used to scrape clothing followed by swabbing of the item. Examples of areas to collect wearer DNA: hat band, inside of gloves, collar of shirt, waistband of pants, etc.
7. Hairs may be collected from an item or left on the item for possible future recovery. If there is a possibility that the hairs may be lost during examination, the hairs should be recovered and packaged. If hairs are left for possible future recovery, the item should be wrapped in the same bench paper on which it was examined and placed in the original packaging.
8. Sample selection will be based on case information and a scientist's experience and training.
9. Written notes, photos, and/or sketches should be made to document the location of sample collection, if applicable.

#### **7.3. Sample Packaging**

1. Wet swabs are air dried prior to final packaging. It is recommended to use swab cartons or paper envelopes/bags to store swabs.
2. Swabs collected from separate sources will be packaged separately.

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3. Paper based packaging is recommended for storage of dry biological samples. Plastic containers may be used for storage, but the item must be completely dry prior to packaging.
4. Label all samples collected with the appropriate lab number, exhibit number, scientist's initials, and description of the sample collected.
5. A petri dish is recommended for collection of hairs or other trace evidence; however other secure packaging may be used as well.
6. Items collected from vehicles or from other large items should be dried, if necessary, and packaged. The package should be labeled with a description, lab number, and initials of the person collecting the sample. The package will be properly sealed and submitted to the evidence receiving unit along with the appropriate request for examination documentation.



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## 8. Blood Casework Analysis

### 8.1. Examination and Testing of Bloodstains

#### 8.1.1. Initial Testing

1. Tetramethylbenzidine and Phenolphthalin (TMB & P) Testing –
  - a. Positive for both TMB & P. Report as “Presumptive chemical testing indicated the presence of blood.”
  - b. If one or both tests are negative, the analyst may stop. Report as “Presumptive chemical testing did not indicate the presence of blood.”
  - c. Positive for both TMB & P but unable to localize stain. Report as “Presumptive chemical testing indicated the presence of blood however, no stain was localized.”
  - d. Inconclusive for TMB & P due to sample interference. Report as “Presumptive chemical testing for the presence of blood was inconclusive due to a component of the sample interfering with the test.”
  - e. If sample size permits, a HemaTrace test should be performed on positive TMB & P stains.
  
2. HemaTrace Testing –
  - a. Positive for TMB & P **and** positive on HemaTrace. Report as “Presumptive chemical testing indicated the presence of blood. Presumptive immunological testing indicated the presence of human hemoglobin, a component of human blood.”
  - b. Positive for TMB & P **but** negative on HemaTrace. Report as “Presumptive chemical testing indicated the presence of blood. Presumptive immunological testing did not indicate the presence of human hemoglobin, a component of human blood.”
  - c. Positive for TMB & P **but** not enough sample for HemaTrace. Report as “Presumptive chemical testing indicated the presence of blood. Presumptive immunological testing for the presence of human hemoglobin, a component of human blood, was not performed due to a limited amount of sample.”



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#### 8.1.2. Additional Testing

1. If sample size permits and a request is received from the District Attorney General or TBI Special Agent in Charge, DNA analysis may be performed.
2. In cases where a subject is not known, reference standards from the victim(s) may be requested prior to DNA testing.

#### Special Policy Note:

1. Use as much sample as is necessary to perform DNA testing procedures, however, the analyst should strive to leave at least half of the sample for re-testing.

#### 8.2. TMB

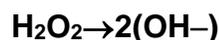
This is used primarily as a screening method for blood and is based on the peroxidase-like activity of hemoglobin. A negative test is conclusive evidence of the absence of blood in quantities sufficient for further examination. Plant peroxidases and/or other substances such as rust or grease can give false positives. However, they can usually be ruled out by careful controls. The test should never be run without positive and negative reagent controls and a negative specimen control when possible. There are multiple references for the reagent make-up. However, the majority of these favor separate solutions of **tetramethylbenzidine** and peroxide.

#### **\*\*Precautions\*\***

TMB is a suspected carcinogen. Gloves should be worn when performing this test.

#### Reaction

Ionic iron forms chelate (ring) structures with many organic compounds and very often such iron-chelates possess catalytic activity in oxidation reactions. An example of such a biological catalyst is peroxidase, which decomposes hydrogen peroxide or organic peroxides to form free hydroxyl radicals:



The heme group of hemoglobin possesses a peroxidase-like activity, which may catalyze this breakdown of hydrogen peroxide. If no other organic oxidizable compound is present, these radicals decompose to form water and oxygen. If



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tetramethylbenzidine is present they will oxidize it to an intense blue-green, tetramethylbenzidine blue.

#### Reagents Needed

TMB: Stock. Doje's 307A- Store refrigerated or at room temperature.

3% Hydrogen peroxide- Store refrigerated or at room temperature.

#### Procedure

1. Gently rub a clean dry filter paper onto a suspect stain.
2. Add 1-2 drops of TMB reagent to the filter paper.
3. After a brief interval to insure that no color change develops, add 1-2 drops of 3% hydrogen peroxide.
4. Read up to 30 seconds for positive results. Do not call positive any color change after 30 seconds.
5. Check controls.

#### Interpretation

The appearance of a blue-green color, appearing immediately to 30 seconds after the addition of hydrogen peroxide, is a positive test. If no color change or production of color after the 30-second interval, this is a negative test.

#### Trouble Shooting

##### 1. False Positives:

- If a color develops before the addition of peroxide this may be due to chemical oxidants.
- The most likely materials to give a positive result other than blood are plant peroxidases. Plant materials usually produce white or green stains and will show cellular debris if a slide is made. The plant peroxidase is rapidly inactivated at high temperature and with time.

##### 2. False Negatives

- If a stain appears washed or weak, it might not give a positive reaction when tested with a dry filter paper. Therefore, repeat the test using damp paper.
- Or, carry out the test as above, directly on a portion of the stain by cutting a small portion of the stained material.

### 8.3. P or Kastle-Meyer Reaction

The ***phenolphthalin*** test is a presumptive test for blood and is therefore used as a screening method. The Phenolphthalin test is conclusive evidence of the



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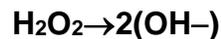
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absence of blood in quantities sufficient for further examination. Plant peroxidases and/or other substances such as rust or grease can give false positives. However, these substances can usually be ruled out by careful controls. The test should never be run without positive and negative reagent controls and a negative specimen control when possible. There are multiple references for the reagent make-up. However, the majority of these favor separate solutions of phenolphthalin, alcohol, and hydrogen peroxide.

#### Reaction

Ionic iron forms chelate (ring) structures with many organic compounds and very often such iron-chelates possess catalytic activity in oxidation reactions. An example of such a biological catalyst is peroxidase which decomposes hydrogen peroxide or organic peroxides to form free hydroxyl radicals.



The heme group of hemoglobin possesses a peroxidase-like activity which may catalyze this breakdown of hydrogen peroxide. If no other organic, oxidizable compound is present, these radicals decompose to form water and oxygen. If phenolphthalin is present, they will oxidize it to a bright pink color.

Prior to its use in the test, **phenolphthalein** must be reduced to phenolphthalin. It is the reduced form which is then used to test for the peroxidase-like activity of hemoglobin. In a positive test, the formation of a pink color indicates the oxidation back to phenolphthalein.

#### Reagents Needed

1. Phenolphthalin: Stock. Doje's 309 (request Army formulation) - Store refrigerated or at room temperature.
2. Ethyl alcohol (Reagent grade) - Store at room temperature.
3. 3% Hydrogen peroxide- Store refrigerated or at room temperature.

#### Procedure

1. Gently rub a clean, dry filter paper onto a suspect stain.
2. Add 1-2 drops of phenolphthalin reagent to the filter paper.
3. After a brief interval to insure that no color change develops, add 1-2 drops of ethyl alcohol.
4. After a brief interval to insure that no color change develops, add 1-2 drops of 3% hydrogen peroxide.
5. Read up to 30 seconds for positive results. Do not call positive any color change after 30 seconds.
6. Check controls.



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#### Interpretation

The appearance of a pink color, immediately to 30 seconds after the addition of hydrogen peroxide, is a positive test. No color change, or production of color after the 30-second interval, is a negative test.

#### Trouble Shooting

##### 1. False positives

- If a color develops before the addition of alcohol, this may be due to partial oxidation of the phenolphthalin reagent due to improper storage.
- If a color develops before the addition of peroxide, this may be due to the presence of chemical oxidants.

##### 2. False Negatives

- If a stain appears washed or weak, it might not give a positive reaction when tested with a dry filter paper. Therefore, repeat the test using damp paper.
- Or, carry out the test as above, directly on a portion of the stain by cutting a small portion of the stained material.

#### **8.4. Bluestar**

BLUESTAR® is a presumptive test designed to reveal fresh, dried or cleaned blood, neat or diluted, in trace or sizeable amounts. This test operates by chemiluminescence, when BLUESTAR® FORENSIC latent bloodstain reagent comes into contact with the iron contained in the heme nucleus of hemoglobin found in blood, it is catalyzed by peroxidase activity and emits a light blue glow. It is of most use at crime scenes which have been suspected of being cleaned to hide or destroy evidence.

#### Principle:

In this test procedure, a suspected bloodstain is lightly misted with a hand-held fine-spray bottle containing BLUESTAR® FORENSIC latent bloodstain reagent. If the iron found in heme (a constituent of hemoglobin found in blood) is present, the BLUESTAR® FORENSIC latent bloodstain reagent is catalyzed by its peroxidase activity. The chemical luminol, a component of BLUESTAR®, is oxidized by hydrogen peroxide, to produce a molecule in an excited state. When the excited electrons in the molecules fall back to their ground states, light is given out. Therefore, if blood is present, an intense light-blue glow will appear when misted with BLUESTAR® FORENSIC latent bloodstain reagent.

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#### Reagents and Materials

1. BLUESTAR® FORENSIC tablets, (Product #BL-FOR-TAB8).
2. Distilled water.
3. Spray bottle (mister).
4. Known blood control.

#### Preparation of Reagent

1. Open the spray bottle and add 125ml of distilled water.
2. Take a white tablet from the white-top tube and close the tube immediately.
3. Take a beige tablet from the orange-top tube and close the tube immediately. Do not switch the caps of the tubes.
4. Add the pair of tablets to the distilled water.
5. Twist the head with its plunger onto the spray bottle firmly.
6. Allow 1 or 2 minutes for the complete dissolution and mixing of the chemicals, swirling gently with your hand in a circular motion.  
\*Note: Do not shake the container upside down.
7. Check the reagent with a positive and negative control. A known blood sample can be used as the positive control.
8. The reagent works best within 3 hours of mixing the tablets in water. The reagent can be used past 3 hours if checked against controls.

#### Method

1. Create optimum lighting conditions:
  - Indoors: Close all the windows and block out outside light sources. Turn off all lights.
  - Outdoors: Wait for night time and turn off all area lights. Screen off distant light sources and work facing away from problematic lights.
2. Spray lightly, horizontally ahead, at least 2 feet away from the target in a side to side sweeping motion.  
\*Note: Do not saturate vertical surfaces; be aware of wind direction.

#### Interpretation

1. Positive. If an intense light-blue chemiluminescence is emitted, the result is positive and indicates the possibility of the presence of blood. A positive Bluestar reaction should be followed by TMB & P testing before reporting an indication of blood. If both Bluestar and TMB & P are positive, report as "Presumptive chemical testing indicated the presence of blood." If Bluestar is positive and TMB & P is negative, report as "Presumptive chemical testing did not indicate the presence of blood."
2. Negative. If there is no chemiluminescence present, the result is negative and indicates an absence of blood. Report as "Presumptive chemical testing did not indicate the presence of blood."



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- Note: "False" reactions can be seen with various materials including cleaners and bleach.

#### NOTE:

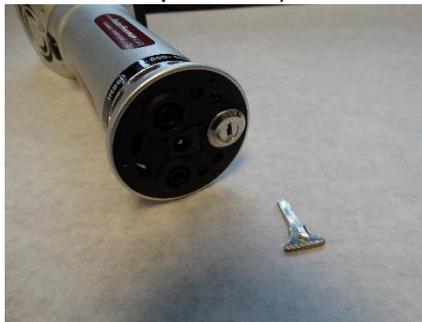
- Photographs of Bluestar-positive areas can be obtained using the *Krimesite Imager* in conjunction with the Bluestar procedure. Using the *Krimesite Imager* and the "blood filter", observe the treated area for increased fluorescence. Photograph as needed.
- Bluestar testing is not a standard procedure used in the lab. It is felt that this test has very limited value. It is left to the individual examiner as to when or if it is used.

#### **8.5. Crime-lite 82S Infrared Alternate Light Source (82S-IR) and Software**

The Crime-lite 82S-IR uses infrared light to aid in the detection of blood on dark fabrics and material. Infrared (IR) light is a non-destructive screening method. Blood absorbs infrared light while many dark fabrics and materials will reflect the light. This makes infrared light useful for screening dark fabrics and materials for the presence of blood.

#### **Operation of the Crime-lite 82S-IR**

- Insert the operating key into the base of the 82S-IR device and turn one-quarter turn until a click is heard and the key is locked in place so that it cannot be pulled out. (NOTE: The device will NOT OPERATE if this key is not in place and locked into position.)



- Attach the battery adapter to the base of the device and then connect to a battery source. Alternately the 82S-IR can be attached to the provided power supply and used with any electrical outlet.

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#### a. Battery Use:



#### b. Unit connected to electrical power supply.



3. Attach the camera to the head of the 82S-IR by sliding the rectangular portion of the camera base into the slits on either side of the LED head of the 82S-IR. The lens portion of the camera should be resting just above the LED head of the 82S-IR and can be tilted towards or away from the light source as needed.



4. Use the mini-USB cord attachment to attach the camera to either a tablet or computer using the Crime-lite Cam software.

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5. Rotate the dial on the side of the camera to the "IIII" position for "IR" as listed on the tag. Make sure the "IIII" portion of the dial appears under this tag. Remove lens cap to camera if attached.



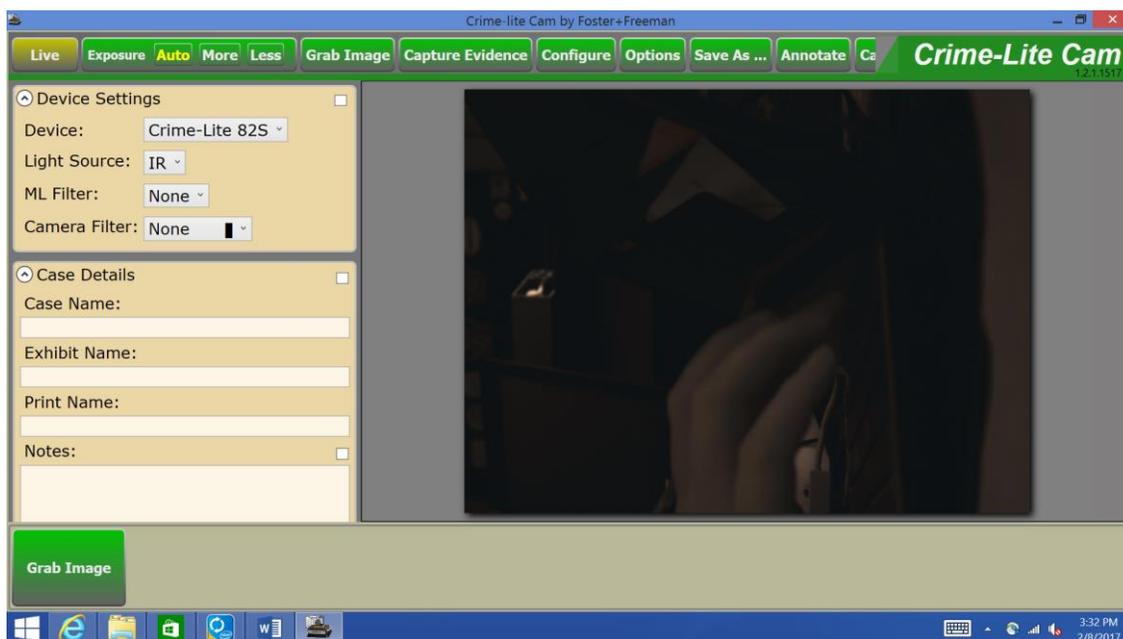
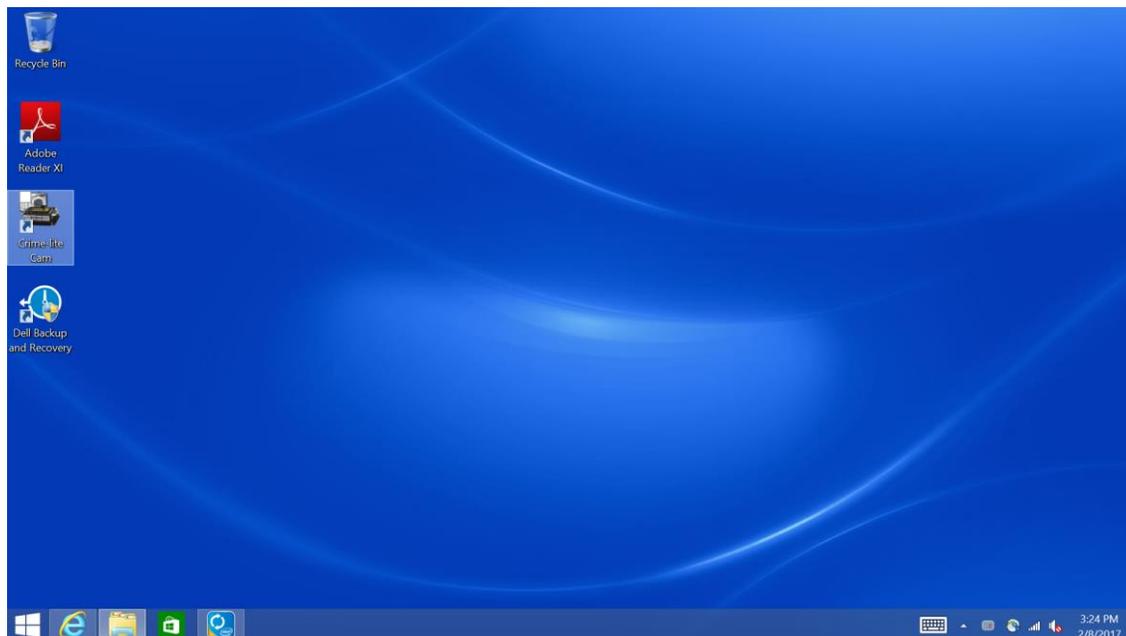
6. Open up the Crime-lite Cam software. Under Device Settings on the left make sure the following selections are made:
  - a. Device: Crime-Lite 82S
  - b. Light Source: IR
  - c. ML Filter: None
  - d. Camera Filter: None

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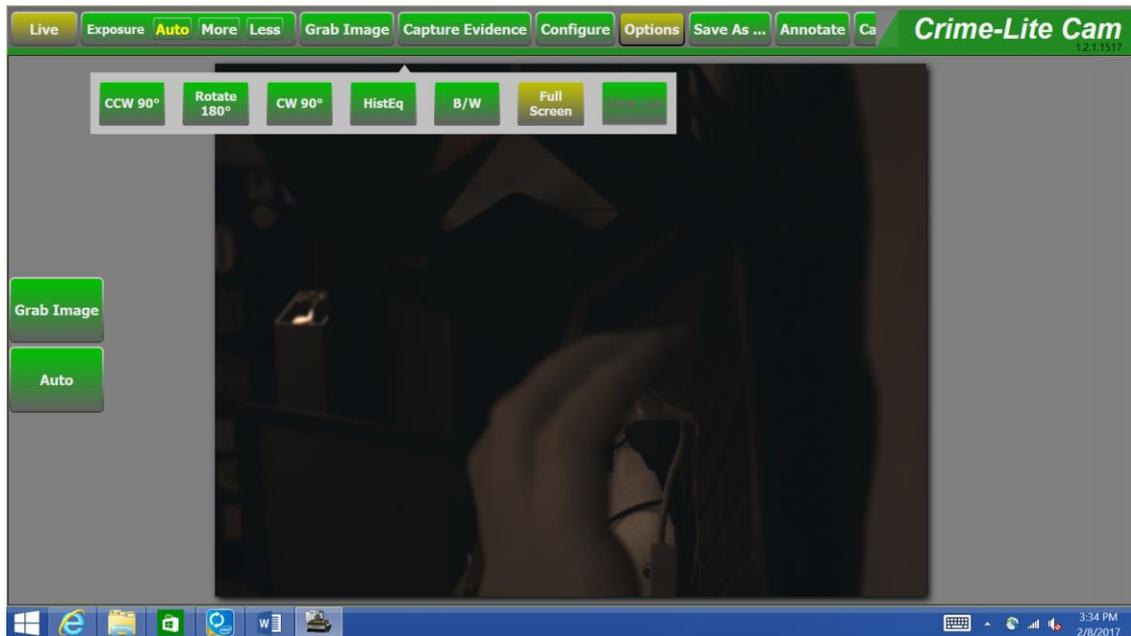
7. In the top left corner, select "Live" and then under Options choose Full Screen. When menu options are selected they turn from green to a gold color.

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8. Turn on the 82S-IR device and wait a few moments until the camera intensity adjusts. There are two LED indicators on the left side of the device. When the device is turned on the lower LED will flash red briefly then the upper LED will turn on a solid yellow. The fan motor in the back of the 82S-IR head will also be running.
9. In “Full Screen” mode there will be an “Auto” selection on the left side of the screen. Select Auto to automatically focus the camera. To test if the 82S-IR is functioning properly point the 82S-IR towards the black control fabric and make sure the fabric reflects the light and turns a white or light grayish color. If the fabric appears dark on the screen in live view then repeat the steps to make sure the device is properly turned on. Note: You MUST utilize the LED indicators and live video feed in determining if the device is on as it does NOT emit any visible light.
10. Scan the area or object in question by slowly moving the device over the area in a search pattern. There may be a delay in response time depending on the computer or tablet being utilized so this should be done at a speed to allow the camera to adjust and focus on the area before moving on to a new area. If the light is too intense or not intense enough choose the “Auto” option on the “Exposure” tab at the top of the screen and wait for the camera to adjust.

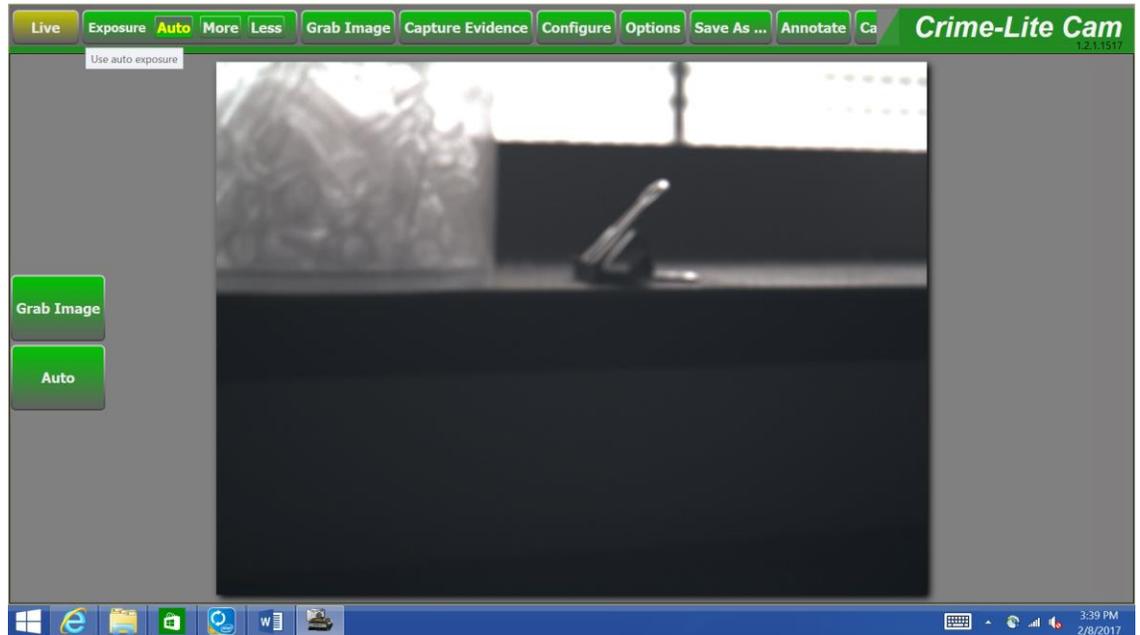
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The camera itself can be tilted towards or away from the IR LED head also to adjust the intensity against a surface.



#### **Interpretation:**

The Crime-Lite 82S will only be used to screen items for the presence of blood. Positive areas from the Crime-Lite 82S will be tested using Tetramethylbenzidine/Phenolphthalein (TMB/P) prior to reporting presumptive blood test results. If the item being screened is negative for staining with the Crime-Lite 82S or all of the stained areas are negative with TMB/P testing, the remaining areas of the item must be screened with TMB/P prior to reporting presumptive blood results. Bluestar may also be used to aid in screening an item that has screened negative using the Crime-Lite 82S.

#### **Controls:**

Positive Control: Blood- stain absorbs light and appears dark

Negative Substrate Control: No absorption/dark stains on an unstained swatch

#### **Reporting:**

Screening with the Crime-Lite 82S must be followed by Bluestar and/or TMB/P; therefore, reporting will be based on the results of the Bluestar and/or TMB/P testing.

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## 8.6. Species Identification

### 8.6.1. Hematrace

The **OneStep ABACard Hematrace** test is designed to detect human hemoglobin, a constituent of blood. The test works by diffusion of antigens through a nylon membrane towards an antibody-dye conjugate.

#### A. Reaction

In this test procedure, approximately 150µl of sample is added to the sample well ("S") and allowed to soak in. If human hemoglobin is present in the specimen, it will react with the mobile monoclonal antihuman hemoglobin antibody and a mobile antigen-antibody complex is formed. This complex migrates through the absorbent device towards the test area ("T"). In "T", a monoclonal antihuman hemoglobin antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. When the human hemoglobin concentration in the sample exceeds 0.05µg/ml, a pink colored band in "T" will form indicating a positive test result. As an internal positive control, human hemoglobin antibody-dye conjugates cannot bind to the antibody in "T", but are captured by an immobilized anti-immunoglobulin antibody present in the control area ("C") forming a complex. The captured pink dye particles will thus form a band in "C", indicating that the test has worked properly.

#### B. Reagents Needed

- (1). Test device+extraction buffer tube (Abacus Diagnostics, catalog #708424).
- (2). Known blood control.

#### C. Procedure

- (1). Cut a portion of the questioned stain or swab and/or control blood sample.
- (2). Place in appropriately labeled kit extraction tubes containing extraction buffer.
- (3). Remove the test device and dropper from the sealed pouch and label appropriately.
- (4). Mix the extract thoroughly by vortexing or pipetting several times in extraction tube.



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- (5). Being careful to avoid the sample cutting, remove approximately 150 $\mu$ l (three to four drops) of sample extract and add to the “S” well of the test device.

D. Interpretation

- (1). Positive. If there are two pink lines, one each in “T” and in “C”, the test result is positive and indicates that human hemoglobin is present at or above 0.05 $\mu$ g/ml. Positive results can be seen as early as one minute, depending on the hemoglobin concentration.
- (2). Negative. If there is only one pink line in “C”, the test result is negative. This may indicate that (a) no human hemoglobin is present above 0.05  $\mu$ g/ml or (b) presence of “High Dose Hook Effect”. Presence of “High Dose Hook Effect” may give a false negative result due to the presence of a high concentration of human hemoglobin in the sample, as for example in undiluted blood or heavily concentrated bloodstain. In such cases the sample may be retested using a 10 to 10,000 fold dilution. For negative results, one must observe the test device for a full 10 minutes.
- (3). Invalid. If there is no pink line visible in “C”, the test is inconclusive. Repeat the test and re-examine the test procedure carefully.

NOTES:

*Following the procedure above, a new lot of the Hematrace cards shall be tested with a positive and negative control prior to use.*

Ferret and higher primates will cross-react with the Hematrace test. Due to the extreme sensitivity of the test, trace levels of hemoglobin may be detected in body fluid samples other than blood (e.g. urine, semen, stool, saliva, vaginal fluid, perspiration).

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**9. Sexual Assault Casework Analysis**

**9.1. Examination and Testing for Semen**

**9.1.1. Testing Sexual Assault Kit Swabs**

- 1) Stain and search slide (slide included or analyst-prepared slide). For slides prepared in-house, swabs may be tested for acid phosphatase (AP) prior to slide preparation in order to determine the best swab or area of a swab to cut for a slide search. If no swabs are submitted with a slide, collect the material from the submitted slide using a sterile swab moistened with DI water prior to staining in order to preserve the biological material for further testing.
  - (a) Positive for spermatozoa – Prepare swabs for differential extraction. Report as “Microscopic examination confirmed the presence of spermatozoa.” When spermatozoa are confirmed, the results of presumptive chemical and/or immunological testing are not required to be reported.
  - (b) Negative for spermatozoa - AP test the swabs if the swabs were not previously tested for AP. If the AP test is negative and the microscopic examination is negative for spermatozoa, report as “Presumptive chemical testing did not indicate the presence of semen and no spermatozoa were observed with microscopic examination.” If the AP test is positive and the microscopic examination is negative for spermatozoa, proceed to p30 testing.
  - (c) Positive PSA (p30) test on swabs- Prepare swabs for differential extraction. Report as “Presumptive immunological testing indicated the presence of semen; however, no spermatozoa were observed with microscopic examination.” The results of presumptive chemical testing for semen are not required to be reported if immunological testing is performed.
  - (d) Negative PSA (p30) test on swabs–Test additional evidence if available. Report as “Presumptive immunological testing did not indicate the presence of semen and no spermatozoa were observed with microscopic examination.” The results of presumptive chemical testing for semen are not required to be reported if immunological testing is performed.



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- (e) If Buccal swabs were submitted as a reference standard and the case facts indicate that an oral assault may have taken place with no more than 24 hours between the assault and collection of swabs, the analyst may perform semen testing on these swabs if 1) the Oral swabs were negative for semen or 2) no Oral swabs were collected for this purpose. Buccal swabs may be used as a reference standard for DNA testing prior to serology screening, but the analyst should conserve sample if additional screening may be necessary.

#### **9.1.2. Testing Additional Evidence**

- 1) Alternate light source (ALS) - ALS is considered a presumptive visual examination.
  - a) Positive ALS– Proceed to AP testing. If no AP testing is performed, report as “Staining was observed with alternate light source screening. No further testing was performed.”
  - b) Negative ALS – No further testing. Report as “Semen staining was not observed with alternate light source screening.”
- 2) AP Testing
  - a) Positive AP – Prepare a slide for a sperm search
    - i) Positive for spermatozoa – Prepare evidence for differential extraction. Report as “Microscopic examination confirmed the presence of spermatozoa.” When spermatozoa are confirmed, the results of ALS and other presumptive chemical and/or immunological testing for semen are not required to be reported.
    - ii) Negative for spermatozoa – Perform PSA (p30) testing on the item of evidence
      - (1) Positive p30 – Prepare evidence for differential extraction. Report as “Presumptive immunological testing indicated the presence of semen; however, no spermatozoa were observed with microscopic examination.”



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- (2) Negative p30- Test additional evidence if available. Report as “Presumptive immunological testing did not indicate the presence of semen and no spermatozoa were observed with microscopic examination.”
- (3) The results of presumptive chemical and/or visual testing for semen are not required to be reported if immunological testing is performed.

b) Negative AP – No further testing. Report as “Presumptive chemical testing did not indicate the presence of semen.”

## 9.2. Acid Phosphatase (AP)

### 9.2.1. AP Overspray Reagents

#### Solution A:

|                         |       |
|-------------------------|-------|
| Brentamine fast blue B  | 1gm   |
| Hydrated sodium acetate | 20gm  |
| Glacial acetic acid     | 10ml  |
| Distilled water         | 100ml |

Mix and store in a brown bottle in the refrigerator at 5° C (lasts about 6 weeks).

#### Solution B:

|                               |       |
|-------------------------------|-------|
| $\alpha$ -Naphthyl phosphate* | 0.8gm |
| Distilled Water               | 10ml  |

Mix and store in a brown bottle in the refrigerator at 5° C (lasts about 6 weeks).

\* $\alpha$ -Naphthyl phosphate is a carcinogen. Gloves must be worn when performing this test.

#### Working Solution (100ml):

|  |      |
|--|------|
| Dye (Solution A)                       | 10ml |
| Substrate (Solution B)                 | 1ml  |
| Dilute to 100 ml with distilled water. |      |

Store in spray bottle in the refrigerator (lasts about one week).

#### Procedure:



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Dampen filter paper with de-ionized water. Wearing gloves, press paper on questioned area. Hang filter paper in fume hood, spray with working solution, and monitor for two minutes.

#### Controls:

A positive semen control and a negative control must be used with each test.

#### Reaction:

Acid phosphatase is a color test consisting of adding chemicals [ $\alpha$ -naphthyl phosphate and naphthanil diazo blue (diazodiorthanisidine)] to the questioned stain. If acid phosphatase is present, a purple color develops. It is a two-step process.

1.  $\alpha$ -naphthyl phosphate  $\rightarrow$  **acid phosphatase**  $\rightarrow$   $\alpha$ -naphthol
2.  $\alpha$ -naphthol couples with diazonium salt to form an azo dye.

#### 9.2.2. AP Dropper Reagents

##### Buffer:

|                           |       |
|---------------------------|-------|
| Sodium acetate trihydrate | 2gm   |
| Glacial acetic acid       | 0.5ml |
| Distilled water           | 100ml |

##### Solution A:

|                                      |                |
|--------------------------------------|----------------|
| Buffer                               | 50ml           |
| Sodium $\alpha$ -Naphthyl phosphate* | 25mg (0.025gm) |

Mix and put in dropper bottle. Store refrigerated (approximate shelf life - one month).

\* $\alpha$ -Naphthyl phosphate is a carcinogen. Gloves must be worn when performing this test.

##### Solution B:

|                          |                |
|--------------------------|----------------|
| Buffer                   | 50ml           |
| Diazo blue (Fast blue B) | 14mg (0.014gm) |

Mix and put in dropper bottle. Store refrigerated at 5° C (approximate shelf life - one month).

#### Procedure:

Cut out questioned sample and place into a depression plate well. The extract from a sperm search can also be used. Add one drop of Solution



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A and one drop of Solution B directly to the sample. A color reaction of deep purple indicates a positive result.

#### Controls:

A positive semen control and a negative control must be used with each test. If controls do not work properly, fresh solutions should be made immediately.

#### Reaction:

Acid phosphatase is a color test consisting of adding chemicals [ $\alpha$ -naphthyl phosphate and naphthanil diazo blue (diazodiorphanisidine)] to the questioned stain. If acid phosphatase is present, a purple color develops. It is a two-step process.

1.  $\alpha$ -naphthyl phosphate  $\rightarrow$  **acid phosphatase**  $\rightarrow$   $\alpha$ -naphthol
2.  $\alpha$ -naphthol couples with diazonium salt to form an azo dye.

### 9.3. Christmas Tree Stains for Slide Searching

#### ***Kernechtrot (Red)***

Stock: Seri, catalog #R540 or Poly Scientific (Fisher catalog #R5463200500)

#### ***Picroindigocarmine (Green)***

Stock: Seri, catalog #R540 or Poly Scientific, catalog #NC9832737

*New lots of the kernechtrot and/or picroindigocarmine stains shall be tested prior to use. This may be accomplished by extracting a portion of a known semen sample and following Procedure #1 for slide preparation. The results will be documented in the QC Log notebook. A successful result for the kernechtrot stain is indicated by nuclear material staining red. A successful result for the picroindigocarmine stain is indicated by cytoplasmic material staining green.*

#### Procedure #1:

1. Place cutting in tube, add 2-3 drops of de-ionized water or p30 extraction buffer and allow to extract for at least 30 minutes. The p30 extraction buffer may be used for slide preparation if the cutting will be used later for p30 testing.
2. Add 1-2 drops of extract to slide and place on slide warmer.
3. Prepare slide to stain by heat fixing (flame).
4. Add the number of drops of red stain necessary to cover stain, using caution not to touch dropper to smear.
5. Allow to stain for a minimum of 10 minutes.
6. Wash stain off with a gentle stream of water.



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7. Rinse slide again using reagent alcohol to quickly dry slide with either natural air-drying, slide warmer or flame.
8. Add the number of drops of green stain necessary to cover stain, using caution not to touch dropper to smear.
9. Allow to stain 15 seconds.
10. Wash stain off with a gentle stream of reagent alcohol.
11. Allow to dry with either natural air-drying, slide warmer, or flame.
12. Add a few drops of mounting media (Cytoseal) to slide and apply cover slip.
13. The slide is ready for viewing under the microscope.

#### Procedure #2:

1. Place 1-2 drops of de-ionized water or p30 extraction buffer on slide surface, add cutting of questioned sample, vigorously smear cutting with tweezers or scissors, and place on slide warmer. The p30 extraction buffer may be used for slide preparation if the cutting will be used for p30 testing.
2. Place cutting in tube and add 4-5 drops of p30 extraction buffer or DI water for possible p30 testing.
3. Follow steps 3-13 above.



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#### 9.4. PSA/p30 Immunological Testing

The OneStep ABACard PSA (prostate-specific antigen, also known as p30) test is designed to qualitatively detect p30 for forensic identification of semen. The test works by diffusion of antigens through a nylon membrane towards an antibody-dye conjugate. P30 is an accepted marker for detecting semen in criminal cases, including vasectomized or azoospermic individuals.

##### Reaction

In this test procedure, approximately 200 $\mu$ l of sample is added to the sample well ("S") and allowed to soak in. If p30 is present in the specimen, it will react with the mobile monoclonal antihuman p30 antibody and a mobile antigen-antibody complex is formed. This complex migrates through the absorbent device towards the test area ("T"). In "T", a monoclonal antihuman p30 antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. When the p30 concentration in the sample exceeds 4ng/ml, a pink colored band in "T" will form indicating a positive test result. As an internal positive control, p30 antibody-dye conjugates cannot bind to the antibody in "T", but are captured by an immobilized anti-immunoglobulin antibody present in the control area ("C") forming a complex. The captured pink dye particles will thus form a band in "C", indicating that the test has worked properly.

##### Reagents Needed

1. Test device (Abacus Diagnostics, catalog #308332).
2. Distilled water.
3. Known semen control.

##### Procedure

1. Cut a portion of the questioned stain or swab and/or control semen sample.
2. Place in tube and add 2-3 drops of p30 extraction buffer or DI water.
3. Allow cutting to extract at room temperature for at least 30 minutes.
4. Add 1-2 drops of extract to slide, place on slide warmer, and prepare slide to stain by heat fixing (flaming is optional fixing method). See slide staining procedures.
5. Add 4-5 drops of p30 extraction buffer or DI water to the cutting and extract an additional 30 minutes.
6. Remove the test device and dropper from the sealed pouch and label appropriately.
7. Mix the extract thoroughly by pipetting several times in tube.



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8. Being careful to avoid the sample cutting, remove approximately 200 $\mu$ l (four to five drops) of sample extract and add to the "S" well of the test device.

#### Interpretation

1. Positive. If there are two pink lines, one each in "T" and in "C", the test result is positive and indicates that p30 is present at or above 4ng/ml. Positive results can be seen as early as one minute, depending on the p30 concentration.
2. Negative. If there is only one pink line in "C", the test result is negative. This may indicate that (a) no p30 is present above 4ng/ml or (b) presence of "High Dose Hook Effect". Presence of "High Dose Hook Effect" may give a false negative result due to the presence of a high concentration of p30 in the sample, as for example in undiluted seminal fluid. In such cases the sample may be retested using a 10 to 10,000 fold dilution. For negative results, one must observe the test device for a full 10 minutes.
3. Invalid. If there is no pink line visible in "C", the test is inconclusive. Repeat the test and re-examine the test procedure carefully.

#### **NOTES:**

*Following the procedure above, a new lot of the Abacus p30 cards or extraction buffer shall be tested with a positive and negative control prior to use.*

PSA (p30) testing is considered a presumptive test. Rhesus monkey semen will cross-react with p30, the only animal found to do so.

#### **9.5. Alternate Light Source (ALS)**

This is a high-intensity light source that is mainly used for the location of semen stains. Positive results are not conclusive for the presence of semen, as other body fluids, such as saliva and urine can also fluoresce. The assay works best when conducted in a dark room. Instruments such as the **Polilight** or **Mini-CrimeScope 400**, are set from a wavelength of 445-455 nanometers. Spots that fluoresce are circled for further testing.

#### Notes and Precautions:

1. A caution sign should be placed on the outside of the dark room.
2. The light should not be pointed directly into anyone's eyes or on highly reflective objects.
3. Orange UV filter goggles must be worn by everyone in the room.



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4. Extremely dark fabrics, or those which absorb light in the 450nm range, may have to be AP-tested, as there may be minimal fluorescence noted.
5. The ALS may be used to aide in locating possible body fluid staining.
6. Controls
  - a. Positive - known semen swatch fluoresces.
  - b. Negative - no fluorescence of unstained swatch areas.
7. Reporting
  - a. Negative ALS- Report as "Semen staining was not observed with alternate light source screening." This may also be reported as "No fluorescence was observed with alternate light source screening." when screening for body fluids other than semen.
  - b. Positive ALS- If no other testing performed, report as "Staining was observed with alternate light source screening. No further testing was performed." If further testing is performed, the results will be reported as to the body fluid testing conducted.

#### 9.6. Rapid Stain Identification Series (RSID™ - Saliva) saliva test

Detection of  $\alpha$ -amylase using the Rapid Stain Identification Series (RSID™ - Saliva) saliva test strips.

The RSID™ - Saliva test is an easy to use and accurate test that utilizes two anti-salivary amylase monoclonal antibodies in a lateral flow immuno-chromatic test strip to detect the presence of  $\alpha$ -amylase proteins. The Universal Buffer provided in the kits is STR free and contains a DNA stabilizer so the remaining extract can be used in DNA analysis.

This test is specific for humans and higher primates. It has been tested against a wide variety of animals with no reaction observed. No high-dose hook effect has been observed either. Furthermore, RSID™ - Saliva is very sensitive, with a reported value of greater than a 1:10,000 dilution factor.

#### Principle

This test is a lateral flow immuno-chromatic strip test that uses two mouse monoclonal antibodies specific for human salivary  $\alpha$ -amylase. One of the antibodies is conjugated to the pad beneath the sample window. The second antibody is located at the test line "T" on the membrane. The area of the membrane containing the control line "C" is bound with the anti-mouse IgG antibody to serve as a positive control. As sample is introduced at the sample well it diffuses through the conjugate area releasing the antibodies. Both the sample and antibodies then travel through the membrane to the test area "T". If



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$\alpha$ -amylase is present in the sample, it will be captured by the immobilized anti- $\alpha$ -amylase antibodies at the test area forming a pink line. The sample and conjugate antibodies then continue moving through the membrane where the anti-mouse IgG on the control line will capture any mouse antibodies flowing past the "T" area forming a pink line and indicating that all components within the strip test are performing correctly. If no  $\alpha$ -amylase is present, the sample and conjugate antibodies move past the test line "T", forming no pink line, then continue on to the control line "C" forming a pink line.

#### Reagents and Materials

1. RSID™ - Saliva test strips with Universal Buffer, (Product #0130).
2. Clean dropper or pipettor.
3. Nuisance mask
4. Neat saliva sample (positive control)

#### Preparation of Reagent

1. The cartridges should be stored at room temperature.
2. The supplied Universal Extraction Buffer must be stored at 2-8°C.
3. Each box is provided with an expiration date, after which all remaining tests should be discarded.
4. Positive and negative controls must be performed for each lot of test strips and universal buffer prior to use.

#### Method

1. Cut a portion of the evidence to be tested ( $\approx$ 3mm x 3mm) and place in 1.5ml tube.
2. Add approximately 200-400 $\mu$ l of supplied Universal Buffer to a sample.
3. Incubate the sample at room temperature for an hour.
4. Add 3 drops (approximately 100-200 $\mu$ l) of extract to the sample well of the test strip.
5. Read the test strip within 10 minutes.

#### Results

1. Positive: If the control line and test line both appear within 10 minutes, the result is positive for  $\alpha$ -amylase and indicates the possibility of the presence of saliva.

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2. Negative: If the control line appears and the test line does not within a maximum of 10 minutes, the result is negative and indicates the absence of saliva.
3. Inconclusive: If only the test line appears or neither line appears, the result is inconclusive and must be repeated.

Reporting

Negative: "Presumptive immunological testing did not indicate the presence of alpha-amylase which is a component of saliva."

Positive: "Presumptive immunological testing indicated the presence of alpha-amylase, which is a component of saliva."

**NOTES:**

1. Urine and feces are also known to contain alpha-amylase. An addendum sentence may also be added to the Positive reporting statement when applicable (i.e. labial swabs, underwear, etc.): "Alpha-amylase is also a component of urine and feces".
2. The universal buffer does not interfere with DNA testing.



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## 10. DNA Analysis Policies

### 10.1. DNA Analysis

#### Sexual Assault Cases:

1. Negative for SEMEN → no STR DNA analysis, but Y STR DNA analysis may be offered or performed for areas of penetration (e.g. vaginal and anal swabs) if all items screen negative and/or no probative STR profile is obtained.
2. Positive for SPERM → DNA analysis
3. Negative for SPERM but Positive for SEMEN → DNA analysis

#### Blood Cases:

1. Negative for BLOOD → no DNA analysis
2. Negative for HUMAN BLOOD → no DNA analysis, except at analyst discretion
3. Positive for HUMAN BLOOD → DNA analysis:
  - a. Presumptive chemical testing indicated the presence of blood. Presumptive immunological testing indicated the presence of human hemoglobin, a component of human blood → DNA analysis
  - b. Presumptive chemical testing indicated the presence of blood. Presumptive immunological testing for the presence of human hemoglobin, a component of human blood, was not performed due to a limited amount of sample. → DNA analysis

#### Epithelial Cell Cases:

Items with epithelial cell DNA may have DNA analysis performed on them without preliminary serological tests.

Examples: cigarette butts, hats, envelopes, fingernail clippings/scrapings, or other item(s) which may be a potential source of epithelial cells.

Cases with KNOWN subjects may not be profiled without subject and victim standards and a prior request from the District Attorney General, a TBI Special Agent in Charge, a Court Order, or an Agreed Order.

#### Y-STR Testing:

Y-STR testing will generally apply to sexual assault cases, but may be considered for other violent crime cases in which an overabundance of female DNA prohibits the detection of an interpretable male profile. Y-STR will generally



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not be considered for property crimes and other non-violent offenses. Y-STR testing will generally not be performed without a subject standard.

#### 10.2. Post Conviction

Post-Conviction DNA Testing will only be performed upon receipt of a Court Order or an Agreed Order. As per TBI Forensic Biology Unit policy, the standards for comparison listed below may be required prior to DNA testing. *It is preferred that both victim and subject standards be submitted prior to DNA Analysis.* Without these standards, final results may not be interpretable and/or statistical assessment may not be possible:

1. Subject(s) standard(s)
2. Victim(s) standard(s)
3. Other elimination standard(s)
4. Biological parental standards.

#### 10.3. Touch DNA Evidence

This type of DNA evidence is defined as evidence with no visible staining and *would likely* contain DNA resulting from transfer of epithelial cells from the skin to an object. Touch evidence does not include items where saliva might occur or articles of clothing submitted to determine the wearer. Touch DNA evidence will not be processed for non-violent cases. If touch DNA evidence is submitted for a non-violent case that will not be processed, the report should state the following:

“The TBI Crime Laboratory does not perform touch DNA analysis on items associated with a non-violent crime. If there is a violent component to this case that was not indicated on the request for examination form, please contact the crime lab to discuss the possibility of DNA testing.”

Touch DNA evidence will be accepted for possible STR DNA analysis in violent cases when there is a high degree of likelihood that the evidence will provide interpretable results or investigative leads. A high degree of likelihood may be established by means of witness corroboration, visual monitoring system, or sound deductive reasoning. Due to the nature and limitations of touch DNA evidence and the possible contamination from DNA obtained from other sources, the following will be considered before items are tested for epithelial touch DNA analysis:

- Items submitted must have been used in the commission of a crime.
- No other probative evidence is available.

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- Known suspects and victims must be submitted for comparison. (Elimination standards including law enforcement personnel when appropriate).
- Items submitted must have the likelihood of being handled by one person.
- Items submitted must have the likelihood of epithelial cell transfer such as rough or uneven surfaces. Casually touched items excluded.
- Items have not been previously handled/examined by a TBI/Police/private crime laboratory.
- Items have not been processed for latent prints.
- The entire item (not swabbings), when possible, should be submitted for touch DNA analysis. Items such as guns should not be swabbed.
- Swabbings of items, such as the exterior of cars, dwellings, businesses, will not be worked unless there is a high degree of likelihood of association of the suspect and the evidence corroborated by witness or visual monitoring system.
- Swabbings from public access areas will not be worked such as public telephones, doors, office objects, and counters, unless there is a high degree of likelihood of association of the suspect and the evidence corroborated by witness or visual monitoring system.
- Cartridge casings will only be analyzed in homicide cases where there is no other probative evidence.
- DNA profiles obtained and eligible to be entered into CODIS must have appropriate elimination standards submitted, including law enforcement personnel when appropriate.
- Touch DNA analysis will not be performed to establish felony possession of a firearm.
- Items submitted for touch DNA analysis will comply with existing policy on number of items submitted by case type.

#### 10.4. DNA Analyses per Case Type

The type of case will determine the number of probative items analyzed for DNA analysis. The most probative items in the case will be examined first. When DNA results are obtained which answer the investigative questions pertinent to the case, additional items may not be tested. DNA analysis will be complete when an association is established from the probative evidence (for example, an association established between the subject and victim, subject and crime scene, etc.). If the most probative items yield negative results, then additional items may be tested.

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Homicides:

Up to 10 probative items + appropriate DNA standards

Sexual Assaults:

Sexual assault evidence kit (victim)

Up to 2 additional probative items (if no SA kit, then up to 5 items) + appropriate DNA standards

Crimes Against Persons:

Up to 5 probative items + appropriate DNA standards

Property Crimes:

Up to 2 probative items + appropriate DNA standards

**TBI Management will have the ultimate authority to approve casework for DNA analysis on a case-by-case basis.**

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**11.1. References**

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**12. Appendices**

**12.1. Forensic Biology Unit Abbreviations**

|                 |                                     |
|-----------------|-------------------------------------|
| '               | foot                                |
| ”               | inches                              |
| #               | number                              |
| (-) or (=) or = | negative                            |
| (+) or pos      | positive                            |
| +               | CODIS obligate allele designation   |
| /               | per                                 |
| @               | at                                  |
| [ ]             | concentration                       |
| ~ or ≈          | approximately                       |
| ≈               | about or approximately equal to     |
| <               | less than                           |
| >               | greater than                        |
| >>              | much greater than                   |
| :::             | additional information not included |
| ♀               | female                              |
| ♂               | male                                |
| 1st             | first                               |
| 2nd             | second                              |
| 3rd             | third                               |
| AA              | African-American                    |
| ABI or AB       | Applied Biosystems Inc.             |
| ADA or ADAG     | Assistant District Attorney General |
| add'l           | additional                          |
| add'ly          | additionally                        |
| adh             | adhesive                            |
| admin           | administrative                      |
| Agt.            | Agent                               |
| AL              | Allelic Ladder                      |
| ALS             | alternate light source              |
| amp             | amplify                             |
| amt             | amount                              |
| Amel            | Amelogenin                          |
| AP              | acid phosphatase                    |
| Approx          | approximately                       |

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|                       |                                     |
|-----------------------|-------------------------------------|
| AR                    | Administrative Review               |
| ASAP                  | as soon as possible                 |
| AT                    | analytical threshold                |
| b/c                   | because                             |
| b/f or b4             | before                              |
| b/t or b/w            | between                             |
| BA                    | blood alcohol                       |
| bact/bac              | bacteria                            |
| Bakit or ba kit       | blood alcohol kit                   |
| bbox(es) or brbox(es) | brown box(es)                       |
| BC or BCL             | bar code/label                      |
| BCS                   | brown crusty stain                  |
| BET                   | blue evidence tape                  |
| bg(s) or b(s)         | bag(s)                              |
| bio. st.              | biohazardous sticker                |
| Bio or biohaz         | biohazardous                        |
| Bio. T                | biohazardous tape                   |
| bl                    | blue                                |
| bld                   | blood                               |
| blk                   | black                               |
| blnk                  | blank                               |
| bp                    | base pair                           |
| bpb(s) or brpb(s)     | brown paper bag(s)                  |
| bpp(s) or bppkg(s)    | brown paper package(s)              |
| brb(s)                | brown bag(s)                        |
| brn or br             | brown                               |
| BS                    | buccal swab                         |
| BST                   | bloodstain                          |
| BSTRB                 | reagent blank-questioned bloodstain |
| burg                  | burgundy                            |
| bus env               | business envelope                   |
| bwn                   | brown                               |
| bx(es)                | box(es)                             |
| buc or bucc           | buccal                              |
| Cauc or Cau           | Caucasian                           |
| CB                    | call back                           |
| CE                    | capillary electrophoresis           |
| cert.                 | certified                           |
| CET                   | clear evidence tape                 |

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|                     |  |
|---------------------|--|
| cl                  | clear  |
| cld                 | called   |
| cntn or ĉ          | contains   |
| cntning or c'ing    | containing   |
| ctrl                | control  |
| CO or Co or C       | COfiler  |
| COC                 | chain of custody                                   |
| CODIS               | Combined DNA Index System                          |
| conc                | concentrate(d)                                     |
| CSP or CP           | consensual sex partner                             |
| ct                  | clear tape   |
| cyl                 | cylinder   |
| cw/                 | closed with  |
| D                   | deep   |
| DI H <sub>2</sub> O | de-ionized water                                   |
| diff                | differential                                       |
| DA or DAG or AG     | District Attorney General                          |
| DAR                 | District Attorney Request                          |
| DBS                 | dried bloodstain                                   |
| DOB                 | date of birth                                      |
| DNA                 | deoxyribonucleic acid                              |
| dr                  | driver   |
| drb                 | differential reagent blank                         |
| E or excl           | excluded   |
| ea                  | each   |
| Ec/d                | epithelial cells and debris                        |
| Epp tube            | Eppendorf tube                                     |
| env(s) or e(s)      | envelope(s)  |
| Epith or epi. or EC | epithelial cells                                   |
| EPG(s) or Egrams    | electropherogram(s)                                |
| ESS or ESNS         | entire slide searched or entire slide not searched |
| ESV or ESNV         | entire slide viewed or entire slide not viewed     |
| et                  | evidence tape                                      |
| ETOH                | ethanol  |
| Evid                | evidence   |
| ESL                 | evidence storage location                          |
| Exp                 | expired/expiration                                 |
| Ex#                 | exhibit number                                     |

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|                |                                   |
|----------------|-----------------------------------|
| FA             | Firearms                          |
| FB             | Forensic Biology                  |
| FedEx          | Federal Express                   |
| FEBox          | FedEx box                         |
| FM             | Forensic mixture                  |
| FP             | Forensic partial                  |
| frt            | front                             |
| FS             | Forensic Scientist                |
| ft             | feet                              |
| FTL            | Fruit of the Loom                 |
| FU             | forensic unknown                  |
| GF or G        | GlobalFiler                       |
| gly            | glycogen                          |
| GMID           | Genemapper ID or ID-X             |
| grn            | green                             |
| gry            | gray                              |
| g/s            | glue sealed                       |
| H              | sperm heads                       |
| haz            | hazard                            |
| HazMat         | hazardous material                |
| HHW            | Hanes Her Way                     |
| H.O.           | human origin                      |
| hs or hosp st  | hospital sticker                  |
| HT             | Hematrace                         |
| Hum or Hu      | human                             |
| HumDNA         | human DNA                         |
| HumOr          | human origin                      |
| IDF            | Identifiler                       |
| ID+ or ID Plus | Identifiler Plus                  |
| in.            | inches                            |
| incl           | Included or including             |
| inc            | incident                          |
| inco           | inconclusive                      |
| ID             | my Identification (same as my ID) |
| in'd           | initialed                         |
| info           | information                       |
| insuff         | insufficient                      |
| init           | Initials or initial               |
| inv            | inventory                         |

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|  |  |
|--|--|
| Inv.   | Investigator                             |
| invsh  | Inventory sheet                          |
| JPD  | Jackson Police Department                |
| Jr.  | Junior                                   |
| K  | known                                    |
| <br>   |  |
| <u>Kind Classification (sperm or epithelial cells)</u> |  |
| Rare   | <10 sperm heads/cells on slide           |
| 1+   | Few; difficult to locate                 |
| 2+   | Some in some fields                      |
| 3+   | Some in many fields; easy to locate      |
| 4+   | Many in most fields                      |
| KLAB   | Knoxville Crime Lab                      |
| KPD  | Knoxville Police Department              |
| Krb or KRxB  | known reagent blank                      |
| lft or lt  | left                                     |
| lg   | large                                    |
| liq  | liquid                                   |
| LP   | latent print                             |
| ltr  | letter                                   |
| LV   | large volume                             |
| m.o.   | modus operandi                           |
| M or med   | Medium                                   |
| mag  | magazine                                 |
| MCL or MLAB  | Memphis Crime Lab                        |
| me(s)  | manila envelope(s)                       |
| ME   | Medical Examiner                         |
| med  | medical                                  |
| Metro  | Metropolitan Nashville Police Department |
| µl or µL   | microliter                               |
| mg   | milligram                                |
| ml or mL   | milliliter                               |
| MP   | Missing person                           |
| MPD  | Memphis Police Department                |
| MNPD   | Metro Nashville Police Department        |
| man env  | manila envelope                          |
| micro  | microscopic examination/microanalysis    |
| MSARC  | Memphis Sexual Assault Resource Center   |
| msg  | message                                  |

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|                       |  |
|-----------------------|--|
| my ID                 | my identification or (ID) or actual initials |
| maj                   | major  |
| min                   | minor  |
| N                     | normalization                                |
| n.m.                  | normal manner                                |
| N/A or NA             | not applicable                               |
| NC                    | not collected                                |
| ND                    | not determined                               |
| neg                   | negative                                     |
| NEP                   | no exam performed                            |
| ng                    | nanogram                                     |
| NLAB                  | Nashville Crime Lab                          |
| NS or NSF             | non-sperm/fraction                           |
| NSRB or nsrb or NSRxB | non-sperm reagent blank                      |
| num                   | numerous                                     |
| OAR                   | Officer and Agency return address            |
| obv                   | obvious                                      |
| occ                   | occasional                                   |
| ODAG                  | Office of the District Attorney General      |
| OET                   | orange evidence tape                         |
| Ofc or ofcr           | officer                                      |
| OIA                   | Officer information and address              |
| O/N                   | overnight                                    |
| Orb                   | organic reagent blank                        |
| P                     | partial locus/obligate allele                |
| pass                  | passenger                                    |
| pa                    | paper  |
| P/E                   | property/evidence                            |
| pc                    | piece  |
| PC                    | positive control                             |
| PCR                   | polymerase chain reaction                    |
| PD                    | police department                            |
| PEV                   | primary evidence vault                       |
| pg(s) or pp(s)        | page(s)                                      |
| phpth or P            | Phenolphthalin or -ein                       |
| Ph                    | possible/probable sperm head                 |
| PHR                   | peak height ratio                            |
| Pk                    | swab pack                                    |
| Pkged                 | packaged                                     |

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|                      |                                      |
|----------------------|--------------------------------------|
| pkg(s)               | package(s) or packaging(s)           |
| pl                   | plastic                              |
| plb                  | plastic bag                          |
| PL                   | Polilight                            |
| plb(s) or plbg(s)    | plastic bag(s)                       |
| Pop.                 | population                           |
| pos                  | positive                             |
| poss                 | possible                             |
| pp or Pro or P or PP | Profiler Plus                        |
| pr                   | pair                                 |
| pres. pos.           | presumptive positive                 |
| prev                 | previously                           |
| prob                 | probability                          |
| prop                 | property                             |
| PSA or p30           | prostate specific antigen            |
| PSL                  | personal storage location            |
| PTMB                 | phenolphthalein+tetramethylbenzadine |
| pu or p/u            | pick up                              |
| pzb or plzlb         | plastic ziploc bag                   |
| Q                    | questioned                           |
| QA                   | Quality Assurance                    |
| QAM                  | Quality Assurance Manager            |
| QF                   | Quantifier                           |
| Quant Trio or QFT    | Quantifier Trio                      |
| QRB or QRxB          | questioned reagent blank             |
| QNS                  | quantity not sufficient              |
| QRBR                 | questioned reagent blank-robotic     |
| QS                   | questioned stain or saliva           |
| Quant                | quantification or quantitation       |
| R or RI              | Reinjection                          |
| RA                   | re-amplification                     |
| r. band              | rubber band                          |
| rb                   | reagent blank                        |
| RBC or rbc           | red blood cell                       |
| RB K                 | reagent blank – known                |
| RB Q                 | reagent blank - questioned           |
| RB NS                | reagent blank - non-sperm            |
| RB Sp                | reagent blank –sperm                 |
| RBS or rbs           | reddish brown stain                  |

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|                     |   |
|---------------------|---|
| RC                  | reagent control                                 |
| RCC                 | Rape Crisis Center                              |
| Rcv                 | receive   |
| RE                  | re-extraction                                   |
| re:                 | regarding                                       |
| re-amped            | re-amplified                                    |
| rec'd or rcvd       | received  |
| req                 | request   |
| RET                 | red evidence tape                               |
| ret'd/rtd           | returned  |
| ret.add.            | return address                                  |
| RFE                 | request for examination form                    |
| RFU                 | relative fluorescence unit                      |
| rpt                 | report  |
| rt                  | right   |
| RXB                 | reagent blank                                   |
| S                   | Sealed or sperm fraction                        |
| sa                  | sexual assault                                  |
| SA                  | Special Agent                                   |
| SF                  | sperm fraction                                  |
| sal                 | saliva  |
| SalRB               | reagent blank-saliva                            |
| sakit               | sexual assault kit                              |
| sbakit              | sealed blood alcohol kit                        |
| sbpb(s)             | sealed brown paper bag(s)                       |
| SBS                 | subject blood standard                          |
| scat or Scatt or sc | scattered                                       |
| Sero                | Serology  |
| SEV                 | secondary evidence vault                        |
| SF or S             | Sperm fraction                                  |
| skit                | sealed kit                                      |
| sm                  | small   |
| sme(s)              | sealed manila envelope(s)                       |
| S.O.                | Sheriff's Office                                |
| sp                  | sperm   |
| splb(s) or szlb(s)  | sealed plastic bag(s) or sealed zip lock bag(s) |
| SRB or srb          | sperm reagent blank                             |
| SSN                 | social security number                          |
| SST                 | semen stain                                     |

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|                        |                                      |
|------------------------|--------------------------------------|
| st                     | sticker                              |
| stats                  | statistics                           |
| std or stnd            | standard                             |
| Stp                    | stapled or staples                   |
| ST                     | stochastic threshold                 |
| STR                    | short tandem repeat                  |
| styro b or bx          | styrofoam box                        |
| Sub or (s)             | subject                              |
| sw or sw/ or s/w       | sealed with                          |
| Swbx(es) or swhibx(es) | sealed white box(es)                 |
| swe(s)                 | sealed white envelope(s)             |
| SWH                    | Southwestern Hispanics               |
| swube                  | swab tube                            |
| sye(s)                 | sealed yellow envelope(s)            |
| szlplb(s)              | sealed ziploc plastic bag(s)         |
| T                      | trace                                |
| t                      | thick                                |
| TBI                    | Tennessee Bureau of Investigation    |
| Tech                   | technical                            |
| TE/GLY                 | TE buffer with glycogen              |
| TL                     | technical leader                     |
| TM                     | technical manager                    |
| TMB                    | tetramethylbenzadine                 |
| TMB+P or TMBP          | tetramethylbenzadine+phenolphthalein |
| TN or Tenn             | Tennessee                            |
| Tox                    | Toxicology                           |
| TR                     | Technical review                     |
| t/s                    | tape sealed                          |
| UBSL                   | unit bulk storage location           |
| UHR                    | unidentified human remains           |
| ul or uL               | microliter                           |
| und                    | underwear                            |
| Undet                  | undetected                           |
| Unk                    | unknown                              |
| uns or unse            | unsealed                             |
| unsub                  | unknown subject                      |
| V stmt                 | victim's statement                   |
| vag                    | vaginal                              |
| Vic or (v)             | victim                               |

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|                       |                               |
|-----------------------|-------------------------------|
| VBS                   | victim blood standard         |
| VCRT                  | Violent Crime Response Team   |
| VM                    | voice mail                    |
| VO                    | victim profile only           |
| vulv                  | vulva or vulvar               |
| W                     | sperm wholes                  |
| wde                   | wide                          |
| WET                   | white evidence tape           |
| w/                    | with                          |
| w/w                   | wrapped with                  |
| WBC or wbc            | white blood cell              |
| wbx(es)               | white box(es)                 |
| we(s)                 | white envelope(s)             |
| wk                    | weak positive                 |
| wpb(s)                | white paper bag(s)            |
| wt, wh, whi, or wht   | white                         |
| x                     | times                         |
| XL                    | extra large                   |
| XXL                   | extra, extra large            |
| Y or yell             | yellow                        |
| Ycs                   | yellow crusty stain           |
| Ye                    | yellow envelope               |
| YET                   | yellow evidence tape          |
| YF+ or YFP or YF Plus | Yfiler Plus                   |
| yme                   | yellow manila envelope        |
| ywcs                  | yellow, white crusty stain(s) |
| zlb or zip bg or zl   | zip lock bag or zip lock      |
| zplb(s) or zplb(s)    | zip lock plastic bag(s)       |

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#### 12.2. Digital Camera Policy

Currently, *Sony* digital cameras and various printers (both dye-sub, inkjet and color laser) are available for use by the Nashville, Knoxville and Memphis Forensic Biology units. The available *Sony* cameras are the *DSC-Hx* family, which are flash media-based digital cameras.

1. The issued *Sony* digital cameras will be used for documentation purposes only. No captured images should be used for analytical purposes.
2. *Sony Memory Stick Pro DUO* flash media (hereafter called memory sticks) will be provided for the *DSC-Hx* family of cameras.
3. Captured images from the memory sticks, or directly from the cameras, can be printed on any compatible printer.
4. The issued digital cameras should not to be used at crime scenes. TBI laboratory policy states that any digital photography taken at a crime scene should be preserved in its original state by archiving the flash media with the TBI Forensic Imaging Specialist (FIS). *Nikon* digital cameras are provided by TBI for crime scene documentation, therefore, issued *Sony* digital cameras should not be used at crime scenes.
5. If an issued digital camera is used at a crime scene, the memory stick will be transferred to the FIS for storage. The FIS will then copy the photos from the memory stick and provide a disc with copies of the original photos to the Forensic Scientist.
6. Captured images do not have to be printed immediately, but the integrity of the images must be preserved. This may be accomplished by either printing the pictures or by transferring the files to a computer's hard drive (C: drive) for storage. The memory sticks may then be formatted and re-used.
7. Any photographic image data may be transferred from a computer hard drive (C: drive) to a standard 12 cm CD-R or external hard drive at the discretion of the Forensic Scientist. It is suggested that this be accomplished at least once a month to prevent loss due to hard disk (C: drive) failure.
8. Those accessories recommended by *Sony* should be used with *Sony* cameras.

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9. There should be no modifications made to the supplied *Sony* cameras or any printers.
10. Only recommended paper and ribbons/ink/toner should be used in the provided printers.
11. Always check the manual for additional information and capabilities of the cameras.
12. Personnel should be trained in proper use of the equipment prior to using the cameras and printers.
13. Follow common photographic techniques when using the cameras to capture images.