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Research article

Validation studies of rapid stain identification-blood (RSID-blood) kit in forensic caseworks

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Abstract

When bloodstains are detected at crime scene using presumptive tests (e.g. luminol, phenolphthalein, leuchomalachite green), it is important to establish the real human nature of each stain. This is possible using confirmatory tests. One of these is rapid stain identification-blood (RISD-blood) a lateral flow immuno-chromatographic strip test format which allows the identification of human blood by detection of glycophorin A, a red blood cell membrane antigen, using two anti-human glycophorin A (GPA) monoclonal antibodies.

The aim of this study is to assess the sensitivity of RSID-blood test in old, degraded bloodstains and in some bloodstains previously treated with BlueStar Forensic, a presumptive test which is often used in crime scene investigations to detect latent bloodstains. The genetic analysis of all bloodstains of confirmed human nature was subsequently performed using the AmpF1STR Identifiler PCR Amplification Kit (Applied Biosystems), to validate the possibility of obtain a consistent and reliable DNA typing results.

Keywords: Forensic DNA typing; Human blood; Hemoglobin detection; Immuno-chromatographic test

1. Introduction

Various biological samples could be found at crime scenes and some of which may not have human origin. The ability to detect and to identify every stain and, at the same time, to confirm the real human nature of each stain, is crucial both for investigative and legal purpose. DNA typing without checking the actual stains' nature and origin can easily leads to make serious mistakes. Blood is one of the most common evidences found at crime scenes. There are many presumptive tests for blood, but these cannot provide any information about human origin of the stain detected so that every time additional confirmatory tests must be performed. Recently, a new human blood confirmatory test (rapid stain identification-blood, RSID-blood) has been proposed.

The aim of this study is to evaluate the sensitivity of RSID-blood test and the feasibility of its application as an alternative method for the detection of human blood. The kit was tested in forensic caseworks involving aged, degraded stains and some bloodstains previously treated with BlueStar Forensic, a luminol-based chemical which is often used in crime scene investigations as presumptive test to detect latent bloodstains. Furthermore, to check the possibility of obtain a consistent and reliable DNA typing, genetic analysis of all confirmed bloodstains was performed using the AmpF1STR Identifiler PCR Amplification Kit (Applied Biosystems).

2. Materials and methods

The RSID-blood test was purchased from Galantos Genetics, Mainz, Germany. The test permits the identification of human blood by detection of a red blood cell membrane antigen, glycophorin A, using two anti-human glycophorin A (GPA) monoclonal antibodies in a lateral flow immuno-chromatographic strip test format.

In this preliminary study we have tested the RSID-blood kit on eleven samples of human blood coming from different forensic cases. All samples have been stored at room temperature and under ambient conditions for 1–10 years. Moreover, we have checked the possible interference with the

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RSID-blood kit of presumptive test reagents frequently used to detect latent bloodstains. To do that, four aliquots of 50 μl of human blood coming from the same subject were diluted with sterile water as the following ratios: 1:20, 1:200, 1:1000 and 1:2000. Afterwards, each dilution were placed on pieces of paper, air dried for 24–72 h and exposed to BlueStar Forensic (Roc Import, Monte Carlo, Monaco). In addition, two replicas of the previous aliquots (50 μl of human blood coming from the same subject diluted with sterile water as the following ratios: 1:20, 1:200, 1:1000 and 1:2000) were mixed with 50 μl of BlueStar Forensic. All samples were tested according to the RSID-blood protocols.

Furthermore, DNA-STR analysis was performed on all bloodstains of confirmed human nature. DNA was extracted directly from the remaining RSID-blood extraction buffer of each stains by DNA IQ System kit® (Promega, Milan, Italy) and PCR amplification was carried out using the AmpF1STR Identifiler PCR Amplification Kit (Applied Biosystems). PCR products were separated and analyzed using an ABI PRISM 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA) and a GeneScan software v. 3.7.

3. Results and conclusions

Only one sample of all aged and degraded bloodstains analysed by RSID-blood gave negative results: pantyhose. From the four aliquots of 50 μl of human blood diluted with sterile water, placed on pieces of paper and exposed to BlueStar Forensic, positive results were obtained only from the samples diluted as the ratios 1:20 and 1:200. Same results were obtained from the four aliquots of 50 μl of human blood diluted with sterile water as the ratios 1:20, 1:200, 1:1000 and 1:2000 and subsequently mixed with 50 μl of BlueStar Forensic. Assuming that all steps were performed correctly, these dilutions correspond to 2.5 μl and 250 nl, respectively. These results are in agreement with the RSID-blood detection limit for human blood which is reported of 250 nl. All samples were also tested with another immuno-chromatographic confirmatory test: Hexagon OBTI. This kit showed positive results for all aged and degraded stains and also for all blood samples which were exposed or mixed with BlueStar Forensic.

DNA extraction and PCR amplification by AmpF1STR Identifiler PCR Amplification Kit (Applied Biosystems) have been possible from all aged and degraded bloodstains of confirmed human nature and the results were excellent. No DNA results were obtained from any of the blood dilution series which were exposed or mixed with BlueStar Forensic. This could be because there was such small amount of template DNA to achieve detectable amplification products.

In conclusion, we observed that RSID-blood test is less sensitive than other immuno-chromatographic confirmatory tests (e.g. Hexagon OBTI test). Anyway, as reported in the RSID-blood technical information sheet, the sensitivity has been adjusted so that when blood is detected, sufficient biological material should be present to provide an STR profile [1]. In our opinion, this could reduce the RSID-blood employment in forensic caseworks, in particular when there are latent bloodstains and the use of presumptive tests as luminol are required. On the other hand, the opportunity to perform both stain identification and genetic analysis using the same share of stain is very important, especially when just a small amount of evidence is available.

Conflict of interest

None.

Reference

[1] Rapid Stain Identification of Human Blood, Technical information sheet, Independent Forensics, Hillside, IL.