Evaluation of the ABAcard HemaTrace for the Forensic Identification of Human Blood

Connie J. Swander, and Jennifer G. Stites
Michigan State Police, Forensic Laboratory
103 S. James
Grayling, MI 49738

ABSTRACT:

This study examined the ABAcard HemaTrace for it's usefulness in the forensic identification of human blood. The goal of this study was to verify and compare the sensitivity of the HemaTrace card to other immunohematological tests currently available. Serial dilutions of human blood were examined as were bloodstains subjected to different conditions encountered at crime scenes, or in the laboratory. The results have shown the ABAcard HemaTrace to be a highly sensitive, convenient, and rapid test for the identification of human blood both in the laboratory and at the crime scene.

BACKGROUND:

The ABAcard HemaTrace is an immunochromatographic 1-step test for the detection of human blood. If human hemoglobin is present in the questioned bloodstain, it will react with the mobile monoclonal antihuman antibody and a mobile antigen antibody complex is formed. This complex then migrates through the HemaTrace's absorbent membrane towards the test area 'T'. In this test area 'T', a polyclonal antihuman hemoglobin antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. When the human hemoglobin concentration is above a certain minimum detection limit (0.05 ug/ml) the purple dye particles present in the device will form a purple band at the 'T' area which is indicative of a positive result for the presence of human blood.

As an internal control, human hemoglobin antibody-dye conjugates cannot bind to the antibody in the test area 'T', but are captured by an immobilized anti immunoglobbulin antibody present in the control area *'C* forming a complex. The captured purple dye particles will thus form a band in the control area *'C* forming a complex, indicating that the test has worked properly and proper procedures have been followed. To interpret results, the presence of two colored bands, one in the test area 'T' and one in the control area *'C'*, indicates a positive result, while a band only in the control area 'C' would indicate a negative result (provided no "high dose hook effect"). If no band occurs at the control area 'C' the test is determined invalid meaning either the HemaTrace card itself was defective or proper procedures were not followed.

MATERIALS AND METHODS:

1. Known human blood.
2. One Step ABAcard HemaTrace (manufactured by Abacus Diagnostics, West Hills, CA) Used according to manufacturer's directions.
3. Titan IV Double Diffusion plates (manufactured by Helena Laboratories, Beaumont, Texas)
4. Anti human sera (manufactured by Serological Research Institute, Richmond, CA)
5. Tetramethylbenzidine solution (TMB)
   3.3,5.5 tetramethylbenzidine (Aldrich Co.) 2 g.
   Glacial acetic acid 100 ml.
   3% Hydrogen peroxide
6. Leucocrystal Violet solution (LCV)
   5-sulfosalicylic acid 10g.
   3% Hydrogen peroxide 500 ml.
   1.1 gms leucocrystal violet
   4.4 gms sodium acetate
7. "Hemastix" test strips (manufactured by Bayer)

Sensitivity:

Known human blood with a hemoglobin concentration of approximately 13 g/dl was used. Serial dilution's of the known blood were made using the extraction buffer provided in the ABAcard HemaTrace kit. The required 150 uls of each dilution were added directly to a HemaTrace sample 'S' well respectively. The results were read and recorded at 10minutes. Ouchterlony double diffusion plates were run on the same serial dilutions. Approximately 5 uls of dilutions 1: 16 through 1 :65,536 were applied to their respective wells and the results were read and recorded at 24 hours.

Conditioned bloodstains:

Known human blood was subjected to various conditions and chemicals commonly encountered at crime scenes and in the laboratory. This was done by applying the blood directly to the condition in question or applying the condition to a bloodstained cotton swatch. A portion of each of the conditioned bloodstains was then tested utilizing the HemaTrace and Ouchterlony double diffusion technique. The results were read and recorded as documented.

Forensic Correlation:

Approximately 100 ul of each of the above serial dilutions was applied to respective 1 cm square white cotton swatches. The swatches were allowed to air dry for 48 hours. The visual color of the swatch was then graded and recorded on a red/brown, yellow/brown, tan, off-white, to white scale. A portion of each of the stained cotton swatches was then tested with Tetramethelbenzidine, Leucocrystal Violet, and "Hemastix" strips (presumptive tests for blood). The results were read and recorded on a 1 + to 4+ scale with 4- being the strongest reaction. The same cotton swatches were tested for species identification utilizing HemaTrace cards. The results were read and recorded as documented.

RESULTS AND DISCUSSIONS:

Sensitivity: *Serial dilutions of known human blood:* Positive results were recorded if a purple band occurred at the 'T' and 'C' areas. Negatives were recorded if a purple band occurred only at the 'C' area, and a test was recorded as invalid if no purple band occurred at the 'C' area. These results were all read. and recorded at 10 minutes. Comparison studies were done using the Ouchterlony double diffusion technique. These results were read and recorded after 24 hours. (See Figure 1)

Human Blood Dilutions	HemaTrace	Ouchterlony
1 :16	+	+
1:32	+	+
1:64	+	+
1 :128	+	+
1:256	+	+
1:512	+	+
1: 1024	+	+
1 :2048	+	0
1 :4096	+	0
1:8192	+	0
1: 16384	+	0
1 :32768	+	0
1 :65536	+	0
1 :262144	+	
1:1048576	+	
1:16777216	+	
1 :33554432	0	
1 :67108864	0	
Tris extraction buffer	0	0

Figure 1: Serial dilution of human blood comparison
0 =negative + =positive

Positive results were obtained with the HemaTrace test up to a dilution of 1: 16,777,216 The Ouchterlony Double Diffusion test showed positive results up to a dilution of 1: 1,024.

Conditioned Blood: *Human blood subjected to conditions commonly encountered at crime scenes, and in the laboratory.* Comparison studies of the conditioned bloods were carried out using HemaTrace, and the Ouchterlony double diffusion technique. Results for each technique were read and recorded the same as the above mentioned results for the comparison of sensitivity. (See Figure 2).

Human Blood Dilutions	HemaTrace	Ouchterlony
Treated w/ luminol (swatch)	+	+
Treated w/ leucocrystal violet (swatch)	+	+
Treated w/ tetramethylbenzidine (swatch)	+	+
Heat fixed (swatch)	0	0
Bleached (swatch)	+	0
Soil debris	+	+
Off of plant material	+	+
Off of leather	+	+
From washed jeans	+	0
1 yr . old frozen lysate	+	+
10 yr. old frozen lysate	+	0
10 yr. old rm. temp. stain	+	0
Rust from vehicle (no blood)	0	0

Figure 2: Human blood subjected to various conditions and chemicals
0 =negative + =positive

Positive results were obtained with both the HemaTrace and Ouchterlony techniques for most of the conditioned blood stains tested. Bleached bloodstains, washed (from denim) bloodstains, and the ten year old blood stains all gave positive results with HemaTrace and negative results with Ouchterlony. It was found that a longer extraction time (up to four hours) of older stains gave a positive HemaTrace reaction (within the 10 minute reading time) whereas Ouchterlony results were still negative.

Forensic Correlation: *An attempt to correlate the visual appearance of the stain dilution to the sensitivity of the presumptive test and the sensitivity of the species(human) identification. (HemaTrace)* This study attempted to compare the visual appearance of the blood stain to its reaction with presumptive and species identification testing. (See Figure 3).

Dilution	Visual	Presumptive			Human HemaTrace
		Hemastix	TMB	LCV	
1:2	Red/brown	4+	4+	4+	+
1: 16	brown/yellow	4+	4+	4+	+
1:64	light tan	4+	4+	4+	+
1: 128	off white	4+	4+	4+	+
1 :256	off white	4+	3+	4+	+
1 :512	off white	3+	2+	3+	+
1: 1024	white	3+	1+	3+	+
1 :2048	white	2+	1+	2+	+
1 :4096	white	1+	0	2+	+
1:8192	white	1+	0	1+	+
1:16384	white	+/0	0	0	+
1 :32768	white	0	0	0	+
1:262144	white	0	0	0	0
1: 1048576	white	0	0	0	0
1:4194304	white	0	0	0	0
1:16777216	white	0	0	0	0
1:33554432	white	0	0	0	0
1:67108864	white	0	0	0	0

Figure 3: Correlation between dilution, color, presumptive, and HemaTrace
0 =negative +=positive

Visual observation detected coloring (staining) of the cotton swatches up to a dilution of 1:512. Presumptive tests were able to detect a positive reaction up to 1:8192 (Hemastix), 1:2048 (TMB), and 1:8192 (LCV), although visually no staining or discoloration of the swatch was noted. The HemaTrace test gave a positive reaction for the presence of human blood up to a dilution of 1:32,768, well past any visual or presumptive determination of the presence of blood.
It should be noted here that the swatch supporting the blood dilutions were extracted using the 2 ml of buffer provided in the HemaTrace kit, thus adding another dilution factor and could therefore explain the difference in sensitivity from the original serial dilutions.

Known animal bloods from deer, cow, pig, horse, dog and cat were also tested utilizing the HemaTrace method with negative results obtained. Human saliva and urine stains also gave negative results.

CONCLUSION:

The ABAcard HemaTrace is a convenient and rapid test for the identification of human blood. It is far superior to the Ouchterlony Double Diffusion technique in terms of sensitivity and tolerance to different conditions. The correlation between the visual appearance of a blood stain and the ability to determine the presence of human blood, makes the ABAcard HemaTrace an acceptable and highly useful tool for the forensic science community both in the laboratory and at crime scenes.

References:

(I) Hochmeister, M., Rudin O., Sparkes, R., Gehrig, C., Schmidt, L. Evaluation of an Immunochromatographic 1-Step Occult Blood Test for the Forensic Identification of Human Blood. J. *For. Sciences.* 1998. Submitted

(2) Spear, T.F. , Binkley, S.A. The HemeSelect test: a simple and sensitive forensic species test. J. *For. Sci. Soc.* V34 (1), P 41-6, 1994.

(3) Fernando, S.A., Wilson, G.S. Studies of the 'hook' effect in the one-step sandwich immunoassay. *J.Imm. Methods.* V151(l-2), p 47-66,1992

(4) Cox, M. A study of the sensitivity and specificity of four presumptive tests for blood. J. *For. Sci.* V36(5), p 1503-11, 1991.

(5) Doherty, P.E. & Mooney, D.J. Deciphering bloody imprints through chemical enhancements. J. *For. Sci* V35(2), p 457-65, 1990.