6 Blood Detection

Please refer to SOP #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines, of the Phenolphthalein Test Kit, Luminol and ABAcard® HemaTrace® Test Devices.

Many samples submitted to the Houston Police Department Crime Laboratory are tested for the presence of blood. Generally, a sample will be subjected to a presumptive phenolphthalein test. If positive, the sample will be subjected to a confirmatory test for the presence of human blood. Testing is typically discontinued on phenolphthalein negative samples.

6.1 Phenolphthalein Test Kit

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. The test is exceedingly sensitive to minute traces of hemoglobin and its derivatives but will produce a false positive reaction in the presence of any of a number of oxidizing substances. Should a color reaction take place, the result only indicates the possible presence of blood; the test is therefore a presumptive test.

Safety

This reagent presents the following hazards:

Solution B: Flammable. Handle with care. Harmful or fatal if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Light sensitive! Avoid light, heat, sparks, and flame. Keep container tightly closed.

Solution C: Handle with care. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Avoid heat, sparks, and flame. Keep container tightly closed.

Wear gloves, lab coat, and mask during preparation and use. Broken skin should be covered.

Equipment, Materials, and Reagents

- Sterile diH_2O
- Sterile cotton swab(s) or filter paper
- Phenolphthalein Forensic Test reagents* - Solutions B and C

* Solution A is also included in the Phenolphthalein Forensic Test. However previous testing showed that using sterile diH_2O in place of Solution A did not affect the outcome of this test. Therefore, sterile diH_2O will be used in place of Solution A.

Procedure

1. Moisten a sterile cotton swab or piece of filter paper with sterile diH_2O.
2. Rub or press down on the suspected bloodstain with a sterile cotton swab or filter paper to allow for any possible blood to transfer.

3. Apply approximately 1 drop of Solution B to the swab or filter paper.
4. Observe briefly to identify color change. A green color is often observed when blood is present; any other color change should be documented.
5. Add approximately 1 drop of Solution C to the swab or filter paper.
6. Observe the swab or filter paper for an immediate pink color change indicating a positive reaction.
7. After interpretation, mark the items/areas tested with the results.
8. Discard the swab or filter paper by placing it in the biohazard trash.

Interpretation

Appearance of an immediate pink color change after the application of Solution C is a presumptive positive result for blood. No color change indicates a presumptive negative result for blood. A negative result indicates that blood is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

6.2 Luminol

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. In the case of luminol, the catalytic oxidation of the substrate compound produces light. Because the reagents are applied as a spray over a large area, the test is primarily used in the visualization of bloodstain patterns. As with similar presumptive blood tests, a number of oxidizing substances will produce a false positive reaction. Should a positive reaction take place, the result only suggests the presence of blood; the test is therefore a presumptive test.

Safety

This reagent presents the following hazards: luminol is an irritant. Sodium perborate and sodium carbonate are toxic and irritating. Avoid breathing dust; do not get in eyes, on skin, or on clothing. Avoid breathing sprayed solution. Wash hands after handling. Gloves must be worn during testing. Broken skin should be covered.

Equipment and Supplies

- Luminol powder: 3-aminophthalhydrazide
- Sodium carbonate
- Sodium perborate
- Sterile diH_2O
- Two darkened or foil wrapped spray bottles
- Scale

Preparation (Reagent A)

1. Measure 250 mL of sterile diH_2O.
2. Add 2.5 g of sodium carbonate.

3. Mix until all of the powder is dissolved.
4. Add 0.5 g of luminol powder.
5. Mix until all of the powder is dissolved.

Preparation (Reagent B)

1. Measure 250 mL of sterile diH_2O.
2. Add 6.5 g of sodium perborate.
3. Mix until all of the powder is dissolved.

Procedure

1. Mix equal volumes of Reagents A and B to create a luminol mixture. The mixture is unstable and must be prepared fresh immediately before use; it is only stable for about one hour.
2. Darken the examination room as completely as possible.
3. Spray luminol mixture onto evidence and observe for luminescence (spraying will deposit a light white film on surfaces).
4. Document or photograph luminescent areas.
5. Re-spray as necessary. The ability to further test the stain decreases with increased or repeated spraying. Excessive spraying will cause stains to run, therefore, excessive spraying of non-porous surfaces is not suggested.
6. Dispose of excess solution in the regular sink, flushing with water.

Interpretation

Faint to strong luminescence shows oxidation of the luminol reagent and represents a positive presumptive result for blood. A negative result (a lack of luminescence) indicates that blood is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

6.3 ABAcard® HemaTrace® Test Devices

Scope

The ABAcard® HemaTrace® test is a qualitative detection method specifically designed for forensic identification of human blood. Heme is a part of the hemoglobin molecule that is characteristic of red blood cells found in blood. The ABAcard® HemaTrace® has been shown to detect as little as 0.05 µg/ml of hemoglobin in 10 minutes.

The sample is added to a sample well where any detectable human hemoglobin (hHb) present in the sample will bind with mobile monoclonal antihuman Hb antibody which has an attached pink dye particle. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where an immobile polyclonal antihuman Hb antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody

sandwich. Conjugated pink dye particles become visible in the area of immobilized antibody (in the test area: "T") when the hHb concentration in the sample exceeds 0.05 µg/ml. The resultant pink band indicates 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 that appropriate procedures have been followed.

Hemoglobin from the Family Mustelidae (ferrets) and higher primates also gives a positive result. The examiner must evaluate the likelihood of blood from either of these sources being present when this test is used as, forensically, the practical implications of this cross reactivity is minimal.

The ABAcard® HemaTrace® test may be skipped if, in the analyst's opinion, consumption for human origin testing will reduce the potential success of subsequent DNA analysis. Samples that are of limited size may be documented as Quantity Not Sufficient For Further Analysis ("QNS"). Only a presumptive screening result will be reported but DNA analysis may be performed.

Equipment, Materials, and Reagents

- ABAcard® HemaTrace® Test device – one per sample
- ABAcard® HemaTrace® extraction buffer
- Pipettes and pipette tips
- Timer

Procedure

1. Allow the sample(s) to warm to room temperature if they have been refrigerated.
2. Extract body fluid by placing suspected bloodstain sample into the extraction buffer provided with the kit and allow to sit for 5-30 minutes. Aged samples may be less soluble, and should therefore be soaked closer to 30 minutes. The extract(s) may be placed at room temperature in an ultrasonic cleaner for 5-15 minutes. The analyst must exercise good judgment regarding the amount of sample to be portioned for each ABAcard® HemaTrace® test device, providing sufficient sample for subsequent DNA analysis. Stain intensity and size are a few characteristics that should be considered. Generally, up to approximately ¼ of a swab or up to approximately $(1\ cm)^2$ of a stain may be consumed for this analysis.
3. For each sample, unwrap <u>and label</u> an ABAcard® HemaTrace® test device.
4. Add 150 µl of supernatant to the sample well "S" of the device.
5. Results may be read up to 10 minutes from application of the supernatant. Positive results can be seen as early as 2 minutes.
6. Record the ABAcard® HemaTrace® test results on the Serology Results worksheet.
7. Have a second qualified analyst examine the ABAcard® HemaTrace® test device and verify the documented test result(s) by initialing and dating the appropriate Serology Results worksheet.

Interpretation

There are two lines that appear on the test device:

1. **Control "C" line:** the presence of this line confirms the integrity of the test components and the test procedure. It is expected for all tests and must be present. Absence of the "C" line is an invalid result and the test should be repeated if adequate sample remains.
2. **Test "T" line:** A line indicates the presence of human hemoglobin and should be documented as a positive result. The absence of a line or a line that appears after 10 minutes is a negative result. A negative result indicates that blood is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

Note: the presence of the "High Dose Hook Effect" may give false negative results due to the presence of a high concentration of human hemoglobin in the sample. When huge amounts of human Hb bind to the antibody to form an antigen-antibody complex but also free Hb migrates towards the test area "T", the antibody in the test area "T" is blocked by this free Hb. As a result, the mobile antigen-antibody complex with the pink color cannot bind to the antibody and the pink line cannot form in the test area "T", even though a lot of Hb is actually present. In such cases where human blood is strongly suspected, the extract may be retested using a 1:10 or 1:100 fold dilution.

6.4 References

1. Abacus Diagnostics, Inc. ABAcard® HemaTrace® For the Forensic Identification of Human Blood Technical Information Sheet.

2. Gaensslen, Robert E. 1983. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* U.S. Department of Justice, National Institute of Justice. Sections 10.3.2 and 10.3.3.

3. Lee, Henry C. 1982. Identification and grouping of bloodstains. *In*: Forensic Science Handbook, Volume 1. Richard Saferstein, ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 273.

4. Lytle, L. T. 1978. Chemiluminescence in the visualization of forensic bloodstains. Journal of Forensic Sciences 23(3): 550-562.

5. Saferstein, R. *Forensic Science Handbook: Identification and Grouping of Bloodstains*, (Prentice-Hall, Inc., 1982), p. 274.

6. Stoilovic, M. 1991. Detection of semen and blood stains using Polilight as a light source. *Forensic Science International* 51: 289-296.