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VALIDATION STUDY OF THE ABACUS DIAGNOSTICS ABAcard® HemaTrace® MEMBRANE TEST FOR THE FORENSIC IDENTIFICATION OF HUMAN BLOOD

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ABSTRACT

The purpose of this validation study was to determine whether the ABAcard® HemaTrace® (Abacus Diagnostics, West Hills CA), a simple immunochromatographic test for the forensic identification of human blood, was suitable for introduction into routine laboratory use at the Centre of Forensic Sciences. In this study we examined the sensitivity and specificity of the kit, as well as the effect of environmental exposure and contamination on the test.

The limit of detection of the kit was determined to be 0.07μg haemoglobin/mL. Tests showed specificity to human, higher primate, and ferret blood. Positive results were unaffected by a variety of contaminants and under a range of conditions, with the exception of stains subjected to a tumble-dryer cycle or prolonged exposure to soil.

The results of this validation support the introduction of the ABAcard® HemaTrace® test for routine use in the forensic identification of human blood.

RÉSUMÉ

Le but de cette validation était de déterminer si le test ABACard® Hematrace® (Abacus Diagnostics, West Hills, CA), un simple test immunochromatographique servant à l'identification de sang humain à des fins médicolégales, pouvait être recommandé pour usage systématique au laboratoire du Centre des sciences judiciaires de Toronto. Dans cette étude, nous avons examiné la sensibilité et la spécificité de la trousse ainsi que les effets sur le test d'une exposition à divers facteurs environnementaux et contaminants.

La limite de détection de la trousse a été déterminée comme étant de 0.07 μg d'hémoglobine/mL. Les tests effectués ont démontrés une spécificité au sang humain, de primates supérieurs et de furet. Une exposition à divers contaminants et conditions environnementales n'a pas affecté les résultats positifs obtenus avec le test, sauf dans le cas de taches soumises à une exposition prolongée à de la terre et au séchage par culbutage.

Les résultats de cette validation supporte l'introduction du test ABACard® Hematrace® pour l'identification de sang humain à des fins médicolégales.

INTRODUCTION

In forensic examinations, the Phenolphthalein test (1), also known as the "Kastle-Meyer" (KM) test, is used for testing suspect stains for blood, however, this test is insufficient to confirm the presence of human blood.

The species identification of blood, in a forensic context, usually relies on the binding of an antibody to a corresponding antigen, resulting in a visual precipitate (1). The technique currently utilized at the Centre of Forensic Sciences, and many other laboratories, for the forensic identifi-

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cation of human blood is the crossed-over electrophoresis method (2). The process of crossed-over electrophoresis is time consuming. Each lot of commercially produced antisera and positive control serum samples must be validated by testing against all control antiserum and animal serum reference samples to test for cross reactivity. In addition, these samples have a limited shelf life. Due to the fact that crossed-over electrophoresis is a relatively labour intensive and time consuming process, there is considerable interest in a fast and efficient alternative that is equally sensitive and specific.

In the ABAcard® HemaTrace® test, human haemoglobin binds mobile antibody dye conjugate, and begins to diffuse along the test membrane as a mobile antigen-antibody complex (Fig.1 – Rows 1&2). As this complex passes along to the test area "T", it binds to an immobile polyclonal anti-human-haemoglobin antibody (Fig.1 – Row 3). Further along the membrane, there exists a control area "C", which contains immobile anti immunoglobulin (Ig)-antibody. In this area, unbound monoclonal antihuman haemoglobin antibody dye conjugates will bind (Fig.1 – Row 3) and serves as a positive control for the test. In the case of a positive test for human haemoglobin, there will be two bands. One appears at the test area, and consists of accumulated antigen-antibody-antigen sandwich, and the second appears in the control area, resulting from the accumulation of excess anti Ig-antibody antihuman haemoglobin antibody complexes (Fig.1 – Row 4). In the case of the ABAcard® HemaTrace®, the dye used is pink in colour, so the bands appear as solid pink lines in the test and control areas of the membrane (3–6). In a situation where there is no human haemoglobin present, there will be no accumulation at the test line, and only one band, at the control line, will be visible.

The purpose of the study was to determine whether the ABAcard® HemaTrace® met validation criteria which would make it suitable for introduction into routine laboratory use at the Centre of Forensic Sciences by determining the sensitivity, specificity and performance in casework of the test kit, and assessing the limitations identified.

MATERIALS AND METHODS

The Kastle-Meyer Test

Swabs and cutouts of all test stains were tested for peroxidase-like activity of haemoglobin using the three-step rub or direct method (7). Dilutions of blood were tested by applying a drop of the dilution to a filter paper, then adding the test reagents directly to the dilution.

Crossed-over Electrophoresis

Crossed-over electrophoresis was performed as originally described by B.J. Culliford (2).

The ABAcard® HemaTrace® Membrane Test for the Forensic Identification of Human Blood

Each ABAcard® HemaTrace® comes individually packaged in a pouch, which contains a dropper. The kit also contains 2 mL aliquots of extraction buffer (pH 7.5).

The sample collection for the ABAcard® HemaTrace® was performed according to the protocol provided by the manufacturer "For Fresh Bloodstained materials" (5) with the following modifications: For stains, a 2 mm diameter cutout (using a Harris Punch) was used, and an equivalent sized portion was used for swabs. Each sample was left for five minutes in the entire volume of buffer to extract before being applied to the kit as outlined in the "Test Protocol". Immediately before the sample was applied to the sample well, the extract was gently swirled. If a negative result was observed and the stain being tested was known to be older than one year, the sample was left to extract for an additional 15 minutes, and the test was repeated. Results were read no more than ten minutes later, after which the kit was discarded.

A positive result is obtained if pink lines in both the test area "T" and in the control area "C" are observed (Fig.2 – Left). A positive result can appear as early as 45 seconds. A negative result is indicated by the presence of only one band, located in the control area "C", after a period of 10 minutes (Fig.2 – Centre) (5). It is possible, if the concentration of blood is too high, that a false negative will be observed due to the Hook Effect (8). In this situation, excess antigen (human haemo-

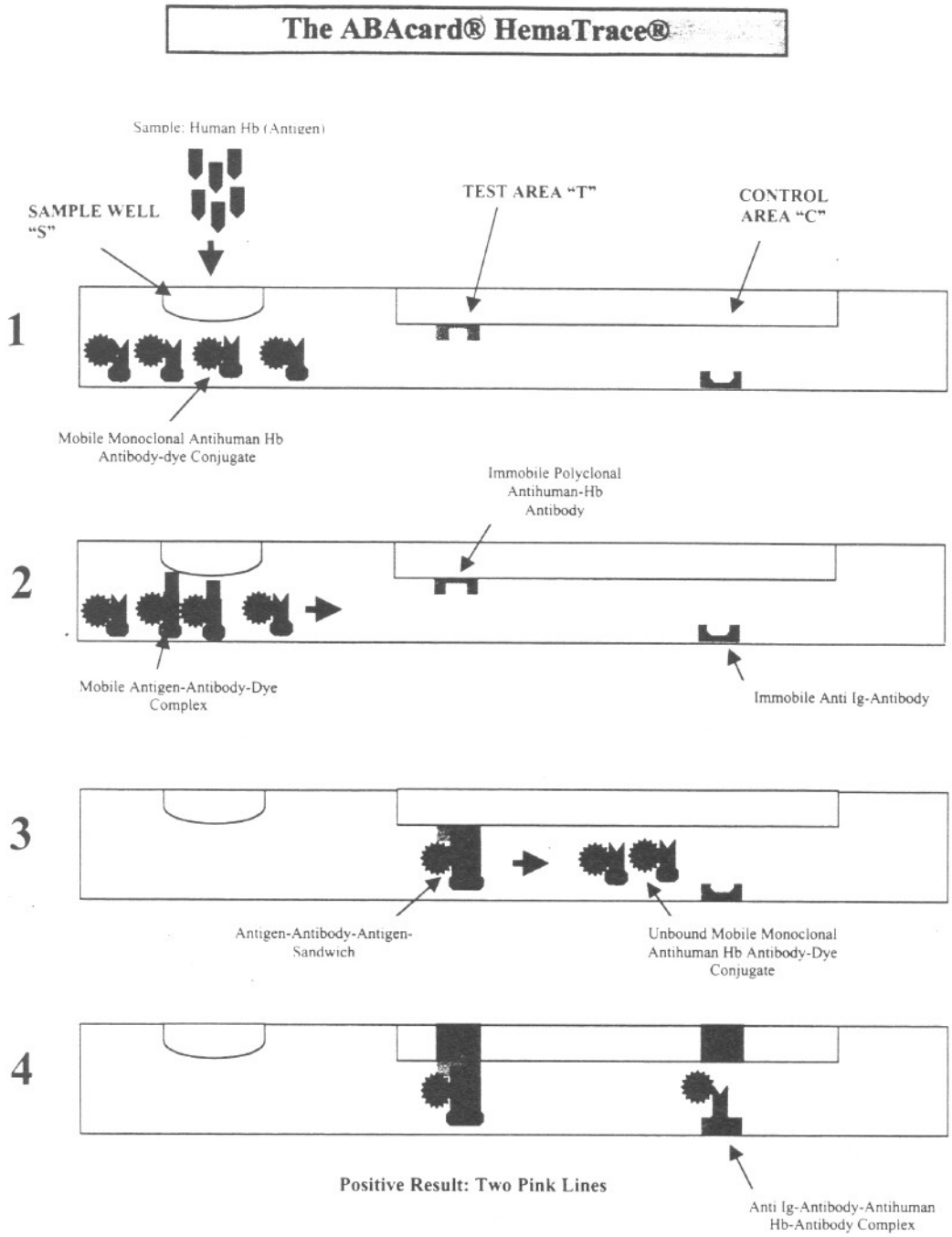

Figure 1. Mobile Human Hb-Antibody-Dye complex binds to immobile polyclonal antibody in test zone. Unbound antibody-dye complex binds to Immobile Anti Ig-Antibody at control area. This results in the formation of two pink bands indicative of a positive result.

globin) binds to the immobile polyclonal antihuman-haemoglobin antibodies in the test zone, preventing these antibodies from reacting with the antigen-mobile antibody complexes (Fig.2 – Right) (9). The test is inconclusive if no bands appear (5).

Sensitivity Study

Whole blood, collected by venipuncture in heparin and EDTA tubes was diluted in doubling dilutions with both sterile water and the Tris buffer (pH 7.5) supplied with the kit. Stains were made using 50 µL of whole blood on white cotton air dried overnight. A 2 mm diameter portion of the stain, extracted in the kit supplied buffer, was used to prepare a series of doubling dilutions in order to test the sensitivity of the kit towards stains.

Aged bloodstains taken from forensic casework samples that had been stored at room temperature for up to 30 years were analysed using the ABAcard® HemaTrace®. Two millimetre punches of stains were extracted in the entire volume of buffer for both five and 30 minutes before being applied to the strip.

Specificity Studies

Whole blood from dog, cat, horse, pig, goat, and cow, was subjected to a doubling dilution up to 1:100 000 with the kit supplied buffer. Whole blood from orangutan was diluted to approximately 1: 4 000 000 with the supplied buffer. Bloodstains of each of these animals, as well as domestic ferret and cockatiel, were also made using 50 µL of whole blood on cotton allowed to dry overnight. Two millimetre diameter punches of blood stains on S&S paper, which were acquired in 1998 and stored frozen, were tested from the following animals: Dall's sheep (*Ovis dalli dalli*), European reindeer (*Rangifer tarandus tarandus*), Barbary ape (*Macaca Sylvanus*), Mara (*Dolichotis patagona*), Wapiti (elk) (*Cervus elaphus Canadensis*), Black footed ferret (*Mustela Nigripes*), Tamandua (*Tamandua Tatradactyla*), Ring-tailed lemur (*Lemur Catta*), Snow leopard (*Panthera Uncia*), Sable antelope (*Hipptragus Niger Niger*), Boa constrictor (*Boa Constrictor Constrictor*), Trumpeter swan (*Cygnus Cygnus buccinator*), Japanese macaque (*Macaca fuscata*), Sumatran tiger (*Panthera tigris sumatrae*), Chamois (*Rupicapra rupicapra*), African cheetah (*Acinonyx jubatus jubatus*), African elephant (*Coxodonata africana*), Grevy's zebra (*Equus grevyi*), Olive baboon (*Papio cynocephalus anubis*), Beaver (*Castor canadensis*), Thomsons' gazelle (*Gazell thomsoni*), and Canadian lynx (*Felis lynx canadensis*). Additional animal bloodstains (on FTA™ paper) from the following animals were provided by the Natural Resources DNA Profiling & Forensic Centre (Trent University, Peterborough, ON) and 2 mm punches were tested: harbour seal (*Phoca VituOna*), striped skunk, racoon, red fox, moose, San Clemente Loggerhead Shrike (*L. I. Mearnsi*), gray jay (*Perisoreus Canadensis*), eastern wolf and black bear.

Two millimetre diameter punches of stains of human vaginal secretions, semen, faeces, menstrual blood, nasal secretions, saliva, urine and mixtures of semen/saliva and saliva/urine from four different laboratory staff donors were tested. Vaginal, oral, and anal swabs that had been collected from four different people were also tested. Both snippets and entire swabs were extracted in the entire volume of supplied test buffer.

Fresh stains of potential Kastle-Meyer false positives including rust, bleach, sodium cobaltic nitrite and soya root nodule were prepared on cotton and 2 mm diameter punches tested.

Substrate Study

Stains were made on a variety of surfaces including soil, fingernails, newspaper, suede, styrofoam, hair, upholstery, carpet, brown paper bag, curtains, leather, a facial tissue, wallpaper, towel, rubber and leather, and left to dry overnight. A 2 mm by 2 mm portion of each were placed directly in the supplied test buffer, with the exception of the soil, fingernails and hair.

Stains made on fingernails, weather treated wood, untreated wood, leather, cement, glass, stucco, metal, plastic knife handle and tile were swabbed using a cotton swab dampened with sterile water. A snippet of each swab was added to the supplied test buffer. Negative controls of unstained areas of the substrates were also tested.

Degradation Studies

Dried stains of 50 µL of blood were prepared on cotton cloth. Samples were subjected to different conditions for a period of one month, including: frozen at approximately –20°C, left outside in

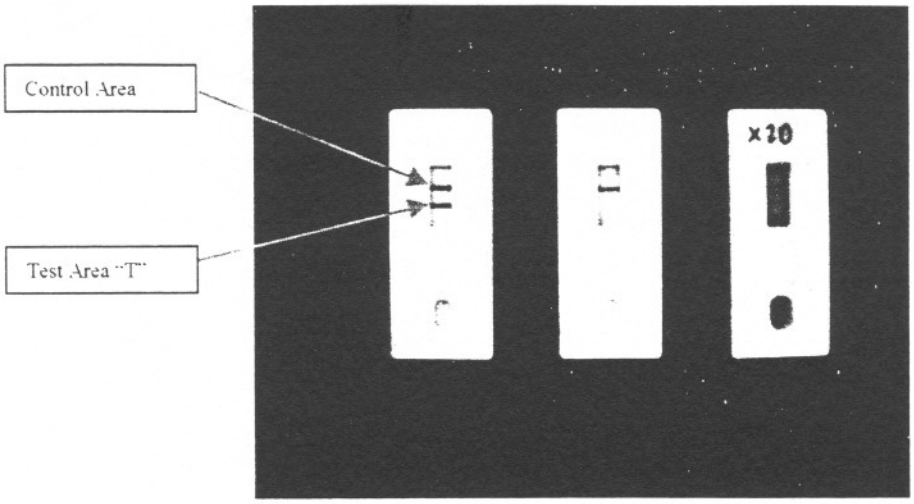

Figure 2. A positive (left), negative (centre), and false negative due to Hook Effect (right) result on the ABAcard® HemaTrace®

an exposed area from mid-July until mid-August (average temperature 21.4°C, UV index moderate to high, and total precipitation approximately 40mm)[2], wrapped in a garbage bag in the same area, buried in soil at a depth of approximately 10 cm, and wrapped in a garbage bag and buried in the same conditions. Two millimetre diameter punches of each were tested as above.

Bloodstains made on cotton cloth were subjected to successive washing cycles using Tide® detergent, then dried in a tumble dryer at high heat. Two millimetre square punches of the stains were tested after one and two wash cycles.

Bloodstains made on cotton were burned and the ashes added to the buffer supplied with the kit.

Contamination Studies

Dried stains of 50µL blood were prepared on cotton cloth. The following contaminants were added, one per stain: Zest® soap, Fantastik® multi-purpose cleaner, Downy® fabric softener, Drano®, Tilex® tile cleaner, Revlon® nail polish, Shout® gel stain remover, Mr. Clean™ bathroom cleaner, Cover Girl® liquid foundation make-up, Vaseline® petroleum jelly, Ombrelle 30SPF sun block, Life Brand® foam shaving cream, Herbal Essences® shampoo, No Name nail polish remover, corn oil and Palmolive® Antibacterial liquid dish soap, Luminol and leucomalachite green. The contaminated stains were left to dry overnight and then tested.

Volumes of the following contaminants were added directly to samples of liquid blood: Javex™, Mr. Clean®, Fantastik®, Palmolive®, luminol, and leucomalachite green such that final concentration of contaminant in the volume of mixture was 1%, 5%, 10%, 25% and 50% and the final dilution of blood in the mixture was 1:2, 1:10, 1:100, 1:1000, 1:10000 and 1:100000. Mixtures were tested after one hour, one day and one week.

Luminol and Leucomalachite Green (LMG) were provided by the Toronto Forensic Identification Services of the Toronto Police Service and made fresh according to the formulae listed below (Table 1).[3]

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2. Monthly Meteorological Summary July and August 2001 for Toronto City. Provided by Brian Smith, Environment Canada. August 14, 2001.
3. Detective Allen Pollard, Laboratory Manager, Forensic Identification Services, Toronto Police Service, Toronto, Ontario, Canada.

Casework Samples

Samples of stains or swabs from forensic casework that tested KM positive and unstained substrate controls, where possible, were collected. The substrate, approximate age and any known contaminants were recorded. Two millimetre square cutouts of stains or snippets of swabs were tested according to the previously outlined protocols. Samples were also tested against anti-human antisera using crossed-over electrophoresis (2,9).

RESULTS AND DISCUSSION

A summary of the results is depicted in Table II.

The ABAcard® HemaTrace®

When the procedure was followed according to the guidelines of the manufacturer, the test was easy to use and reliable. A 2 mm diameter punch of the stain proved to be sufficient size to give a positive result.

The "high dose Hook Effect" that the manufacturers describe was only observed in the experiments in which blood was mixed with detergents. This occurred at concentrations where the corresponding dilutions with water were too viscous to migrate along the test membrane. In these cases the detergent improved the mobility of the sample such that it was migrated along the membrane and a negative result was observed. It was found that in order for whole blood to be sufficiently mobile, it had to be diluted to a minimum of 1:8. Ideally, the dilution should be, at maximum, a clear light red colour before being applied to the test kit.

It was also vital that the test result was read at a maximum of ten minutes. In nearly all experiments, the positive result was visible within the first six minutes. If left longer than ten minutes, there could be "flowback" of the antibody-dye conjugate in the excess buffer. This could result in the appearance of a band at the point where the complex stops moving, which could be confused with a positive band.

Very rarely, negative tests showed what could be described as a "ghost band". This band appeared as a white band in the test area where a positive band would be expected to appear. In all cases where this band was observed, it appeared between nine and ten minutes. It was noted in the cases of the "ghost band", the rest of the membrane was a very pale pink colour, and the control band was intense pink. The appearance of this band bore no resemblance to a positive band so that it was considered that this anomaly was of no concern. This phenomenon has not been reported in the literature, but personal communications with other users confirmed that the phenomenon has been observed by others.[4]

Sensitivity Study

The ABAcard® HemaTrace® proved to be more sensitive than the KM test, and gave a positive result down to the concentration of 0.07 µg haemoglobin/mL blood (based on a level of 130g haemoglobin/L blood, which is average for a healthy female).[5] This is comparable to the sensitivity of 0.05ug/mL claimed by the manufacturer (5). Using the supplied buffer as the diluent, the kit proved to be more sensitive than when using distilled water.

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4. Personal Communications: (i) Abacus Diagnostics Technical Branch—Sushil Madhogarhia, West Hills CA, U.S.A.; (ii) Christine Crossman, Biology Section, Royal Canadian Mounted Police Forensic Laboratory, Vancouver, British Columbia, Canada; (iii) Jeffrey G. Modler, Biology Section, Royal Canadian Mounted Police Forensic Laboratory, Halifax, Nova Scotia, Canada; (iv) Michele Bobyn, Laboratory Agent, Colorado Bureau of Investigation, Department of Public Safety, Pueblo, Colorado, U.S.A.; (v) Adriana Kristaly, Forensic Biology Section, Crime Laboratory Bureau, Miami-Dade Police Department, Miami, Florida, U.S.A.
5. John Wilson, Forensic Technologist, Biology Section, Centre of Forensic Sciences, Toronto, Ontario, Canada

Table I

Preparations of Luminol and Leucomalachite Green (LMG)

Luminol	Leucomalachite Green
• Pour 50mL distilled water into clean container • Add 0.35g of sodium perborate, shake until dissolved (15 seconds) • Add 0.05g Luminol and 2.5g of sodium carbonate. • Stir until chemicals dissolve as much as possible • Let stand about 5 minutes • Decant into plastic spray bottle and use immediately	Container #1 –60mg Leucomalachite Green –200mg sodium perborate Container #2 –20mL methanol –18mL glacial acetic acid Conainer #3 –100mL IDENTISOL • Take container #1 and add to container #2, shake vigorously until crystals are completely dissolved • Add to container #3 and shake lightly to mix • Use immediately

Table II

Summary of Results

Experiment	Result
Sensitivity: Dilutions of liquid blood (water)	Positive to 1:500 000
Sensitivity: Dilutions of liquid blood (supplied buffer)	Positive to 1: 2 000 000
Sensitivity: Dilutions of fresh human blood stain (water)	Positive to 1:256
Sensitivity: Dilutions of fresh human blood stain (supplied buffer)	Positive to 1:256
Sensitivity: Aged bloodstains from forensic casework	KM positive stains up to 31 years old gave a positive result
Specificity: Different animal species	All animal species tested negative with the exception of the higher primates, which tested positive to a dilution of approximately 1: 2 000 000, and ferret blood (insufficient amount to perform dilution)
Specificity: Body fluid stains/swabs	Two of the four semen and faeces stains and all vaginal, oral, anal and rectal swabs tested positive
Casework Simulation: Substrate studies	None of the substrates tested interfered with a positive result, or gave a false positive
Casework Simulation: Degraded human bloodstains	Buried (no bag) and burnt samples gave negative results on both the ABAcard® and crossed-over electrophoresis. Stains that had been machine washed and dried tested negative on the ABAcard®, but were identified as human by crossed-over electrophoresis
Casework Simulation: Contaminated human bloodstains	All positive
Casework Simulation: Contaminated liquid human blood	Contaminants gave positive results to an average of 1:10 000 dilution of blood with 25% contaminant, with the exception of Javex™ Bleach, Palmolive® Dish Detergent, and LMG
Casework samples	Positive for all human bloodstains, negative for all non-human as confirmed by crossed-over electrophoresis

Positive results were obtained from bloodstains aged as long as 25 and 30 years. Extractions of longer time periods (30 minutes) improved the success for aged stains.

Specificity Study

Specificity Against Blood and Bloodstains of Other Species

Experiments for species specificity showed that only human, higher primate and ferret blood gave positive results with the assay. The fact that humans and primates share a close phylogenetic relationship explains why the kit is primate specific (10). When selecting a target sequence within

haemoglobin that the assay will recognize, one that provides maximum discrimination between species is optimal.

A search of protein sequence databases revealed that the positive result from ferret blood can be explained by the fact that the alpha chain of human haemoglobin shares a common sequence with a majority of higher primates and ferrets (11–13). The amino acid sequence TNAVAHV, which spans from residues 67–73, is optimal for use as an epitope to be recognized by monoclonal antibodies, as it shows maximum variation between human and commonly encountered animal haemoglobins. The sequence also shows sufficient variation from the corresponding sequence in rabbit and mouse such that it can cause an immunogenetic reaction, making the production of monoclonal and polyclonal antibodies, such as those exploited in the ABAcard® HemaTrace®, possible (11–13).

Specificity Against Body Fluid Stains and Swabs

When stains of various body fluids were tested, some semen stains gave a positive result. This is due to the fact that semen can contain trace amounts of haemoglobin, which the highly sensitive kit was able to detect (3–6). Some stains of faeces also tested positive, which is not surprising given the fact that kits such as the ABAcard® HemaTrace® were originally designed to detect occult blood in faeces (14).

All of the oral, vaginal, anal and rectal swabs tested gave a positive result for human blood. This can also be explained by the high sensitivity of the kit. None of the swabs or body fluid stains gave a positive KM reaction. The false positives, therefore, could be eliminated by setting the criteria that a stain or swab must first have a positive KM result before being identified as human blood by testing with the ABAcard® HemaTrace®.

Specificity Against KM Test False Positives

When possible false positives were KM tested, only freshly prepared soya root nodule gave a true false positive reaction. None of the possible false positives, however, gave a positive result when tested with the ABAcard® HemaTrace®.

Non-Probative Casework Study

Substrate Study

All substrates tested could be directly added to the test extraction buffer without interfering with a positive result. Swabs that were taken from substrates that were difficult to cut also gave a positive result with the ABAcard®. It was noted that for the best results, a snippet of a swab was ideal, but that stained fibres taken from a swab would also suffice.

Degraded Human Bloodstains

The results of the analysis of degraded human bloodstains are depicted in the table below (Table III).

Of the degraded blood samples, only those buried exposed in soil and those that were burned gave negative results. The fact that the sample contained in a garbage bag, which was then buried, gave a positive result indicates that the micro-organisms present in the soil could have been responsible for the degradation of the haemoglobin. In each case, the samples that tested negative also tested negative for human serum albumins using crossed-over electrophoresis.

The only case in which crossed-over electrophoresis was able to identify a sample as human when the ABAcard® gave a negative result was in the case of laundered bloodstains. Research has shown that heat fixing stains can prevent a positive result from being obtained[6, 7]. This could be due

6. C. J. Swander, and J. G. Stites. Evaluation of the ABAcard HemaTrace for the Forensic Identification of Human Blood. Michigan State Police, Forensic Laboratory publication. Provided by Abacus Diagnostics, West Hills, CA. August 14, 2001.
7. A. Kristaly, and D. Smith. Validation of the OneStep ABAcard HemaTrace for the rapid forensic identification of human blood. Forensic Biology Section, Crime Laboratory Bureau, Miami-Dade Police Department publication. Provided by Abacus Diagnostics, West Hills, CA. August 14, 2001.

Table III

Results of degradation experiments

Condition	KM result	ABAcard Result @ 10 min.	Crossover Result
Frozen (approx.–20°C)	Positive	Positive	Human
Outdoor exposure	Positive	Positive	Human
Outdoors in Black Garbage Bag	Positive	Positive	Human
Buried (approx. 10cm depth)	Positive	Negative	Negative
In Black Garbage Bag and Buried (approx. 10cm depth)	Positive	Positive	Human
Exposed to Fire	Negative	Negative	Negative
1 Regular Machine Wash and Dry Cycle	Positive	Negative	Human
2 Regular Machine Wash and Dry Cycles	Positive	Negative	Human

to the conformational changes or degradation that the epitopes undergo upon heating, which would prevent them being recognized by the antibodies for which they are targets. It is possible that the crossed-over electrophoresis result was unaffected due to the fact that the antibodies of the antiserum were polyclonal, versus the monoclonal antibodies used in the kit, and sufficient recognition sites remained after heating to allow the formation of antibody-antigen complexes for a precipitin reaction (9).

Contaminated Human Bloodstains and Contaminated Human Blood

When contaminants were applied to dried bloodstains, none interfered with the testing. Detergents mixed directly with blood showed varying results. In general, contaminants that increased the viscosity of blood-contaminant mixture, such as Javex bleach, LMG, and Palmolive liquid dish detergent, prevented the blood mixture from migrating through the test membrane, ending in a "no result". When contaminants reached a concentration such that the pH was greater than 9, the maximum level recommended by the manufacturer, a "no result" was also obtained. In all cases, a final concentration of blood of 1:2 did not give a result, regardless of the concentration of the contaminant. In the case of contaminants that did not increase the viscosity of the blood-contaminant mixture, such as Mr. Clean™, Fantastik®, and Luminol, dilutions of 1:10 of blood gave a positive result regardless of the concentration of contaminant.

Fantastik mixed with blood of a final dilution of 1:2 provided the only situation in which the phenomenon of "high dose Hook Effect" was observed. The high dose effect, which is the result of an excess of antigen, was normally not seen due to the correlation between excess haemoglobin and high viscosity of the blood solution being applied to the sample well of the test kit. When Fantastik® was mixed with blood, the result was not as viscous as the Mr. Clean™ or Luminol mixtures, and the mixture was able to flow through the test membrane. Although blood was present, a negative result was observed. When 1:2 blood solution contained 25% or 50% Fantastik®, the Hook Effect was observed. When these samples were further diluted, positive results were observed at the same concentration of contaminant.

These results are similar to those found by Hochmeister *et al.* (14). Negative results that were not due to viscosity or high pH can be explained by the fact that the cleaners denature the proteins or inhibit the immunoassay. Some contaminants, such as soapy detergents, can interfere with protein-protein interactions, making the formation of the antigen-antibody complexes necessary to give a result on the assay, impossible (14).

Casework Studies

All casework samples that were submitted had tested KM positive, with the exception of the human charred muscle sample. Of these samples, the only samples that did not give a positive

Table IV

Results of analysis of casework samples

SAMPLE NUMBER	ABAcard Result @ 10min.	1/2 hour extraction result @ 10min.	Crossover Result
1	Negative	N/A	Negative
1uc	Negative	N/A	Negative
2	Negative	N/A	Negative
3	Negative	N/A	Negative
4	Positive	N/A	Positive
5	Positive (weak)	Positive	Positive
5uc	Negative	N/A	Negative
6-1	Positive	N/A	Positive
6-2	Positive	N/A	Positive
6uc	Negative	N/A	Negative
7-1	Positive	N/A	Positive
7-2	Positive	N/A	Positive
8-1	Positive	N/A	Positive
8-2	Positive	N/A	Positive
9-1	Positive	N/A	Positive
9-2	Positive	N/A	Positive
9-3	Positive	N/A	Positive
9-4	Positive	N/A	Positive
10-1	Positive	N/A	Negative
10-2	Positive	N/A	Negative
10-3	Positive	N/A	Negative
10-4	Positive	N/A	Negative

Legend:

Sample	Unstained Control (UC)	Approx. Age	Substrate	Contaminants
1. cutout	Y	>3months	Cotton sock	Outside
2. tissue	N/A	>3months	N/A	Outside
3. swab	N/A	Unknown	Metal blade	N/A
4. cutout	N	1 month	Yellow cotton	N/A
5. cutout	Y	~31 yrs.	Lt. Blue mesh	Urine
6. cutout—faint and obvious	Y	~1yr.	Denim jeans	None
7. cutout	N	~11 months	Grey cotton T	Dirt (ravine beating)
8. cutout	N	~11 months	Pale blue denim	Dirt (ravine beating)
9. snippets	N	~7 months	I/S house (scene)	Unknown
10. tissue	N/A	~7 months	N/A	burnt
-10-1=burnt blood				
-10-2=liver				
-10-3=burnt muscle				
-10-4=charred muscle				

ABAcard® HemaTrace® result also gave a negative result using crossed-over electrophoresis. Samples that were known to be contaminated with urine and dirt were unaffected. Stains were aged between one month and 31 years (Table IV).

RECOMMENDATION

The ABAcard® HemaTrace® proved to be highly sensitive and when used in conjunction with a KM positive test result, the ABAcard® HemaTrace® was specific for blood. A positive result indicates that blood is of human, higher primate or ferret origin. Since the possibility of encountering higher primate or ferret blood in routine casework is minimal and can be considered on a case by case basis, the fact that the kit cross reacts with these animals is not of great concern. If any doubt exists, the results can be confirmed by DNA analysis.

With the limitations of the ABAcard® HemaTrace® test identified, it proved to be a highly effective and efficient test that will help to improve laboratory operations. In the majority of routine casework samples, the ABAcard® HemaTrace® will be able to provide a fast and reliable result requiring much less time and resources than current methods. Based on the results of this validation study, the Biology Section of the Centre of Forensic Sciences has implemented the ABAcard® HemaTrace® for the identification of human blood.

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