ABAcard® HemaTrace®

Background

The ABA Card HemaTrace is an indicator test for human (primate) blood. This immunochromatographic test offers extremely high sensitivity and specificity and is capable of detecting trace levels of human hemoglobin. This is only an indicator test because cross reactivity has been observed with ferret and mink blood. See **ABAcard HemaTrace Validation Notebook**.

Summary of Procedure

Sample is added to sample well "S." If human hemoglobin (hHb) is present it will react with the mobile monoclonal antihuman Hb antibody-dye conjugate. A mobile antigen antibody complex is then formed. The antigen antibody complex will migrate through the absorbent filter toward test area "T." An immobilized antihuman Hb antibody is present in the test area. The immobilized antibody will capture the mobile antigen antibody complex and conjugated pink dye particles will concentrate in the narrow test window when the hHb concentration in the sample exceeds 0.05µg/ml. The pink band in the test window indicates a positive test result.

Sample Handling

Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling**.

Warnings and Precautions

The high dose hook effect occurs when the hHb concentration is too high. The hHb will bind to the hHb antibody-dye conjugate. Additionally, free hHb will migrate towards the test area. The free hHb will block the test area preventing the mobile antigen antibody-dye conjugate complex from binding. This results in the lack of a pink band in the test area (false negative). In this case a new test may be performed with a dilution of the sample.

The following stains have been tested by the manufacturer and determined to have no interference: Luminol treated, Coomasie blue treated, ninhydrin treated, soil debris, tide detergent treated, stains off of plant material, stains off of leather, stains from washed jeans and bleached stains.

Reagents and Materials

See **Appendix B** for reagent preparation
10% Bleach
MBG Water
100% Ethanol
5% ammonia
ABAcard® HemaTrace® test device
ABAcard® HemaTrace® extraction buffer
PH paper/meter

Lab Coat
Kimwipes
Gloves
Disposable bench paper

Protective Eyewear
Sample handling tools (scissors, scalpel blades, forceps, etc.)
Vortex
20-200µl pipette
20-200µl pipette tips

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Evidence Examination Worksheet** or other appropriate worksheet is required for documentation. All information must be completed.

All results must be verified by a second qualified analyst. If a second qualified analyst is unavailable photo documentation of the result is acceptable.

Positive Control

An internal positive control is included in this test. The hHb antibody-dye conjugate can not bind to the immobilized antihuman Hb antibody in the test area due to the lack of hHb. The control area, "C," contains an immobilized anti immunoglobin antibody. The hHb antibody-dye conjugate is captured by the anti immunoglobin antibody in the control area resulting in a pink band.
If a pink band appears in the control area "C", the test is valid. If a pink band does not appear in the control area "C", the test is not valid and a new test should be performed.

Procedure

1. Allow the sample to warm to room temperature if it has been refrigerated.

2. For fresh bloodstained material:
    a. Make a cutting of the fresh bloodstained material.
    b. Soak the cutting for 1-5 minutes in the entire volume of extraction buffer.

    For bloodstains that have been stored at room temperature up to five years:
    a. Make a cutting of the bloodstained material.
    b. Soak the cutting for 30 minutes in the entire volume of extraction buffer.

Note: For aged/difficult bloodstains consult manufactures package information:

3. Remove the device from the sealed pouch and label with the sample ID/date/initials.

4. Add 150µl of extracted sample to sample well "S."

5. Read result in 10 minutes. Positive results can be seen as early as two minutes. Negative results must be read after the full ten minutes.

Results and Conclusions

The antihuman Hb antibody-dye conjugate is captured by the anti immunoglobin antibody in the control area resulting in a pink band. A pink band in the control area, "C," indicates the test worked properly. If no pink line is present in the control area "C", the test failed.

Positive Result: The presence of two lines, one in the test area, "T," and the control area, "C," is indicative of a positive test result. This would be reported as human blood is indicated on the appropriate report.

Negative Result: If there is only one pink line in the control area "C" and no pink line in the test area, "T," the test is negative. This would be reported as no human blood is indicated on the appropriate report.

Inconclusive Result: If the line in the test area, "T" is not conclusive or decisive, the test is inconclusive. This would be reported as the test for the indication of human blood was inconclusive.

Technical Assistance

For information and assistance regarding the performance or applications, contact Abacus Diagnostics, Inc Phone (877) 225 - 9900

Reference

Abacus Diagnostics, Inc., Technical Information Sheet, ABAcard® HemaTrace®.