FBS03- ABAcard® HemaTrace® Test for the Identification of Human Blood

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1. Scope

1.1. This procedure is used to confirm the presence of blood.

2. Background

2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.

2.2. Hemoglobin is an iron-containing protein located in red blood cells that primarily serves to transport oxygen and carbon dioxide within the body. It is one of the most abundant proteins in blood, with concentrations approximately 120-160 mg/ml and 140-180 mg/ml for women and men, respectively.

2.3. The ABAcard® HemaTrace® kit test is an immunochromatographic test for the forensic identification of human blood. If human hemoglobin is present in a sample, it will react with the dye-conjugated mobile monoclonal antihuman hemoglobin antibody forming a mobile antigen-antibody complex. This antigen-antibody complex migrates through the absorbent test strip toward the test "T" region of the test card. In the "T" region an immobilized polyclonal antihuman hemoglobin antibody is present. This immobilized antibody captures the mobile antigen-antibody complex resulting in an antibody-antigen-antibody sandwich

complex. When the human hemoglobin concentration in a sample is greater than 0.05 µg/ml (minimum detection level), the antibody-antigen-antibody complex forms a pink colored band in the "T" region indicating a positive result, thus confirming the presence of blood.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.

3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

4.1. HemaTrace® test cards

4.1.1. Note: Each lot of HemaTrace® test cards must be evaluated prior to use. See FBQ19 for the information regarding the procedure for evaluation.

4.2. HemaTrace® Extraction Buffer (supplied with the kit)

5. Standards and Controls

5.1. The Positive and Negative Controls are cut after all the questioned stains. Control extracts should be the last samples added to the cards in a sample set. One set of controls may be tested with each sample set. Record the results in the casework documentation.

5.1.1. ~3 mm x 3 mm cutting of a known bloodstain (FBR02) is extracted in 300 µl of HemaTrace® Extraction Buffer and labeled as a Positive Control. This control should exhibit a solid pink line at the "T" (test) region and the "C" (control) region. A positive result may be recorded at any time within the 10 minute development period.

5.1.2. ~3 mm x 3 mm cutting of a sterile, unstained piece of cotton sheeting is extracted in 300 µl of HemaTrace® Extraction Buffer and labeled as a Negative Control. This control should exhibit a solid pink line at the "C" (control) region. A negative result is valid if a card remains negative at the "T" (test) region for the full 10 minute development period.

6. Calibration

6.1. Not applicable

7. Procedures

7.1. Take ~ 3 mm x 3 mm cutting or ~1/4 swab cutting from a suspected blood stain and place it in an appropriately labeled 1.5 ml or 2.0 ml microcentrifuge tube. Note: A larger cutting, ~ 5 mm x 5 mm may be taken for less concentrated stains.

7.2. Add 300 µl of buffer to samples followed by the controls.

7.3. Allow the samples to incubate at room temperature for at least ~ 30 minutes or overnight at 4°C. Samples may be placed on an orbital shaker during the incubation time.

7.4. Remove the HemaTrace® card from packaging. Take care to confirm that the lot number being used has previously been approved for casework and has not expired.

7.5. Label the card with the appropriate sample identification number or control name.

7.6. Add 150 µl of each sample's extract to the sample well of card. Controls should be added last.

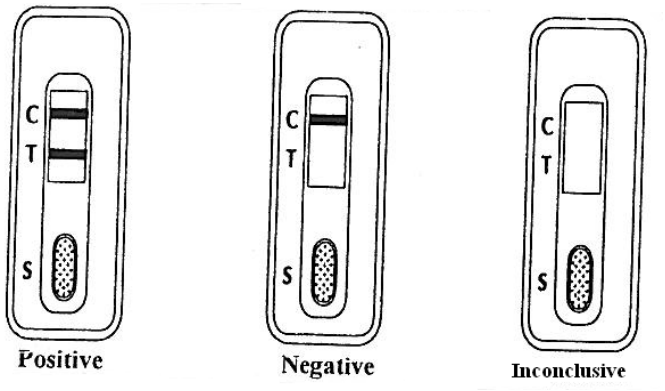
7.7. Allow the card to remain at room temperature for 10 minutes.

7.8. Read and record the results on the designated HemaTrace® worksheet. A positive result may be recorded at any time within the 10 minute period, however an inconclusive or negative result is not confirmed until the full 10 minutes has elapsed. Inconclusive results should be retested if possible.

HemaTrace® Results

Card Result	**Recorded Result**

Pink lines at C and T	Positive
Pink line only at C	Negative
Pink line only at T	Inconclusive
No Pink line at C and T	Inconclusive



7.9. A photograph of the cards is taken and included in case documentation.

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6).*

11. Limitations

11.1. No test result should be recorded after the 10 minute development period has elapsed.

11.2. Insufficient sample quality and/or quantity could limit the development of a positive reaction.

11.3. Samples that are weak positive or do not appear clearly in the photograph require a second reader (qualified analyst) whose initials are recorded on the HemaTrace® worksheet.

11.4. The samples must be properly diluted in order to avoid the High Dose Hook Effect. If there is an excess amount of hemoglobin in the sample, hemoglobin will bind both to the antibody to form an antigen-antibody complex but also free hemoglobin migrates toward the test area 'T". The antibody in the test area "T" is blocked by this free hemoglobin. Thus, the mobile antigen-antibody-antibody complex with the conjugated pink dye cannot bind to the antibody. The formation of the sandwich complex is repressed and no pink test result line is formed, resulting in a false negative.

11.5. If the High Dose Hook Effect is suspected, the sample maybe retested using a 1:10 and/or 1:100 dilutions. To avoid the High Dose Hook Effect, ideally the stain extract should be a straw colored solution.

11.6. Positive results may be obtained in body fluids other than blood due to the sensitivity of the HemaTrace® test to detect trace amounts of hemoglobin in other body fluids. However, coupled with the results of a presumptive test, this is not an issue to confirm the presence of blood.

11.7. Positive results may be obtained from blood samples from primates (i.e. chimpanzee, monkey and orangutan) and ferrets.

12. Documentation

12.1. FBU Serology Examination Worksheet

12.2. FBU HemaTrace® Worksheet

12.3. FBU Diagram/Photo Worksheet

12.4. FBU Report of Results

13. References

13.1. Reynolds, Mark. The ABAcard® HemaTrace® A confirmatory identification of human blood located at crime scenes. IABPA News. v20(2), p 4-10, 2004.

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13.3. Kristaly, A., Smith, D.A.S. Validation of the ABAcard® HemaTrace® for the rapid forensic identification of human blood. 1999. Unpublished.

13.4. Abacus Diagnostics. ABAcard® HemaTrace® for the Forensic Identification of Human Blood, Technical Information Sheet, Rev: April 2005.

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13.6. Onondaga County Center for Forensic Sciences. Validation of the OneStep ABAcard® HemaTrace®. Unpublished.

13.7. Spear, T.F., Brinkley, S.A. the HemSelect test: A Simple and Sensitive Forensic Species Test. J. For. Sci. Soc. V34(1), p41-46, 1994.

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13.9. Seratec Diagnostica. Seratec® HemDirect Hemoglobin Assay, Product Literature Insert, Rev: June 2009.

13.10. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)

13.11. *FSL Departmental Operations Manuals* (Current Versions)

13.12. *FSL Laboratory Operations Manuals* (Current Versions)