Journal of Forensic and Legal Medicine 38 (2016) 101–105

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Applicability of three commercially available kits for forensic identification of blood stains

Ivana Horjan [a, *], Lucija Barbaric [a], Gordan Mrsic [a, b]

[a] Ivan Vucetic Forensic Science Centre, General Police Directorate, Ministry of Interior, Ilica 335, 10 000 Zagreb, Croatia
[b] University Centre for Forensic Sciences, University of Split, Rudjera Boskovica 31, 21000 Split, Croatia

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form
3 November 2015
Accepted 29 November 2015
Available online 18 December 2015

Keywords:
Forensic science
Blood tests
Sensitivity
Specificity

ABSTRACT

Various commercially available one-step immunoassays for detection of human (primate) blood have been developed. This study evaluated two hemoglobin tests, ABAcard® HemaTrace® and HemDirect Hemoglobin against glycophorin A test-RSID™-Blood for following parameters: sensitivity, specificity, effectiveness using various substrates, stain remover and aged blood stains. The highest blood detection limit was observed if HemaTrace® was used. When compared with HemaTrace®, ten times lower sensitivity was observed for HemDirect Hemoglobin test. No false positives were obtained for HemDirect Hemoglobin while ABAcard® HemaTrace®, probably due to its extreme sensitivity, showed high percent of false positives with saliva. The lowest sensitivity and 40% of false positives with saliva was exhibited by RSID™-Blood. In addition, this test encountered the lowest efficacy if aged blood-stains or blood treated with stain remover were used. As expected, none of the tested substrates (wood, metal, brick, and soil), influenced on blood testing, although soil substrate affected STR amplification. Conducted studies established HemDirect Hemoglobin test as more reliable for evaluated parameters than ABAcard® HemaTrace® and RSID™-Blood.

1. Introduction

After a crime has occurred, various traces of human origin connected with the crime scene can be used to link potential suspects to the victim or the crime scene. Traces or stains of different body fluids may be invisible to the naked eye, look similar in appearance to other fluids or substances, and even be degraded due to environmental conditions.[1,2] Regarding traces of blood, one of the most commonly found human body fluids, an important issue studied by forensic scientists is the detection of blood stains that have been deliberately removed with various cleaning agents (e.g., bleach, stain remover, detergents) and their influence on degradation of blood proteins and subsequent DNA analysis.[3–5] In addition, substrates on which blood stains are found may also influence the efficiency of subsequent DNA analysis.[3,4,6] A well-established procedure used at different forensic laboratories includes the examination of potential blood traces by means of one or more presumptive tests and, in the event of a positive reaction obtained by a presumptive test, the use of a confirmatory test to definitively establish the origin of the sample. Over the years, several one-step immunochromatographic assays based on the determination of hemoglobin have been developed, enabling fast and easy detection of human blood for forensic purposes. Compared to presumptive tests, which have been thoroughly studied to determine their sensitivity, specificity, and potential negative effects on subsequent DNA analysis, immunochromatographic hemoglobin kits are generally less sensitive but more specific.[6–12] Their main disadvantages are their susceptibility to "high-dose hook effect" – i.e., the occurrence of false negative results observed when a very high level of the target antigen is present in the tested sample – and the possibility of cross-reaction with ferret, skunk, and primate blood, and even with other human body fluids, such as urine, stool, semen, vaginal fluid, saliva, and perspiration.[13–16] Lately, RSID™-Blood, a lateral flow immunochromatographic strip test that detects human glycophorin A, has been proposed as a confirmatory test and a possible alternative method for the detection of human blood.[17,18]

The objective of the present study was to evaluate two hemoglobin tests, HemDirect Hemoglobin Assay (SERATEC®, Göttingen,

* Corresponding author. Tel.: +385 13788620.
E-mail addresses: ihorjan@mup.hr (I. Horjan), lbarbaric@mup.hr (L. Barbaric), gmrsic@mup.hr (G. Mrsic).

http://dx.doi.org/10.1016/j.jflm.2015.11.021
1752-928X/

Germany) and the ABAcard® HemaTrace® test (Abacus Diagnostics, Inc., West Hills, CA, USA), their sensitivity, specificity and effectiveness using various substrates, stain remover, aged blood stains, as compared with the human glycophorin A test RSID™-Blood (Independent Forensics, Lombard IL, USA), in order to implement these kits in the current laboratory workflow of human blood identification.

2. Materials and methods

Commercially available HemDirect Hemoglobin Assay (SERATEC®, Catalog Number: HbF07), ABAcard® HemaTrace® (Abacus Diagnostics Inc., Catalog Number: 708424), and RSID™-Blood (Independent Forensics, Catalog Number: 0300) were evaluated.

The fresh, liquid blood and saliva used in the sensitivity and specificity studies were contributed by staff volunteers with their written consent. The positive control was prepared from the blood of the same donor as used in the testing. For the study involving aged samples, casework blood stains deposited on paper and stored at room temperature were used. The samples were prepared under equal conditions and tested according to the manufacturer's protocols.[14,15,20]

2.1. Sensitivity

Three aliquots of twenty μL of blood from a same donor were placed into a 1.5 ml sample tubes, serially diluted ten folds in extraction buffer of each test (starting at 1:10 down to 1:10 000 000), and tested in triplicate.

2.2. Specificity - cross-reactivity with saliva

Fifty μL samples of saliva contributed by five female donors were each diluted in four hundred and fifty μL of ultrafiltered water, incubated for five minutes at room temperature on a shaker, vortexed for 10s, and tested with the HemDirect Hemoglobin and ABAcard® HemaTrace® tests in triplicate. For the RSID™-Blood test, saliva from the same five donors (50 μL) was extracted at room temperature on a shaker in four hundred and fifty μL of RSID™-Saliva extraction buffer, for one-and-a-half hours and vortexed for 10s. Twenty μL of the extracted sample solution was placed into a new tube with eighty μL of RSID™-running buffer and vortexed for 10s. One hundred μL of prepared mixture was placed in the sample well of the test strip and tested in triplicate.

Every test that showed at least one positive result was additionally tested with saliva obtained from an additional five female donors and ten male donors, prepared as previously described.

2.3. Conditioned samples with various substrates and stain remover

Twenty μL of blood was deposited on various substrates in triplicate and allowed to dry overnight. Wood, metal, brick, and soil substrates were tested to investigate their possible influence on blood testing. The samples deposited on wood, metal, and brick were collected using sterile cotton swabs moistened with ultrafiltered water and each one was placed into a 1.5 ml test tube, while the blood deposited on soil ($m = 0.1 \pm 0.05$ g) was extracted together with the substrate. Twenty μL of blood on a sterile cotton swab was left to dry overnight at room temperature and was used as a positive control. Substrates without blood were used as negative controls.

The samples along with positive control were extracted in one thousand μL of extraction buffer of each test, incubated for two hours on a shaker at the room temperature. Solid substrates were separated using spin baskets. Following centrifugation, each supernatant was used for blood testing in triplicate. Swabs were added to pellet, resuspended and used for genomic DNA extraction.

In addition, the effect of commercially available stain remover containing active oxygen (Procter and Gamble, Italy) on blood testing was investigated. Due to established reproducibility of all three tests, following two experiments were conducted in monoplicate. Three blood samples (20 μL) were deposited on separate white cotton fabrics, left to dry overnight, and treated as follows: the first sample was soaked in 50 mL of warm water (40 °C) and mixed with the stain remover (final concentration 2% v/v), the second sample was soaked in 50 mL of cold water and the stain remover (final concentration 2% v/v), and the third sample was soaked in 50 mL of warm water (40 °C) without added stain remover. All samples were soaked for 2 h, rinsed in running water, and allowed to dry overnight. Blood (20 μL) deposited on white cotton fabric without soaking was used as a positive control. White cotton fabric without blood (0.5 cm^2), was used as a negative control. An approximately equal size cutting (0.5 cm^2) of each of the blood stains and positive control was cut and extracted in one thousand μL of extraction buffer of each test, incubated for two hours on a shaker at room temperature. Fabric was separated using spin baskets. Following centrifugation, supernatant was used for blood testing. Fabric was added to pellet, resuspended and used for genomic DNA extraction.

2.4. Aged casework blood stains

Five 19- to 28-year-old casework blood stains stored at room temperature were tested. An approximately equal size cutting (0.5 cm^2) of each of the blood stains was cut, extracted in the extraction buffer of each test according to manufacturer's protocols, and tested in monoplicate.

2.5. Interpreting the results

For the ABAcard® HemaTrace® test, one hundred and fifty μL of the extracted sample (one hundred μL for the HemDirect Hemoglobin and RSID™-Blood tests) was pipetted into the sample well of the test strip, and the results were recorded after exactly 10 min. The presence of two red lines (HemDirect Hemoglobin/RSID™-Blood), or two pink lines (ABAcard® HemaTrace®), one at the test "T" position and one at the control "C" position, indicates a positive result ("+"). A colored line at the control "C" position only, indicates a negative result ("−"). If there is no line visible at the control "C" position or only at the test "T" position, it indicates that the test is invalid.[14,15,20]

A clearly visible line at the control "C" position indicates that each tested strip has worked correctly. The results of the body fluid specificity testing were classified as positive, weak positive, trace positive, and negative, based on the test line intensity visualized at the test "T" position when compared to the colored line of the control "C" position. As none of the tested kits are quantitative in nature, this highly subjective scoring was used for this study only. In casework samples, the results of all three tests should be recorded as either positive (+) or negative (−), in accordance with the manufacturer's recommendations.

2.6. DNA testing

Genomic DNA was extracted using Chelex®100 with the addition of proteinase K.[19,21] Following quantification using a Quantifiler® Human DNA Quantification Kit and amplification with an AmpF*l*STR®NGM™ Amplification Kit, the PCR products were analyzed on a 3130*xl* Genetic Analyzer using GeneMapper® *ID-X* software (all of the above from Applied Biosystems, USA).[21] The