Forensic Biology Methods Manual

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1 Use of the Alternate Light Source for Detection of Stains

1.1 Introduction

Dried stains such as saliva, semen, urine and perspiration often produce a bright fluorescence when exposed to ultraviolet light. This property can be utilized in the non-destructive examination of items for the presence of body fluids.

The fluorescence in dried semen stains is thought to be due to non-proteinaceous compounds produced by a strain of the bacterium *Pseudomonas fluorescens*. Not every semen or saliva stain will fluoresce, so further testing of negative stains may be warranted. Also, other materials such as foods, cosmetics, etc., may fluoresce, so additional tests may be performed. Finally, depending on the color and/or fabric of the substrate, strong background fluorescence may be produced. In this case, experimentation with different wavelengths and colors of eye protection is warranted. BCI laboratories utilize three types of alternate light sources (ALS), the Spex Crimescope CS-16, the Crime-lites 80S and 80L, and Crime-lite ML.

1.2 Safety Considerations

High intensity xenon light source, ultraviolet light - Wear protective goggles, do not look directly at the emitted light
High voltage - Disconnect unit before servicing
Do not set the remote on clothing or bedding, emitted light may cause a fire.

1.3 Crime-lites 80S and 80L

The Foster + Freeman Crime-Lite 80S is a high intensity forensic light source comprised of light emitting diodes (LED) and associated beam conditioning lenses. BCI uses the blue/green light with a wavelength of 460-510 nm.

The Crime-Lite 80L is a linear, high intensity white light source that delivers broad, low angle illumination to assist in the examination of large items for biological stains. The 80L utilizes colored filter strips that attach magnetically to the unit and provide contrasting wavelengths that can be used to locate biological stains.

Both the 80S and 80L units operate on either a DC Power unit or a rechargeable battery. The DC Power unit plugs into an outlet and directly into the back of both the 80S and 80L units.

CAUTION: Switch off the 80S or 80L units before connecting the adaptor.
Read the instructions supplied with the battery charger before charging a battery. Failure to do so may result in shortening the life of the battery.

1.4 Crime-lite ML

The Crime-lite ML combines the versatility of multi-wavelength, high intensity LED illumination and the comfort of wide area bi-ocular magnification to provide an effective bench top search and examination tool for the forensic scientist. BCI uses the violet light with a wavelength of 395-425 nm and the blue light with a wavelength of 430-470 nm.

1.5 Operation of Crime-lites

Place the item to be examined on an unused sheet of brown paper on a clean laboratory bench in a room that can be darkened. Use the 80S unit plugged into either the DC Power unit or the battery holder and orange colored goggles. Larger items such as sheets, comforters, blankets, etc. can be laid out on a large table or hung over metal racks and processed with the 80L or ML units.

The 80L unit can be used without a filter for the examination of dark clothing and bedding for the presence of minute bloodstains.

Where no potential body fluid stains are detected by visual examination and no chemical body fluid testing is performed: No stains for analysis.

Where chemical body fluid testing is performed but clarity is needed regarding other fluids of interest, use the wording for the fluid tested as well as:

No additional stains for analysis

Ex.: For ALS θ, TMB ⊕ results, use:
Presumptive positive for blood.
No additional stains for analysis.

1.6 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, National Institute of Justice, Washington, D. C., 1983, p. 178.
2. Kobus H, Silenieks E, Scharnberg J, Improving the effectiveness of fluorescence for the detection of semen stains on fabrics, *J Forensic Sci*, 47(4), 2002, pp. 819-823.
3. http://www.fosterfreeman.sinstr.ru/buklet/CL_ML.pdf

2 Wurster/Laux Paper for Saliva

2.1 Introduction

The Wurster/Laux (W/L) test is a rapid, non-quantitative presumptive test used to identify the presence of possible saliva stains. It is based on the ability of amylase, a component of saliva and other body fluids, to hydrolyze starch.

2.2 Safety Considerations

Potassium iodide – Irritant
Iodine – Irritant

2.3 Preparations

Amylase Diffusion Buffer (PBS pH 6.9)
Dissolve premix two containers of amylase diffusion buffer (SERI) in 1000 ml of deionized water. This solution should be stored in the refrigerator.

Starch Solution			While stirring, add the starch to the amylase diffusion buffer in a flask and bring to a boil on a stir plate. When all of the starch granules are in solution, allow the solution to cool to a warm temperature.
Amylase diffusion buffer	1000	ml	
Soluble starch	1.0	g	

Wurster/Laux Paper

1. Cut sheets of thick chromatograph paper (Fisher 05-714-4) into desired sizes.
2. Pour warm starch solution into a large ~~plastic~~ container.
3. One at a time, place the filter paper pieces into the plastic container and saturate the paper with the starch solution.
4. Place the saturated filter paper on sheets of aluminum foil and allow to dry. Aluminum foil may be tented over paper if necessary to protect paper while drying.
5. Wrap the paper in the aluminum foil, then a manila envelope, then a sealed plastic bag to avoid moisture and store in the freezer.

Iodine Stock Solution			
Potassium iodide	1.65	g	Dissolve the potassium iodide in the deionized water. Add the iodine and stir for approximately 5 minutes, then filter. Store refrigerated in a dark-stoppered bottle.
Iodine	2.54	g	
Deionized water	30	ml	

Low Level Amylase Standard

Stock Solution	α-Amylase (from human saliva) (Sigma A1031)	5000	units
	ddi H_2O	10	ml
Working Solution	Stock solution	40	µL
	ddi H_2O	2	mL

Add the water directly to the stock bottle and vortex. Store in freezer as Stock Solution. Working Standard can be prepared by mixing 40 µl Stock Solution with 2 ml ddi H_2O. Aliquot 20 µl into 200 PCR tubes. Store tubes frozen in a pipette tip rack. To use, thaw a tube and add the aliquot to a designated area of the W/L paper. The sample equates to .2 units amylase/20 µl. An average neat saliva stain is equivalent to ~100 units per ml. This minimal amylase standard equates to a ~1/10 dilution of saliva.

2.4 Procedure

1. Lightly moisten the W/L paper with deionized water and place it over the suspected stain.
2. Press evenly for approximately 10-15 seconds.
3. Remove the Wurster/Laux paper and place it on a watch glass, in a weigh boat, or other container. Add the amylase standard to a designated labeled area on the paper. Document the preparation date for the paper *and note the result of the controls using (+) or (-)* in the case notes.
4. Incubate the W/L paper for approximately 10 minutes at room temperature.
5. Gently pour a ~1:100 dilution of Iodine Stock solution over the W/L paper. Do not rinse. *The dilution may be increased if the diluted iodine solution appears too dark.*
6. Photograph positive results from maps with the paper next to the item.

2.5 Interpretation of Results

The positive control must be positive. A white area on a blue background indicates a positive result for amylase. A ⊕ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. Results that are unable to be documented in a photograph are reported as negative for amylase. Refer to Section 11 for report wording.

If the positive reaction is speckled in appearance, the following comment may be used in the Remarks section of the report or may be included as a table footnote:
The positive amylase area is similar to a mist or spatter pattern. Coughing and sneezing cannot be ruled out as possible origins of the stain.

2.6 References

1. Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, National Institute of Justice, Washington, D. C., 1983, pp. 183-189.
2. Wurster, J.W. and Laux, D.L. 1990. A rapid amylase mapping procedure. MAFS Newsletter 19:48-49.

3 Acid Phosphatase for Semen

3.1 Introduction

The acid phosphatase (AP) test is an enzymatic reaction. It is a qualitative assay and uses alpha-napthyl phosphate as the substrate. The acid phosphatase test is a preliminary color test and is only a presumptive test for semen. It is not a confirmatory test since acid phosphatase is found in other substances, including other body fluids.

3.2 Safety Considerations

Sodium α-naphthyl phosphate- Irritant
Brilliant Blue B (Ortho-Dianisidine) - Carcinogen
Weigh out reagent in a hood.

3.3 Preparations

AP reagent from SERI			
AP spot test premix reagent (PMR)	0.26	g	Mix the acid phosphatase spot test PMR and deionized water. The AP reagent should be stored in a dark bottle and refrigerated when not in use.
Deionized water	10	ml	

Low Level AP Standard			
Acid phosphatase (from potato) Sigma P-1146	50	units	Add water directly to a freshly opened bottle of acid phosphatase and vortex gently. Add the solution to an 8cm x 10cm piece of Whatman #3 filter paper and allow to dry. Cut the paper into 3mm x 3mm squares (makes approximately 860 squares at a concentration of 20 units acid phosphatase/ml). Store in the freezer. To use, remove a test square from the freezer, place onto a small piece of filter paper and add 1 drop of the apPMR test reagent. If a purple color develops within 10 minutes, the reagent can be used. A neat semen stain is equivalent to ~250 units acid phosphatase/ml. The standard equates to a ~1/10 dilution of semen.
ddi H_2O	2400	µl	

3.4 Procedure

1. Each day, before using the AP reagent on evidence, test it with the known acid phosphatase standard and negative control consisting of a blank swab or filter paper. If the acid phosphatase standard gives a positive result and the negative control gives a negative result the reagent may be used. Document in the case notes the lot number and *note the result of the controls using (+) or (-). The initials of the analyst that performed the QC check must be in the case file or the daily QC check form.* ~~that the reagent is working properly~~.
2. Moisten a swab or filter paper with deionized water and rub or blot ("map") over the stain.
3. Add the AP reagent and look for a color change to purple/pink. Alternately, if large sheets of paper are used for mapping, the AP reagent can be sprayed onto the paper in a hood.
4. Results can be read at 10 minutes.
5. Photograph positive results from maps.

3.5 Interpretation of Results

A purple/pink color change is a positive result. A ⊕ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. If case facts and circumstances indicate the potential for a false negative, a second semen test may be performed at the discretion of the forensic scientist. Refer to Section 11 for report wording.

3.6 References

1. Metropolitan Police Forensic Science Laboratory Biology Methods Manual, 3-17 to 3-19, London, England (1978).
2. Schiff, A.F. 1978. Reliability of the acid phosphatase test for the identification of seminal fluid. JFS 23:853-844.
3. Laux, Dale L. 2003. Forensic Detection of Semen I: The acid phosphatase test, Midwestern Association of Forensic Scientists Newsletter 2:6-10.

4 Kernechtrot-Picroindigocarmine Stain for Spermatozoa

4.1 Introduction

The presence of semen in questioned specimens can be confirmed by the observation of one or more spermatozoa. A biological stain such as Nuclear Fast Red (Kernechtrot)-Picroindigocarmine stain, also known as the Christmas Tree stain, may be used on slides to enhance the appearance of the spermatozoa. With this stain, nuclear material stains red and background materials, including cellular material, stain green. The spermatozoa head stains red with the acrosomal cap remaining clear, while the tail (if present) stains green. Spermatozoa should be identified with a 10x objective or greater on the compound microscope.

4.2 Safety Considerations

Aluminum sulfate – Severe irritant
Indigocarmine – Slightly toxic; irritant
Ethanol – irritant
Methanol – Flammable; slightly toxic
Picric acid- Severe Irritant; explosive
Permount – Flammable; irritant
Nuclear Fast Red – Slightly toxic; irritant

4.3 Preparations

Nuclear Fast Red Stain (Kernechtrot)			Dissolve the aluminum sulfate in the hot deionized water and add the nuclear fast red. Stir the solution overnight, allow it to cool, and then filter. Store refrigerated.
Aluminum sulfate	5	g	
Hot deionized water	100	ml	
Nuclear fast red	0.1	g	

Saturated Picric Acid **Do not let the stock dry out**
Prepare by adding deionized water directly to the bottle of reagent, stir, let settle, and decant into a graduated cylinder.

Picroindigocarmine Stain (PICS)			Combine the indigocarmine and saturated picric acid. Stir overnight, and then filter. Store in the refrigerator.
Indigocarmine	0.33	g	
Saturated Picric Acid	100	ml	

Note: Nuclear Fast Red and Picroindigocarmine Stain can be obtained pre-prepared from SERI

4.4 Instrumentation

A compound microscope with 10x, 20x and 40x objectives is required

4.5 Procedure

4.5.1 Method 1

1. Use a previously prepared slide (from SAEC kit) or prepare a slide from an extract.
   a. Extracts may be made from cuttings of swabs or fabric placed into a tube. For swabs, cut approximately 1/8-1/16 of each swab supplied, regardless of AP result. For fabric, cut 5 mm x 5 mm samples if the AP reaction was weak, consider cutting 10 mm x 10 mm.
   b. Add between 50-100 µl, depending on the sample, of water.
   c. Vortex the extract for 20 seconds and let the sample sit for 5 minutes.
   d. Vortex the extract again for 20 seconds and spin briefly to remove droplets from the top and sides of the tube.
   e. Remove 15 µl of the extract to a microscope slide.
   f. Air dry the slide to fix the sperm. A heat block may be used to speed drying. A grease pencil may be used to circle the stain.
2. Add enough Nuclear Fast Red stain to cover the sample. A clean wooden stick may be used to spread out the stain. The stain should stay on the slide for at least 15 minutes, but not until the stain dries. Rinse the stain off with deionized water.
3. Add enough PICS stain to cover the sample. Allow the stain to stay on the slide for approximately 10 – 15 seconds. Rinse the stain off with reagent grade ethanol. Caution: Over staining may occur if the stain is left on for more than 20 seconds.
4. Allow the slide to air dry or use a heat block, then add a small amount of Permount or Cytoseal mounting medium and apply a cover slip. Using a microscope, observe the slide with a 10x objective or greater and rate using the ++++ system. In the case notes, rate and record each examined slide. For slides that receive a rating of few, record in the case notes the serial number or other unique identifier of the microscope used and coordinates for four sperm cells, if possible. A second forensic scientist *that is currently proficient in biology must* confirm a "few" or single sperm cell rating by *independently documenting the results of the verification,* initialing and dating in the notes. *If the verification is not consistent with the original observation, the final resolved rating will be clearly recorded in addition to the initial observations.* ~~the coordinates of the sperm cell they confirmed~~.
5. The following are the guidelines of the ++++ rating system:

4+	Many sperm cells in every field.
3+	Many or some sperm cells in most fields.
2+	Some sperm cells in some fields, easy to find.
1+	Some sperm cells in some fields, hard to find.
Few	Small numbers of sperm cells on the entire slide.
Single sperm cell	One sperm cell on the entire slide
θ	No sperm cells present on slide.

4.5.2 Optional method

1. If a swab is used to test the AP reaction of a stain and the reaction is strong, the extraction procedure may be skipped and a slide prepared by pressing the swab onto a microscope slide while still damp.

2. Prepare and examine a slide as in 4.5.1 beginning at step 1f.
3. If no sperm cells are identified, make an extract (see 4.5.1).

4.6 Interpretation of Results

Human spermatozoa are stained red, oval, contain an acrosome, and are smaller than the nuclei of epithelial cells. Tails, if present, will stain green and should be noted.

Some cells may resemble a sperm cell without having all the characteristics of one. This cell can be referred to as a QNC (Questionable, Not Called). This should be documented in the case notes when no sperm are observed *with reasoning why the cell was QNC*. A second forensic scientist *that is currently proficient in biology* should confirm by initialing and dating the QNC they have observed *and also documenting as "QNC".* A QNC result will be reported as inconclusive for semen unless seminal fluid is detected in further testing. *If the hospital smears have not been examined and the analyst prepared slide has sparse cells or only QNCs, the corresponding smear should be stained and evaluated.*

Refer to Section 11 for report wording.

4.7 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry U.S. Government Printing Office: Washington, DC, 1983, pp. 149-154.
2. Federal Bureau of Investigation, *Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence*, U. S. Government.
3. Stone, I.C., Staining of Spermatozoa with Kernechtrot and Picroindigocarmine for microscopic identification, SWIFS Criminal Investigation Laboratory, September 1972.

5 Seratec PSA Semiquant Kit

5.1 Introduction

Prostatic specific antigen (PSA or p30), is a protein that was isolated and characterized by Sensabaugh in 1978. Antisera to the protein quickly became utilized in the forensic field for the detection of semen. The Seratec PSA Semiquant is an immunochromatographic one-step test for the detection of PSA. A 120 µl volume is added to the chamber that equates to a detection limit of 0.4 to 0.8 ng PSA. Although a strong PSA test result is highly indicative of semen, recent publications have documented the presence of PSA in body fluids other than semen. Positive PSA test results have also been obtained from lubricants and/or spermicides. This test is considered to be a presumptive test for semen.

5.2 Positive Reactions for PSA with the Seratec Kit

During the test, PSA from the fluid reacts with the gold labeled monoclonal anti-PSA-antibody and forms a PSA-gold labeled anti-PSA-antibody-complex. Through the capillary effect of the membrane, the reaction mixture including the complex is carried across with the fluid. Upon positive findings, the PSA-gold labeled anti-PSA-antibody-complex binds to the fixed monoclonal mouse anti-PSA-antibody on the membrane by creating a red line at the test region. Both monoclonal anti-PSA-antibodies detect different epitopes of the PSA. The intensity of the red lines depends on the concentration of PSA.
The colored gold labeled anti-PSA-antibody binds to the anti-mouse-antibody at the control region and region of the internal standard by developing a red line in each region. The two lines are independent of the existence of PSA in the fluid. The internal standard line has been adjusted to a color intensity of 4 ng PSA/ml.

5.3 Safety Considerations

No known safety hazards.

5.4 Procedure

1. Use the smallest possible amount of the stain (*e.g*. 0.25 x 0.25 cm or ~1/8 swab). Extract the sample for at least 2 hours in 1.0 ml of the PSA Buffer provided with the Seratec Kit.
2. Centrifuge at 6000-8000 rpm for 3 minutes.
3. Add 120 µl of the supernatant to the sample well.
4. Read the results at 10 minutes. Photograph positive results at exactly 10 minutes. Write the case number, item number, and initials on the test kit (or document in the photograph) and the lot number and date performed in the case notes.

5.5 Interpretation of Results

- A pink line in both the test and control areas is a positive result.
- A faint pink line that cannot be reproduced in the photo or copy is reported as negative. No filter enhancements are to be performed for documentation.
- A pink line in the control area and the absence of a pink line in the test area is a negative result, provided there is no high dose hook effect. [High dose hook effect occurs at concentrations above 500 µg PSA/ml. If this is suspected, dilute the sample and re-test].
- Any test without a control line is considered invalid and should be repeated.
- Refer to Section 11 for report wording.

5.6 References

1. Hochmeister M.N., et al., Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid, J Forensic Sci 1999 Sep;44(5):1057-60.
2. Laux, Dale L., Tambasco, Anthony and Benzinger, Elizabeth A. Forensic Detection of Semen II : Comparison of the Abacus Diagnostics OneStep ABAcard p30 Test and the Seratec PSA Semiquant Kit for the Determination of the Presence of Semen in Forensic Cases, Midwestern Association of Forensic Scientists Newsletter, Vol. 32, Fall 2003, pp. 11-18.
3. Laux, Dale L. and Custis, Sarah E. Forensic Detection of Semen III: Detection of PSA Using Membrane Based Tests: Sensitivity Issues with Regards to the Presence of PSA in Other Body Fluids, Midwestern Association of Forensic Scientists Newsletter, Vol. 33, Winter 2004, pp. 33-39.
4. http://www.seratec.com/docs/user_instructions/psa_in_body_fluids (accessed on 03/21/13)
5. http://www2.cedarcrest.edu/academic/bio/hale/biostatsummaries/haubois.html (accessed on 03/21/13)
6. Bitner, S.E., False Positives Observed on the Seratec PSA SemiQuant Cassette Test with Condom Lubricants, J Forensic Sci 2012 Nov;57(6):1545-48.
7. Snead, M.C., et al., Effect of topical vaginal products on the detection of prostate-specific antigen, a biomarker of semen exposure, using ABAcards, Contraception, In Press.

6 Creatinine for Urine (Jaffe Test)

6.1 Introduction

Creatinine is present in body fluids such as serum, saliva and sweat, but it is one hundred times more concentrated in urine than in other body fluids. The Jaffe test is used to reveal the presence of creatinine.

When picric acid is added to creatinine in the presence of NaOH, creatinine picrate forms an orange-colored product confirming the presence of creatinine, a component of urine.

6.2 Safety Considerations

Picric Acid – Severe irritant; explosive

Sodium Hydroxide – Corrosive, hygroscopic; causes eye and skin burns; causes digestive and respiratory burns

6.3 Preparations

1.25 M Sodium Hydroxide (5% Sodium Hydroxide)			
4.0 M Sodium hydroxide	31	ml	Store at room temperature
Deionized water	69	ml	

Saturated Picric Acid
Add deionized water directly to the picric acid reagent bottle and stir. Allow the solution to settle prior to use. Store at room temperature.

Creatinine Standard			
Creatinine, anhydrous, Sigma C4255	0.1	g	Add 100 ml ddiH_2O to 0.1 g creatinine in a small beaker and stir until dissolved. Pour the solution into a 2L beaker and add cotton-tipped swabs. Soak swabs to saturation and dry. Store frozen. Prepares several hundred swabs (1.0 mg creatinine/ml). This equates to undiluted urine. To use, add a drop of concentrated picric acid followed by a drop of 5% NaOH. If an orange color develops within 5 minutes, the reagent can be used.
Deionized H_2O	100	ml	

6.4 Procedure

1. Before using the picric acid and sodium hydroxide on evidence, test with the known creatinine standard and deionized water. If the known creatinine standard gives a positive result and the deionized water gives a negative result the reagents may be used. Document *the result of the controls* in the case notes. ~~that the reagents are working.~~
2. Swab, map or cut a portion of the suspected stain area (~2-3 cm^2). If possible, cut so that the stain covers about ½ of the cutting and the remaining portion is unstained. *A separate sample from a non-stained area may also be used.* If the sample is still in liquid form, add a couple drops to a clean weigh~~t~~ boat.
3. Place the cutting on a piece of Whatman filter paper and add ~~a~~ *1-3* drops of saturated picric acid solution to the cutting at the junction of the stained and unstained areas. Alternatively,

extract cuttings of the stained and unstained areas in test tubes and add the picric acid to the tubes.

4. Add ~~a~~ *1-3* drops of 5 % NaOH and view results after 5 minutes.
5. If a swabbing of the stain is negative, repeat the analysis on a cutting from the item.

6.5 Interpretation of Results

An orange coloration on the stained area and no orange coloration on the unstained area of a sample is a positive result for the presence of creatinine. A ⊕ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. Refer to Section 11 for report wording.

6.6 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology and Biochemistry, National Institute of Justice, 1983, pp.191-195.

7 Urobilinogen for Feces

7.1 Introduction

Tests for urobilinogen are used to indicate the presence of feces and are considered presumptive. Fecal material consists of water and solid waste materials made up of mucus, undigested food residue, and microorganisms. Fecal material is usually brown in color due to urobilinogen that is formed from bilirubin by reduction processes in the intestine. Fecal material may also be green, black, or red depending on diet, drugs, and pathological conditions. There is no confirmatory test for feces.

7.2 Safety Considerations

Zinc Chloride – Irritant; moderately toxic
Ethanol – Irritant

7.3 Preparations

Zinc Chloride Solution

1. In a small beaker, add enough zinc chloride ($ZnCl_2$) to EtOH to prepare a saturated solution (e.g., 1 g $ZnCl_2$ to 10 ml EtOH).
2. Allow the solution to settle prior to use.
3. Store the solution at room temperature.

7.4 Procedure

1. Before using the $ZnCl_2$ reagent on evidence, test it on a known feces sample and ethanol. If the feces sample gives a positive result and the ethanol gives a negative result the reagents can be used. Document *the result of the controls* in the case notes. ~~that the reagents are working properly~~.
2. Extract the sample in one or two drops (~100 µl) of ethanol in a test tube for at least 30 minutes.
3. Add two drops of $ZnCl_2$ to the test tube and gently shake.
4. Examine the liquid with an ALS.

7.5 Interpretation of Results

Use a yellow shield to evaluate samples with the 430-470 nM wavelength filter and an orange shield with the 460-510 nM wavelength filter. An apple green fluorescence occurs if urobilinogen is present. A $\oplus$ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. Refer to Section 11 for report wording.

7.6 References

1. Lloyd, J.B.F. and Weston, N.T., "A Spectrometric Study of the Fluorescence Detection of Fecal Urobilinoids", *Journal of Forensic Sciences*, Vol. 27, No. 2, April 1982, pp. 352-365.

8 Tetramethylbenzidine for Blood

8.1 Introduction

Tetramethylbenzidine (TMB) is a presumptive chemical color test for blood that is based on the peroxidase-like activity of the heme portion of hemoglobin. The IR camera may be helpful in identifying potential blood stains on dark items.

8.2 Safety Considerations

3, 3', 5, 5'-tetramethylbenzidine – Irritant
Ethanol – Irritant
Hydrogen peroxide - Eye irritant

8.3 Preparations

TMB reagent			Mix the TMB with the ethanol, and then add the glacial acetic acid. Store in a dark colored bottle in the refrigerator. Expires after 12 months.
3,3', 5,5'- tetra-methylbenzidine	0.5	g	
Ethyl alcohol	*350*	ml	
Glacial acetic acid	3	ml	

3% hydrogen peroxide (H_2O_2) Purchase from drug store. Store refrigerated.

Low Level Hemoglobin Standard (0.05 mg hemoglobin/ml)			**Stock:** Add 100 ml ddiH_2O to a bottle of hemoglobin and gently vortex. Place the solution in a graduated cylinder and bring to 1 L with ddi H_20. Store frozen.
Hemoglobin, human (Sigma H7379)	1	g	**Working solution:** Prepare a 1:20 dilution (10 ml stock + 190 ml ddiH_2O) in a 2 L beaker and add cotton-tipped swabs (~300). Soak swabs to saturation and dry. Repeat. Store frozen. This equates to a 1 in 3000 dilution of blood.
ddi H_2O	1	L	**To use:** Add a drop of TMB followed by a drop of 3% H_2O_2. If a blue-green color develops within 10 seconds, the reagent can be used.

8.4 Procedure

1. Before using the TMB reagent and H_2O_2 on evidence, test them with the hemoglobin standard and negative control consisting of a blank swab or filter paper. If the hemoglobin standard gives a positive result and the negative control gives a negative result, the TMB reagent and H_2O_2 may be used. *Document in the case notes the lot number and note the result of the controls using (+) or (-). The initials of the analyst that performed the QC check must be in the case file or the daily QC check form.* ~~Record these results and the lot number in the case notes.~~
2. Small stains should be photographed before testing.
3. Moisten a swab or filter paper with deionized water and rub or blot the stain. A dry swab may be rubbed against a substantial stain.
4. Add a few drops of the TMB reagent. Wait a few seconds while carefully observing to ensure no color change develops at this time.

5. Add a few drops of the 3% H_2O_2 and wait up to 10 seconds for a color change.

8.5 Interpretation of Results

A rapid greenish-blue color change after addition of the H_2O_2 is a positive result. If the swab or filter paper does not turn greenish-blue within 10 seconds, it is a negative result. A $\oplus$ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. If there is a color change after adding the TMB reagent but prior to the H_2O_2, the result is inconclusive for blood. Refer to Section 11 for report wording.

8.6 References

1. Holland, V.R., *et. al.* 1974. A substitute for benzidine in the detection of blood. Tetrahedron 30:3299.
2. Gasner, D.D., *et. al.* 1976. An evaluation of tetramethylbenzidine as a presumptive test for blood JFS 21:816.
3. Tetramethylbenzidine: a reported noncarcinogenic analog of benzidine. 1989. Aldrichimica Acta 22:48.
4. Cox, M. 1991. A study of the sensitivity and specificity of four presumptive tests for blood. J. Forensic Sci. 36:1503-1511.

9 HemDirect for Human Blood

9.1 Introduction

Hemoglobin (Hb) is located in the erythrocytes and predominantly serves the transport of oxygen and carbon dioxide within the body. The HemDirect test is an immunochromatographic one-step test for the detection of human blood. This test is considered to be confirmatory for human blood.

9.2 Positive Reactions with the HemDirect test

If human hemoglobin is present in a sample, the human hemoglobin reacts with a mobile monoclonal antihuman hemoglobin antibody forming a mobile antigen-antibody complex. This antigen-antibody complex migrates through the absorbent device toward the test area. In the test area there is an immobilized monoclonal anti-human hemoglobin antibody. This immobilized antibody captures the above complex resulting in an antibody-antigen-antibody complex. If the human hemoglobin concentration is greater than 0.05 μg / ml the pink dye particles form a pink colored band in the test area, indicating a positive result. The HemDirect test incorporates an internal positive control consisting of gold labeled rabbit antibodies that are captured by an immobilized polyclonal goat anti-rabbit antibody present in the control area, forming a complex. High dose hook effect is a false negative that occurs if there is more human hemoglobin (hHb) than can be bound by the mobile monoclonal anti-human hemoglobin antibody. The excess hHb binds to the immobilized polyclonal anti-human hemoglobin antibody, thus blocking binding to the mobile antigen-antibody-dye complex in the test area. This test cross-reacts with ferret and higher primate blood.

9.3 Safety Considerations

No known safety hazards.

9.4 Preparations

Make sure that the sample is at room temperature prior to analysis.

9.5 Procedure

1. Use the smallest possible sample (e.g. ~5x5 mm or ~1/8 swab) depending on the intensity of the stain.
2. Allow the sample to extract for approximately 30 minutes in the chamber supplied with the kit. For old or degraded samples, add 2-3 drops of 5% ammonia to the stain and allow the ammonia to evaporate (~5 minutes) prior to adding the extraction buffer. Overnight extraction may help older samples. If the extract is cloudy or contains debris, centrifuge to clarify.
3. Add 150 μL (three drops from the chamber) of the extract to the sample well, “S”, of the HemDirect test. Record both buffer and card lot numbers and date performed in notes.
4. Read the results at 10 minutes. Photograph positive or inconclusive results or copy the results at exactly 10 minutes. Write the case number, item number, and initials on the test cassette.

9.6 Interpretation of Results

1. A pink line in both the test and control areas is a positive result.
2. A faint pink line that cannot be reproduced in the photo or copy is reported as no human blood identified.
3. A pink line in the control area and the absence of a pink line in the test area is a negative result, provided there is no high dose hook effect. [If this is suspected, dilute the sample and re-test]
4. Any test without a control line is considered invalid and should be repeated.
5. Refer to Section 11 for report wording.

9.7 References

1. Hochmeister, M., Rudin O., Sparkes, R., Gehrig, C., Schmidt, L. Evaluation of an Immunochromatographic 1-Step Blood Test for the Forensic Identification of Human Blood. *J. For. Sciences* May 44(3), 597-602, 1999.
2. Misencik, A., and Laux, D.L. Validation Study of the Seratec HemDirect Hemoglobin Assay for the Forensic Identification of Human Blood, MAFS 2005 Fall Meeting, Oct. 7, 2005, St. Louis, Missouri
3. Spear, T. F., Binkley, S. A. The HemeSelect test: a simple and sensitive forensic species test. *J. For. Sci. Soc.*34 (1), p 41-6, 1994.
4. Fernando, S.A., Wilson, G.S. Studies of the 'hook' effect in the one-step sandwich immunoassay. *J. Imm. Methods* 151 (1-2), pp. 47-66, 1992.

10 Hair Examination

10.1 Introduction

Cases such as hit skips, assaults, and homicides may be submitted requesting hair examination. If no body fluid is detected, the analyst may examine the trace evidence with the objective to identify clearly relevant apparent hairs with a root for DNA testing. Hair evidence submitted in a rape kit is routinely examined for the presence of body fluids. No hair comparisons will be performed.

10.2 Procedure

10.2.1 Examination of non-rape kit evidence

If a body fluid suitable for DNA testing is detected in the case and a clearly relevant apparent hair is observed, then the apparent hair is retained for potential future analysis as follows.

- A single/few apparent hair(s) are observed:
  - Attach the apparent hair to a Post-It™ note
  - Place the Post-It™ note in a coin envelope
  - Re-package the coin envelope within the original evidence item package.
- Many apparent hairs are observed:
  - Leave the hairs on the evidence item and document in the notes
  - The examination paper should be folded and packaged with the original evidence item package to prevent loss.

Should the DNA testing not yield probative results, a request may be made by the DNA analyst to have the apparent hair analyzed to determine if a root is present for DNA testing. If a root is present, cut off approximately a 1 cm section or less containing the root and place in a tube. Retain the remainder of the hair.

10.2.2 Examination of rape kit evidence

Using an ALS or stereoscope, examine apparent hairs for potential semen stains. If body fluids are indicated and additional traditional screening is not performed, use a single SEB swab to collect a sample for DNA testing. If additional screening procedures will be performed, samples may be collected with a single swab with water.

If trace debris is observed on a swab, try to remove it prior to screening the swab and report the trace debris as a separate line in the report. If the debris cannot be removed from the swab, then screen the swab and list the debris in the report with the sample on the same line.

For DNA testing, cut off approximately a 1 cm section or less containing the root and place in a tube. Retain the remainder of the hair.

10.3 Documentation

1. When the objective is to determine the source of a hair, case notes should include characteristics such as color, texture, length, and if a root is present.
2. Refer to Section 11 for report wording.

11 FB Report Wording Guidelines

The following document is a guideline intended to help achieve uniform content and style in technical reports. Departures from the statements below will at times be necessary to accommodate specific circumstances. Reporting that is highly accurate, grammatically correct, and easily understood by non-scientific readers shall be acceptable.

FB body fluid testing results table statements	
Result is…	**Finding**
ALS- /(visual exam neg for bld)	No stains for analysis
ALS- /(visual exam pos for bld)	*No stains for semen analysis*
AP +	~~Presumptive positive for semen~~ *Positive for acid phosphatase activity**
AP-	~~No semen identified~~ *Negative for acid phosphatase activity**
** AP Footnote*	**Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria*
SS+	Semen identified
AP +, SS-	*Positive for acid phosphatase activity*, no sperm identified*
SS-	No semen identified
SS+ (one cell)	Single sperm cell identified
SS+ (few) – typically for testing of non-orifice swab evidence	Trace amounts of semen identified
SS+ (few)	Semen identified
SS-/PSA+	Presumptive positive for seminal fluid, no sperm identified
SS-/PSA-	No semen identified
SS QNC	Inconclusive for semen due to cell(s) lacking definitive morphological sperm cell characteristics
SS QNC/PSA-	Inconclusive for semen due to cell(s) lacking definitive morphological sperm cell characteristics
SS QNC/PSA+	Presumptive positive for seminal fluid, inconclusive for semen due to cell(s) lacking definitive morphological sperm cell characteristics
SS-/PSA unable to document	~~Inconclusive for seminal fluid due to insufficient information obtained from the testing, no sperm identified~~ *No semen identified*
W/L+	Amylase identified
W/L-	No amylase identified
W/L unable to document	No amylase identified
TMB +	Presumptive positive for blood
TMB -	No blood identified
TMB inconclusive	Inconclusive for blood due to insufficient information

	obtained from the testing
HemDirect +	Human blood identified
HemDirect -	No human blood identified
Creatinine +	Creatinine identified
Creatinine -	No creatinine identified
Urobilinogen +	Urobilinogen identified
Urobilinogen -	No urobilinogen identified
Inmate case, no circled area	No circled area(s) for analysis
Inmate case with unknown fluid thrown; creatinine negative, amylase test results in speckling only	No creatinine identified Amylase identified Use creatinine/amylase footnotes and add the following to the Remarks: The positive amylase area is similar to a mist or spatter pattern. Coughing and sneezing cannot be ruled out as possible origins of the stain.

Miscellaneous FB results table statements	
Situation	**Findings**
DNA standard retained	No analysis
Item swabbed/retained, no testing	No analysis
Item not examined	Not examined
Orifice swabs not in kit	Not found in kit

Hair statements for FB results table	
Note: Mito referrals for violent cases only. Discuss individual case circumstances with your supervisor prior to writing report. Include report wording for body fluids as needed.	
Description	**Findings**
Pubic hair/Head hair standard	No stains for analysis Found to be empty Not examined
Pubic hair combings	Trace debris present No trace debris present No stains for analysis Not examined
Trace debris from Item X	Not examined Suitable for nuclear DNA analysis Not suitable for nuclear DNA analysis Not suitable for nuclear DNA analysis; may be suitable for mitochondrial DNA analysis

Analytical Detail section	
A description of the methods used for testing should be included in the Analytical Detail section of the report. Select the appropriate Method Statements based on the testing performed. The combined statements should summarize the methods used for all samples.	
Analysis	**Method Statement**
Semen (AP/PSA/SS)	Presumptive analysis for semen was performed using [visual examination and] chemical testing. [Presumptive analysis for seminal fluid, the liquid component of semen, was performed using immunological testing.] [Microscopic analysis was used for confirmation of semen.]
Blood (TMB/HemDirect)	Presumptive analysis for blood was performed using visual examination and chemical testing. [Confirmation of human blood was performed using immunological testing which may cross-react with higher primate and ferret blood.]
Wurster/Laux	Analysis for amylase, a component of saliva and other body fluids, was performed using [visual examination and] a chemical test.
Creatinine	Analysis for creatinine, a component of urine and other body fluids, was performed using [visual examination and] a chemical test.
Urobilinogen	Analysis for urobilinogen, a component of feces and other body fluids, was performed using [visual examination and] a chemical test.
Trace debris (examined)	Microscopic examination of trace debris was performed to determine if a hair root is present for nuclear DNA analysis.

Findings in Remarks section (no table in report or items not in table)	
Situation	**Remark**
Item not examined	Item x was not examined.
Hair collected from clearly probative item but being returned to agency because we have something else we will try first	Trace debris collected from Item x is being returned.
All items are negative	Additional items of evidence may be submitted.

No suspect, hair without root, case is one we might send forward for mito if a suspect is developed	Not suitable for nuclear DNA analysis (in Remarks, add: The hair from Item X may be suitable for mitochondrial DNA analysis. If a suspect is developed, please resubmit the hair along with a reference standard consisting of two oral swabs.)

QA remarks for FB

Situation	**Remark**
QA reanalysis – same findings	The above listed items were re-examined for quality assurance purposes. The findings concur with those outlined in the report issued by XX, dated May 1, 2005.
QA reanalysis – different findings	Varies - consult your supervisor or QAA

Disposition of items

Sample was…	**…and from a rape kit**	**…and not from a rape kit**
Collected*	Sample collected	To be returned Sample collected
Not collected	To be returned	To be returned
***The following may be used in the Remarks section of FB reports when samples are collected for DNA testing:** Samples are being forwarded for DNA analysis.		

Discrepancies

Situation	**Action**
Item is different than stated on submission sheet – BCI error	Contact ER – correct submission sheet and mail new submission sheet to department. Use corrected information in report.
Item different than stated on sub sheet and/or packaging	Leave item description as submitted followed by: **found to contain/be [actual item found] OR** **found to contain ___ labeled ___**
Item labeled differently than on submission sheet	Leave item description as submitted followed by: **found to be labeled [how labeled on packaging]**
Item quantity: description specifically states a # of swabs different than the # found	Leave item description as submitted followed by: **found to contain [actual quantity found]**
Item contains evidence stated on sub sheet along with other items	Leave item description as submitted followed by: - **also found to contain** [list only the additional items found]
Standards – not labeled with name on any packaging	Leave item description as submitted followed by: - **found to contain unlabeled swabs** - Subsequent reports will refer to them as unlabeled swabs - In Remarks, request new standard ("Please submit…", see p. 5)
	OK as is. **Does not** include standards that are labeled: "Suspect – John

Standards – labeled on inner and/or outer packaging	Doe, Victim – Jane Doe" with no clarification on packaging as to which one the sample originated from – these are considered unlabeled
	If discrepancy in the name itself use: - **found to be labeled…**
Do NOT discrepancy	- Minor spelling differences – just correct the error - Swab vs. swabs (no specific quantity specified at submission) – just add/remove the "s" as needed - Different # of reference standard swabs (remove the reference to the number of swabs from the Item description)

FB Remarks for cases with suspects	
Situation	**Remarks**
Samples collected	A sample has been collected from Item X. Samples have been collected from Items X and Y. Samples have been collected from Items X-Z. Samples are being forwarded for DNA analysis. *Samples have been collected from Item X for possible future DNA analysis. All evidence will be returned to the submitting agency.*
Need standard	DNA comparisons can be made if a reference standard consisting of two oral swabs from the [suspect, victim] is submitted. *It is our opinion that the DNA analysis procedure most likely to produce data foreign to (victim) is Y-STR analysis. Submission of a reference standard consisting of two oral swabs from (suspect) and resubmission of container(s) xxx are required before DNA analysis will begin. Samples are not being forwarded for DNA analysis at this time.*
Need multiple standards	DNA comparisons can be made if reference standards consisting of two oral swabs each from the suspects (or suspect and victim) are submitted.
Need elimination standard from car owner, homeowner; employees that handle the safe, etc.	DNA comparisons can be made if an elimination standard consisting of two oral swabs from the [primary operator of the vehicle] is submitted.
Need consensual partner standard	DNA comparisons can be made if an elimination standard consisting of two oral swabs from the consensual partner is submitted.
If case will go to DNA even if we don't get a standard	Please submit a reference [elimination] standard consisting of two oral swabs from the suspect [consensual partner]. Or Please submit reference standards consisting of two oral swabs each from the consensual partner and any suspects.
Need consumption authorization or unknown if capital case	It is our opinion that the DNA analysis most likely to produce a DNA profile from Item X requires consuming the entire sample. Written authorization from the prosecutor ~~is~~ *may be* required before testing can begin on this sample. Please contact this office for additional information. Samples are not being forwarded for DNA analysis at this time.
Container(s) to be returned - we cannot proceed without…	All evidence items are being returned to the submitting agency.
A) … standard	Upon submission of the requested standard, please resubmit Container 1.
B) … consumption authorization	Upon submission of consumption authorization, please resubmit Containers 1 and 2.
C) … standard and consumption authorization	Upon submission of the requested standard and consumption authorization, please resubmit Container 1.
Example: Samples have been collected from Items 1 and 2. It is our opinion that the DNA analysis most likely to produce	

a DNA profile from these samples requires consuming the entire sample. Written authorization from the prosecutor ~~is~~ *may be* required before testing can begin.

All evidence items will be returned to the submitting agency. DNA comparisons can be made if reference standards consisting of two oral swabs each from the suspect and the primary operator(s) of the vehicle are submitted. Upon submission of the requested standards and consumption authorization, please resubmit Container 1.

Note: Also see Report Example 2.

Remarks for unknown suspect (CODIS) cases; combine with options A-C (above) as needed	
Situation	**Remarks**
Item(s) eligible for entry without elimination standard and based on connection to crime	Should a suspect be developed, please submit a reference standard consisting of two oral swabs.
Need elimination standard(s) before any profile obtained could be entered	DNA comparisons can be made if an elimination standard consisting of two oral swabs from [the victim, occupants of the house, primary operators of the vehicle] is submitted.
All submitted items cannot be entered (no definite link between the submitted item(s) and the listed crime)	*Based on the current guidelines regulating the CODIS database and the information currently provided to the lab regarding the submitted item, any forensic DNA profile that may be developed from this item is not eligible for database entry. DNA analysis has not been performed and the item is being returned to your department. *If additional investigative information is available that directly links the evidence to the listed offense, please provide this information to the lab and resubmit the evidence. *If a suspect is developed, please resubmit the evidence along with a reference standard consisting of two oral swabs.
Some items CODIS eligible (X, Y), some not (Z).	Samples have been collected from Items X and Y. No examination was performed on Item Z. Followed by same wording as above * but specific for item Z.
Consent partner status unknown but profile will be entered	Please ensure any available elimination standards are submitted.

12 Report Example—FB

12.1 Example 1

To:	Ottawa Hills Police Department Detective Bob Jones 2125 Richards Road Ottawa Hills, OH 43604	BCI Lab Number: Date:	05-00000 March 29, 2005
Re: Subject: Victim:	Rape Robert Smith Jane Doe	Agency Number:	A100

Submitted by Officer Mark Johnson on March 3, 2005

1. Rape kit from Jane Doe
2. Shirt from Jane Doe
3. Hair brush from Jane Doe residence
4. Underwear from Jane Doe residence

	Item	Findings	Disposition
1	Rape kit – Jane Doe		To be returned
	Vaginal samples	Semen identified	Samples collected
	Anal samples	No semen identified	To be returned
	Oral samples	Not found in kit	---
	Skin stain swabs	Amylase* identified No semen identified	Samples collected
	DNA Standard - Jane Doe	No analysis	Samples collected
	Head hair standard	Not examined	To be returned
	Pubic hair standard	Not examined	To be returned
	Pubic hair combings	Not examined	To be returned
	Fingernail scrapings	Not examined	To be returned
2	Shirt	Not examined	To be returned
3	Hair brush	Not examined	To be returned
4	Underwear	Presumptive positive for semen	To be returned Samples collected

Remarks

Samples are being forwarded for DNA analysis.

DNA comparisons can be made if reference standards consisting of two oral swabs each from the suspect and the consensual partner are submitted.

Analytical Detail

Presumptive analysis for semen is performed using visual examination and chemical testing. Presumptive analysis for seminal fluid, the liquid component of semen, is performed using immunological testing. Microscopic analysis is used for confirmation of semen.

Analysis for amylase, a component of saliva and other body fluids, is performed using a chemical test.

_____

Robert Jones
Forensic Scientist
(330) 659-4600
Robert.Jones@ohioattorneygeneral.gov

Based on scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

12.2 Example 2 (Optional Administrative Close Letter)

To:	Agency Detective 409 E. Street City, OH 45402-1374	BCI Laboratory Number:	15-12345
		Date:	December 4, 2015
		Agency Case Number:	15-123
Offense:	Crime		
Subject(s):	Robert Smith		
Victim(s):	Jane Thompson		

Submitted on December 1, 2015 by Detective:

1. Evidence item

Based on the current guidelines regulating the CODIS database and the information currently provided to the lab regarding the submitted item(s), any forensic DNA profile that may be developed is not eligible for database entry without additional required information.
DNA testing has not been performed and the item is being returned to your department. Please provide the selected information below to our agency and resubmit the above item(s) for testing.

□ Synopsis/brief narrative of case facts to include:
- Description of incident
- Where the items of evidence were located
- Who the items of evidence belong to
- How the evidentiary items relate to the crime in question

□ Reference standards from subject and any individuals for elimination
- □ Victim
- □ Individuals with known contact to the crime scene evidence
- □ Other: _____

Please contact me by telephone or email if you have any questions.

_____
Robert Jones
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(330) 659-4600
Robert.Jones@ohioattorneygeneral.gov