Forensic Biology Section

HemaTrace Human Blood Detection

1. Scope:

1.1 The *OneStep* ABAcard HemaTrace is an immunochromatographic test specific for primate hemoglobin and is a reliable confirmatory test for human blood.

1.2 If primate hemoglobin is present, it will react with the antihuman hemoglobin antibody and a mobile antigen-antibody complex is formed. The complex migrates along the membrane to the Test ('T') area where a polyclonal hemoglobin antibody is waiting. The mobile antigen-antibody complex and the polyclonal hemoglobin antibody join, forming an antibody-antigen-antibody sandwich. The pink dye is impregnated into the membrane. When the hemoglobin in the sample exceeds 0.05 µg/ml, the pink dye particles and the antibody-antigen-antibody sandwich will form a pink band in the 'T' area.

2. Safety:

2.1 Treat all biological specimens as potentially infectious. Gloves and a laboratory coat must be worn at all times. Follow Universal Precautions.

3. Specimens:

3.1 Suspected bloodstain or liquid blood.

4. Reagents and Special Supplies:

- ABAcard HemaTrace test device.
- TE Buffer.
- Optional: Extraction Buffer, pH buffer test strips, and 5% ammonia.

5. Procedural Notes:

5.1 The age and intensity of a bloodstain can affect the ability to obtain a positive result.

5.2 A positive control (human blood) and a negative control (or reagent blank) will be analyzed at the same time as the unknown samples.

5.2.1 The positive control will be human blood taken from a volunteer, spotted onto a stain card and air dried, and then stored in a freezer (-10 ^{o}C or below).

5.2.2 The negative control will be the TE buffer, extraction buffer, or reagent blank.

5.3 A negative result does not preclude the possibility that a stain is a human bloodstain. DNA profiling may still be attempted at the discretion of the Analyst.

5.4 There is a Control ('C') area on the test membrane to demonstrate the test is performing correctly and to prevent false negative results due to test failure.

5.5 The in-house validation of HemaTrace tested its reactivity to blood from a variety of animals. The validation did show cross reaction with members of the Mustelidae family, including weasels, ferrets, and mink. Considering the rare occurrence of encountering these blood types at a crime scene, HemaTrace can be considered a reliable confirmatory test for human blood with forensic samples.

6. HemaTrace Extraction Buffer:

6.1 One test device and one extraction buffer tube are needed for each stain along with test devices for the positive and negative controls.

6.2 Remove the test devices from the sealed pouches and label each extraction tube and test device with unique identifiers (e.g. case number and item number; "positive control"; "negative control" or "reagent blank").

6.3 Place a small amount of stain in the tube of extraction buffer for testing. Sample size will be dependent on the size and heaviness of the suspect stain. Often a 1 mm square cutting is adequate.

6.4 Stain should be allowed to extract until the stain dissolves and the buffer takes on a red color, or for 15 minutes or more. In some instances, the stain may not dissolve.

6.5 Add approximately 80 µl of the extract to the sample well marked 'S' on the test device.

6.6 Determine results after a reaction time of 10 minutes. Positive results can be apparent as early as 2 minutes; negative results must be allowed to react for 10 minutes.

7. TE Buffer Extraction:

7.1 One test device is needed for each stain along with test devices for the positive and negative controls.

7.2 Remove the test devices from the sealed pouches and label each extraction tube and test device with unique identifiers (e.g. case number and item number; "positive control"; "negative control" or "reagent blank").

7.3 A positive control should be extracted with the same TE Buffer under the same conditions as the samples.

7.4 An aliquot of the reagent blank can serve as the negative control.

7.5 Add approximately 80 µl of the supernatant from the first room temperature TE Buffer incubation from suspected bloodstains, the positive control, and the corresponding reagent blank or negative control to the sample well marked 'S' on the test device.

7.6 Determine results after a reaction time of 10 minutes. Positive results can be apparent as early as 2 minutes; negative results must be allowed to react for 10 minutes.

8. Alternative Extraction for Aged Stains:

8.1 Soak the sample in 2 to 3 drops of 5% ammonia for approximately 2 to 5 minutes.

8.2 Allow the ammonia to evaporate.

8.3 Add 2 to 3 drops of the HemaTrace Extraction Buffer.

8.4 The pH of the resultant liquid must be between 1 and 9 (check with pH test strips).

8.5 The extract (up to 80 µl) may be added to the sample well immediately.

9. Results Interpretation:

9.1 **Positive results** are indicated by the presence of a pink line in the 'C' and 'T' areas of the test device. This indicates that higher primate hemoglobin is present in the stain at 0.05 µg/ml or above.

9.2 **Negative results** are indicated by the presence of a pink line in the 'C' area of the test device and no reaction in the 'T' area of the test device. This may indicate there is no human hemoglobin present in the stain, the level of human hemoglobin is below 0.05 µg/ml, or the negative result could be possible due to the 'High Dose Hook Effect'.

9.3 **Cross Reactions**: The in-house validation of HemaTrace did show cross reaction with members of the Mustelidae family, including weasels, ferrets, and mink. Considering the rare occurrence of encountering these blood types at a crime scene, HemaTrace can be considered a reliable confirmatory test for human blood with forensic samples.

9.4 **High Dose Hook Effect**: In immunoassays such as this, samples with extremely high concentrations of hemoglobin may give false negative results (known as 'High Dose Hook Effect'). If a false negative due to saturation of the immunoassay is suspected, retest the stain after diluting the extraction buffer (e.g. one hundred-fold dilution of extract).

9.5 **If no pink line develops in the 'C' area of the test device**, the test is **invalid** and must be repeated. Also, if the results from the human positive control or the negative control are not appropriate, that set of tests are invalid and all of the samples and controls should be re-tested.