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The forensic identification of human blood is one of the most important serological tests performed. Disadvantages for the crime scene examiner at the crime scene, are that current identification methods can prove to be unreliable or non-specific with confirmatory results only available following laboratory testing. Through the examination of blood samples on differing substrates and of variable stain age, cross reactivity mixtures and subject to chemical and environmental insults, this paper examines the sensitivity and specificity of Abacus Diagnostic’s AbAcard® HemaTrace® “species of origin” test for blood.

Results, using replicated crime scene samples, indicate that the HemaTrace® “species of origin” test for blood is sensitive and specific to human (higher primate) hemoglobin. Simple to perform and requiring a minimum of equipment, the HemaTrace® can easily be implemented by the crime scene examiner at the crime scene, as a reliable confirmatory test for the detection of human blood.

INTRODUCTION

The timely “confirmatory” identification of blood, in association with species determination, can be of significant investigative importance. Current methods available to the crime scene examiner for the forensic identification of blood, at the crime scene, are generally limited to visual or presumptive chemical means.

The AbAcard® HemaTrace® is an immuno-chromatographic assay for the forensic identification of human hemoglobin (Hb). The testing procedure allows for a questioned sample to be added to the test kit following hemolysis with buffer solution. If human Hb is present within the questioned bloodstain, it will react with a mobile monoclonal antihuman antibody impregnated in the absorbent test strip (stationary phase) forming a mobile antibody-antigen complex. This mobile antibody-antigen complex then migrates (mobile phase) through the test strip to a test window where a polyclonal antihuman Hb antibody is immobilized.
This immobilized antibody captures the mobile antibody-antigen complex so that an antibody-antigen-antibody sandwich is formed. When the human Hb concentration in the sample exceeds 0.05 µg/ml (minimum detection level), this immobilized antibody captures the antigen-antibody complex and a pink precipitin line is formed as a result of the conjugated pink dye antigen-antibody complex being concentrated in this test region (1). As an internal control, human Hb antibody-dye conjugates cannot bind to the antibody in the positive result test area.

The antigen-antibody complex continues to migrate further along the strip and is captured by a second set of immobilized polyclonal anti-immunoglobulin antibodies, thus forming a second pink precipitin line (internal control line) (1). 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 the visualization of only one band in the control area, would indicated a negative result (provided no “High Dose Hook” effect) (2).

Figures 1 to 3, below, show the HemaTrace® in operation with the solution front migrating along the test strip towards the test indication band areas (Figure 2) and a “two band” positive result (Figure 3).

![Figure 1](image1)

![Figure 2](image2)

![Figure 3](image3)

**High Dose Hook Effect**

The High Dose Hook Effect occurs when the human hemoglobin concentration is too high for the test; it is therefore possible to obtain a false negative result from samples containing very high concentrations of hemoglobin (3). Where the High Dose Hook Effect is suspected, the questioned sample should be diluted and re-tested (4).
EXPERIMENTAL MATERIALS AND METHODS

The HemaTrace® test kit, along with a pipette, comes contained within a plastic satchel. The buffer solution is also supplied in a screw cap plastic tube separately. The HemaTrace® testing unit is a small sized plastic component measuring approximately 6.5cm in length by 2.5cm in width (see Figure 4).

One hundred (100) HemaTrace® tests were completed using an array of blood samples on various substrates exposed to a variety of environmental conditions and chemical insults. Cross reactivity was also examined using various blood and fluid types. All test solution samples were extracted using Hematrace® extraction buffer.

Figure 4: HemaTrace® Test components

Blood Sample Volume

While the sensitivity of the HemaTrace® was not critically examined in this study, care was taken regarding maintenance of a bloodstain sample volumetric standard of 0.5ml.

Sample Collection Method

Samples on semi or non-porous substrates (all stain ages) were collected by COPAN cotton swab and de-ionized water. For fresh and aged stains on cloth substrate, sample collection was by both, swabbing and excising a 5mm² swatch. For both collection methods (swab or swatch), the swab head or cloth swatches were immersed completely in the extraction
buffer solution for a period of time (dependent on stain age) ensuring the adequate hemolysis of any hemoglobin contained in the questioned sample.

**Sample Test Method**

The testing procedure involves adding 150µl (4 drops) of the suspect sample into the sample well marked ‘S’ on the testing unit and allowing it to soak in. The solution front then migrates along the absorbent test strip until a band visualizes at the control area marked ‘C’. Positive results were indicated by band visualization at the testing area marked ‘T’. If no band was visualized at the control area marked ‘C’, the test was ruled invalid and repeated. A positive or negative result is read within 10 minutes. For obvious bloodstains, results were often obtained in under 2 minutes.

**RESULTS**

**Environmental Insult / Variable Substrate Study**

Testing of bloodstains on differing substrates exposed to a variety of conditions was carried out in order to simulate crime scene variability. Human blood was deposited on cloth, concrete paving, asbestos and wooden fences and several metal implements. The samples were subjected to a range of environmental conditions such as exposure to rain, sunlight and variable daily temperatures. Positive results were obtained for all obvious bloodstains with a demonstrated need to extend buffer hemolysis time due to decreasing Hb solubility, as stain age increased. The porosity of the substrate in association with sample dilution due to rain appeared to be the major factors influencing results (4).

**Miscellaneous and Chemical Insult Study**

Human blood was subject to a range of mechanical (substrate washing) and chemical insults prior to being sampled and tested. Neat blood was also placed directly into the test sample well. This testing was completed in order to examine the possibility of inducing invalid results (Hook Effect) or “false negatives”. The Hook Effect was not observed and positive results from cloth, following machine washing, were obtained. Chemical insult by a number of strong household cleaning agents on sample stains provided negative results, probably through the denaturing of the Hb.

**Cross Reactivity Study (Animal Hb)**

The HemaTrace® is specific for human hemoglobin and hemoglobin derived from higher primate (2, 5). No cross reactivity to hemoglobin from Canine (dog), Porcine (pig), Equine (horse) or Feline (cat) species was detected. Importantly, Human Hb, in combination, with variant animal Hb was detected (4).
Cross Reactivity Study (Human body fluids other than blood)

Due to the sensitivity of the test, trace levels of hemoglobin can be detected in “other than blood” body fluid samples. Representative samples of various human body fluids were analyzed to assess the presence of hemoglobin. Hemoglobin was detected in neat male and female saliva and in neat male urine (4).

PRACTICAL “CASE WORK” APPLICATIONS

Case I

Prior to the identification of this testing method, the author assisted in the forensic investigation of an apparent homicide. Obvious projected bloodstains were observed within the scene and a bloodstained hammer was located adjacent to the bloodstains (see Figure 5). The event was not witnessed, and the victim was removed alive from the scene but died soon afterwards from severe burns. Following the expenditure of considerable investigative resources over a period of several hours, the relevance of a deceased cat located within the scene was acknowledged. Had the HemaTrace® test been available and utilized, the murder (feline) / suicide (human) may have been recognized earlier.

Figure 5: Feline murder / human suicide scene

Case II

The author assisted in the forensic investigation of a homicide scene where the offenders had partially cleaned the residence, including removal of the deceased, and then allegedly contaminated the scene using animal (sheep) blood. A partial component of the forensic
investigation included an analysis and interpretation of bloodstains and bloodstain patterns within the residence (see Figure 6). The HemaTrace® test was used to separate animal and human bloodstains (general perspective) and to validate human stain selection as part of the overall BPA examination. Further, forensic investigators were able to detail preliminary examination results, in real time, with a high level of confidence.

![Projected bloodstain over possible “cleanup” rivulet](image)

**Figure 6:** Projected bloodstain over possible “cleanup” rivulet

**Case III**

The author recently attended a rural road scene where pooled blood, items of clothing and vehicle safety glass were located. The HemaTrace® test confirmed the blood to be of human origin. Inquiries by detectives, strengthened by the timely HemaTrace® findings, resulted in the incinerated remains of a homicide victim being located on a farm 20 kilometers from the scene where the blood was located.

**Case IV**

In a recent homicide investigation, the author attended a domestic residence and as a partial component of the forensic investigation, conducted an analysis and interpretation of bloodstains present in the residence. Large portions of fresh meat and associated blood from a butchered animal were also present within various areas of the premises. Historical bloodstains of unknown origin and not associated with the incident, were also observed. Using the HemaTrace® test the author was able to distinguish animal, co-mingled (human/animal) bloodstains and identify fresh and historical human bloodstains. Subsequent laboratory DNA examination of scene samples confirmed the HemaTrace® findings at the scene.
CONCLUSIONS

This evaluation study indicates that the ABAcard® HemaTrace® test is a sensitive, reliable and timely “species of origin” testing process suitable for the confirmatory identification of human blood at the crime scene. Simple to use and requiring a minimum amount of equipment, it produces results that require little specialist training to interpret.

Evaluation of sample collection methods, test preparation and operation requirements and the subsequent result interpretation demonstrates that the ABAcard® HemaTrace® test is suitable for operation and interpretation by persons with limited serological knowledge. Importantly, the integration of this assay for the forensic identification of human blood at the crime scene, can be achieved without excessive procedural change or exhaustive training of personnel.

REFERENCES

1. ABAcard® HemaTrace® test kit – Technical Information Sheet (rev. 01/2001). Abacus Diagnostics Inc. USA