1.3 Blood Detection

1.3.1 Foster + Freeman Crime-lite® 82S Infrared (IR) Alternate Light Source

1.3.1.1

The Foster + Freeman Crime-lite® 82S infrared (IR) alternate light source may aid in the detection of blood on dark-colored and patterned substrates. Viewing evidence items with infrared wavelengths (800 - 900 nm) may assist in muting a dark background or pattern, causing bloodstains to appear darker in contrast (light absorption).

1.3.1.2 Safety Considerations

The Crime-lite® 82S IR must be used with the appropriate IR safety filter and/or goggles (light green), per specification.

1.3.1.3 Procedure

Using the associated 5MP UV-Vis-IR multi-spectral camera, Crime-lite® Cam software, and appropriate camera filter for infrared wavelengths, possible bloodstains may be visualized and photographed.

1.3.1.4 Results and Interpretation

The reaction should appear as a dark stain against a lighter background.

1.3.1.5 Limitations

The Crime-lite® 82S IR is a visualization technique, and any stains located through its use must be followed by additional body fluid testing.

If bloodstain patterns (e.g. impact spatter, cast-off, etc.) or impressions are visualized using the Crime-lite® 82S IR, one representative stain from the pattern or impression should be subjected to body fluid testing. A bloodstain pattern analyst should be consulted to determine the stain on which to conduct body fluid testing. Additional stains may be tested if deemed necessary, and in consultation with the bloodstain pattern analyst.

Materials and substances other than blood may absorb light under infrared wavelengths. In addition, substrates may interact differently with the infrared light, causing interference that may hinder visualization of bloodstains that may be present. The Crime-lite® 82S IR should not be the sole determination of the absence or presence of blood.

1.3.1.6 References

The Use of Polilight in the Detection of Seminal Fluid, Saliva, and Bloodstains and Comparison with Conventional Chemical-Based Screening Tests, Vandenberg et al, Journal of Forensic Science Vol. 51 #2, March 2006

Foster + Freeman Crime-lite® 82S: Powerful Alternate Light Sources for Crime Scene and Laboratory

Foster + Freeman Crime-lite® Cam Software Guide

1.3.2 Phenolphthalein Test for Blood (Kastle-Meyer Test) (FBI Method)

1.3.2.1

The peroxidase activity of the heme unit of hemoglobin in blood catalyzes the oxidation of reduced phenolphthalein by peroxidase. The sensitivity is estimated at 1:1 million. Only direct oxidizing substances such as copper, nickel, or potassium ferricyanide seem to give false positive reactions.

1.3.2.2 Safety Considerations

Phenolphthalein - CAUTION - Avoid contact and inhalation

1.3.2.3 Reagents

Phenolphthalein Stock Solution
100 ml Distilled Water
20 g Sodium Hydroxide
2 g Phenolphthalein
20 g Granulated Zinc
Reflux above solution until clear. Store at 4ºC in presence of granular zinc.
Working phenolphthalein solution
Dilute stock solution 1:5 in ETOH and store in dark bottle
3% Hydrogen Peroxide (store in dark bottle)

1.3.2.4 Controls

Positive and negative controls shall be used for function verification. These will be run at the beginning of processing for the day. The positive control shall be a known blood sample. The lot numbers, control results, and the date of QA/QC shall be documented in STACS-CW.

1.3.2.5 Procedure

1.3.2.5.1

A portion of the stain is cut out or removed onto a cotton tipped applicator or piece of filter paper (mapping) that has been moistened in deionized water.

1.3.2.5.2

Add the phenolphthalein working solution followed by the hydrogen peroxide solution in a 1:1 ratio.

1.3.2.6 Alternate Testing Procedure

When a bloodstain is limited in size and/or when non-human blood is highly suspected, a combined phenolphthalein-HemaTrace® procedure may be utilized. The stain should be extracted according to the HemaTrace® procedure. Prior to applying the extract to a test card, however, the 200µl of needed extract must be reserved in a separate extraction tube or appropriate receptacle. The phenolphthalein reagents are then added to the remaining extract.

1.3.2.7 Results and Interpretation

Positive reaction: Pink color
Negative reaction: No color development
Inconclusive reaction: Development of a color other than that specified for a positive or negative reaction or a failure of the controls is considered inconclusive. A color change after the addition of phenolphthalein and prior to the addition of hydrogen peroxide is also an inconclusive reaction.

1.3.2.8 References

Cox, M., "A Study of the Sensitivity and Specificity of Four Presumptive Tests For Blood", Journal of Forensic Sciences, JFSCA, Vol. 36, No. 5, Sept. 1991, p. 1503-11.
Higake, R.S. and W.M.S. Philip, "A Study of the Sensitivity, Stability, and Specificity of Phenolphthalein as an Indicator Test for Blood", Can.Soc. Forensic Science J., 9 (1976) p. 97.

Lundquist, F, Methods of Forensic Science, Volume 1, 1962, pp 254-255.

1.3.3 Tetramethylbenzidine Test for Blood

1.3.3.1

The peroxidase activity of the heme unit of hemoglobin in blood catalyzes the oxidation of reduced tetramethylbenzidine by peroxidase. The sensitivity is estimated at 1:1 million. This is highly sensitive to minute traces of hemoglobin but it can give false positives with any other strong oxidizing agents and metallic salts.

1.3.3.2 Safety Considerations

1.3.3.2.1

Tetramethylbenzidine - CAUTION - Harmful if swallowed, inhaled, or absorbed through skin. Emits toxic fumes under fire conditions.

1.3.3.2.2

Glacial Acetic Acid - CAUTION - Corrosive. Flammable.

1.3.3.3 Reagents

Tetramethylbenzidine Solution (TMB)3,3'5,5'-Tetramethylbenzidine
Glacial Acetic Acid
Prepare a 2% solution of 3,3', 5,5'-tetramethylbenzidine in glacial acetic acid. Keep this solution at room temperature in a dark bottle.
3% Hydrogen Peroxide (store in dark bottle).

1.3.3.4 Controls

Positive and negative controls shall be used for function verification. These will be run at the beginning of processing for the day. The positive control shall be a known blood sample. The lot number, control results, and the date of QA/QC shall be documented in STACS-CW.

1.3.3.5 Procedure

1.3.3.5.1

A portion of the stain is cut out or removed onto a cotton tipped applicator or piece of filter paper (mapping) that has been moistened in deionized water.

1.3.3.5.2

Add the TMB solution followed by the hydrogen peroxide solution in a 1:1 ratio.

1.3.3.6 Results and Interpretation

Positive reaction: Green-blue color
Negative reaction: No color development
Inconclusive reaction: Development of a color other than that specified for a positive or negative reaction or a failure of the controls. A color change after the addition of TMB and prior to the addition of hydrogen peroxide is also an inconclusive reaction.

1.3.3.7 References

Cox, Milton, "A Study of the Sensitivity and Specificity of The Four Presumptive tests for Blood", Journal of Forensic Sciences, JFSCA, Vol. 36, No. 5, Sept. 1991, 1503-1511.

Garner, D.D. et al, "An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood", Journal of Forensic Sciences, February 1976, 816 - 821.

Lee, Henry C., Ph.D., "Identification and Grouping of Blood Stains", Forensic Science Handbook, Saferstein, R., Ph.D., Editor, Vol. 1, Chapter 7, pp. 267-337.

1.3.4 HEMASTIX®

1.3.4.1

The test is based on the peroxidase-like activity of hemoglobin that catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3', 5,5'-tetramethylbenzidine. This test is highly sensitive to minute traces of hemoglobin but it can give false positives with any other strong oxidizing agents.

1.3.4.2 Reagents

Hemastix® (Bayer Corporation)

1.3.4.3 Controls

Positive and negative controls shall be used for function verification. These will be run at the beginning of processing for the day. The positive control shall be a known blood sample. The lot number, control results, and the date of QA/QC shall be documented in STACS-CW.

1.3.4.4 Procedure

A swab or piece of filter paper moistened with deionized water is rubbed against the questioned stain and then applied to the Hemastix®. To conserve reagent, the Hemastix ® can be cut into several strips using sterile technique (see 1.7.1).

1.3.4.5 Results and Interpretation

Positive reaction: Green-blue color
Negative reaction: No color development
Inconclusive reaction: Development of a color other than that specified for a positive or negative reaction or a failure of the controls.

1.3.4.6 References

Bayer Hemastix® Reagent Strips for Urinalysis Test for Blood in Urine Technical Information Sheet.

Tobe, Shannon S. et, al, Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecualr-Weight DNA, Journal of Forensic Science, January 2007, Vol. 52, No. 1, pp. 102 - 109.

1.3.5 Luminol

1.3.5.1

Luminol is a preliminary blood screening test to be used when no visible blood is present. It is a well-known chemiluminescent compound and is used as a presumptive, catalytic test for the presence of blood utilizing the peroxidase-like activity of heme for the production of light as an end product rather than a true color reaction. Luminol will react with vegetable peroxidase, some metals such as copper and copper alloys, and interfering catalysts such as bleach**.**

1.3.5.2 Safety Considerations

1.3.5.2.1

May be harmful if swallowed

1.3.5.2.2

May cause irritation

1.3.5.2.3

Avoid breathing vapors or dusts

1.3.5.2.4

Use with adequate ventilation

1.3.5.2.5

Avoid contact with eyes, skin, and clothes

1.3.5.2.6

Wash thoroughly after handling

1.3.5.2.7

Keep container closed

1.3.5.3 Reagents

- Sodium Perborate Tetrahydrate $NaBO_2 \cdot H_2O_2 \cdot 3H_2O$ (purchase commercially)
- Sodium Carbonate Na_2CO_3 (purchase commercially)
- Luminol $C_8H_7N_3O_2$ (purchase commercially)
- Deionized H_2O

1.3.5.4 Chemical Recipe

- 3.5 grams of Sodium Perborate (#1)
- 25 grams of Sodium Carbonate and 0.5 grams of Luminol (#2)
- 500 ml of diH_2O in a capped container

Note: All components listed above can be weighed out in advance and placed in appropriately labeled containers. The dry chemicals (#1 and #2) should be stored protected from light at room temperature.

1.3.5.5 Chemical mixing order (mix immediately prior to use)

1.3.5.5.1

Add Sodium Perborate (#1) to the capped diH2O container

1.3.5.5.2

Shake well for 30-45 seconds

1.3.5.5.3

Add Sodium Carbonate and Luminol (#2)

1.3.5.5.4

Shake well until contents appear dissolved (1-2 minutes)

1.3.5.5.5

Allow undissolved contents to settle to bottom of container

1.3.5.5.6

Transfer Luminol solution into a spray mist bottle

Note: Mixing the chemicals within the misting bottle is acceptable if the design size allows for chemical entrance, adequate product distribution, and blending.

1.3.5.6 Controls

Known blood and a copper penny should be included as positive controls when using the luminol spray as indicators of the success and degree of relative intensity of the chemiluminescence reaction. These will be run every time a new batch of luminol reagent is prepared. The lot numbers, control results, and the date of QA/QC shall be documented in STACS-CW.

1.3.5.7 Procedure

Under darkened conditions, immediately after mixing chemicals, mist the area of interest. Refrain from over-spraying which can dilute the sample and thus negatively affect subsequent DNA testing. Mark luminescent areas for collection and/or subsequent testing.

1.3.5.8 Results and Interpretation

Positive reaction: Chemiluminescence observed
Negative reaction: No chemiluminescence observed or chemiluminescence observed as a result of a false positive reaction
Inconclusive reaction: Obtain result other than that specified for a positive or negative reaction or a failure of the controls.

1.3.5.9 References

Grispino, R.R.J. (1990). The Effect of Luminol on the Serological Analysis of Dried Human Bloodstains. Crime Laboratory Digest, 17(1), 13-23.

Gross, A. M., Harris, K. A., Kaldun, G. L. (1999). The Effect of Luminol on Presumptive Tests and DNA Analysis using the Polymerase Chain Reaction. Journal of Forensic Sciences, 44(4), 837-840.

Hochmeister, M.N., Budowle, B., Baechtel, F.S. (1991). Effects of Presumptive Test Reagents on the Ability to Obtain Restrictive Fragment Polymorphism (RFLP) Patterns from Human Blood and Semen Stains. Journal of Forensic Sciences, 36(3), 656-661.

Lytle, L.T., Hedgecock, B.A. (1978). Chemiluminescence in the Visualization of Forensic Bloodstains. Journal of Forensic Sciences, 28, 550-562.

MSDS obtained from an Internet search:
Luminol. Retrieved on 3/12/07 from http://www.sciencestuff.com/msds/C2003.html

Royal Canadian Mounted Police Procedures. Retrieved 2/16/07 from http://www.rcmp-grc.gc.ca/firs/recipes/luminol_e.htm

Virginia Department of Forensic Science. Retrieved 4/10/07 from http://www.dfs.virginia.gov/services/forensicBiology/manuals/procedures/02%20-%20II-Presumptive%20and%20Confirmatory%20Tests/05%20-%20Chapter%201%20-%20DETECTION%20OF%20BLOOD.pdf.

1.3.6 ONESTEP ABAcard® HemaTrace® For The Indication Of Human Blood

1.3.6.1 Principle

The OneStep ABAcard ® Hematrace ® is an immunochromatographic 1-step test for the detection of human blood. 150 microliters of sample is added to the sample well 'S' and allowed to soak in. If human hemoglobin (hHb) is present in the specimen, it will react with the mobile monoclonal antihuman Hb antibody and a mobile antigen antibody complex is thus formed. This mobile antibody-antigen complex migrates through the absorbent device towards the test area 'T'. In the test area 'T', a polyclonal

antihuman Hb antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the hHb concentration in the sample exceeds 0.05 microgram/ml the pink dye particles will form a pink colored band in the test area 'T' indicating a positive result. As an internal positive control, hHb antibody-dye conjugates cannot bind to the antibody in the test area '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 the control area 'C', indicating that the test has worked properly and procedures have been followed.

1.3.6.2 Materials

OneStep ABAcard ® HemaTrace ® from Abacus Diagnostics
Manufacturer-provided buffer vials

1.3.6.3 Controls

Positive and negative controls shall be used for function verification. These will be run prior to the use of the kit lot. Future shipments of the same lot number will still need controls run for function verification to account for potential differences in shipping conditions. The positive control shall be a known human blood sample. The lot number, control results, and the date of QA/QC shall be documented in STACS-CW.

1.3.6.4 Procedure

1.3.6.4.1

Extract the questioned sample using one of the recommended extraction techniques below.

1.3.6.4.1.1

For fresh bloodstained materials:

Extraction of specimens from fresh bloodstained materials should be performed in an extraction tube.. Soak a 2–5 mm^2 cutting of the specimen in 250µl of the manufacturer-provided buffer for 1 to 5 minutes, or until a straw-colored extract is obtained. A red extract should be avoided due to the potential for the high dose hook effect, which could give a false negative result. If a red extract is obtained, a dilution of the extract should be used to conduct or repeat the test.

1.3.6.4.1.2

For bloodstains that may be aged, laundered, or exposed to environmental insults (e.g. exposure to direct sunlight, weathering):

Extraction of specimens from aged bloodstained materials, items that may have been laundered, or items that have been exposed to environmental insults should be performed in an extraction tube. Soak the specimen for 30 minutes or longer in 250µl of the manufacturer-provided buffer. Some specimens may require hours to extract, or even an over-night extraction, if very aged or compromised by environmental insults for an extended period of time. These samples may also require a larger cutting for extraction.

1.3.6.4.1.3

Small Bloodstains:

If the stain is limited in size only a portion of the extract may be used, but a minimum of 200 µl should be applied to the test card (more than 4 drops). Discretion should be used in determining whether a small bloodstain should undergo HemaTrace® testing or be preserved for DNA analysis without further body fluid testing.

1.3.6.4.1.4

Chemically treated bloodstains (such as TMB or other presumptive tests):

Results may be obtained from chemically treated bloodstains. However, it is important to make sure that the pH of the diluent with the chemically treated bloodstain is between 1 and 9. pH strips may be dipped directly into the sample to test/adjust the pH. Do not use sodium hydroxide gel to adjust the pH.

1.3.6.4.2

Remove the card from the sealed pouch and label card and tube accordingly.

1.3.6.4.3

Add 4 drops or 200µl of sample extract to the sample well 'S' on the card.

1.3.6.4.4

Read results at 10 minutes. Positive results may be observed as early as 2 minutes, depending upon the hHb concentration. For negative results, one must wait for the full 10 minutes. Positive results read after 10 minutes should be interpreted with caution, as non-specific reactions may occur.

1.3.6.5 Storage

1.3.6.5.1

Specimen collection tubes containing human hemoglobin can be stored or transported for 13 days between 2-8ºC, for 10 days between 14-30ºC and for 3 days at 37ºC.

1.3.6.5.2

HemaTrace® kits should be stored at room temperature (below 82°F) until the expiration date as printed on the kit/test. Do not use the kit after the expiration date. Avoid freezing.

1.3.6.6 Results and Interpretation

Positive reaction: If there are two pink lines, one each in the test area 'T' and in the control area 'C'. A positive reaction indicates the presence of hemoglobin. A positive reaction in conjunction with a positive presumptive test (i.e. phenolphthalein, TMB, Hemastix) may be reported as indicative of blood. On occasion, a positive Hematrace reaction may be observed even when the presumptive tests are negative. These results must be interpreted with caution. Hemoglobin may be present in body fluids

other than blood in low concentrations and should be reported as inconclusive when presumptive tests for blood are negative.

Negative reaction: If there is only one pink line (in the control area 'C'). Note: The "high dose hook effect" may give false negative results due to the presence of high concentration of hHb. In such cases the sample may be retested using a 1:100 dilution.

Invalid reaction: If there is no pink line visible in the control area 'C'. (The test should be repeated if determined to be invalid.)

1.3.6.7 Limitations

1.3.6.7.1

ABAcard ® HemaTrace ® is only for in vitro detection of human hemoglobin (primates) for forensic use. The test must be performed in strict accordance with the instructions to obtain accurate and reproducible results.

1.3.6.7.2

Positive results may be obtained from whole blood from the domestic ferret. Therefore, this test is used only to indicate the presence of human blood.

1.3.6.8 References

Johnston, S. et al, "Validation Study of the Abacus Diagnostics ABAcard® HemaTrace® Membrane Test for the Forensic Identification of Human Blood, Can. Soc. Forens. Sci. J., Vol 36 No. 3 (2003), pp 173 - 183.

Abacus Diagnostics ABAcard® HemaTrace® for the Forensic Identification of Human Blood Technical Information Sheet.