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mRNA profiling in casework analyses

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ABSTRACT

Our work involved the application of mRNA profiling to three bloodstains previously processed by the laboratory, in which routinely methods (immunochromatography) used for specific blood identification showed negative results.
Analyses were accomplished on a sweater worn by the suspect. No bloodstains were found during the routine inspection. In order to identify possible latent bloodstains, luminol was applied. After the reagent vaporization, three distinct areas of luminescence appeared. In order to confirm the presence of the human hemoglobin, luminescent surfaces were collected using 4N6FLOQSwabs® and tested using HemDirect Hemoglobin test (SERATEC®). All samples analysed showed negative results.
Due to the merging blood-specific markers mRNA profiling, the areas previously identified were sampled newly and tested using the three blood specific markers HBB, ALAS2 and CD93, together with two housekeeping genes represented by ACTB and 18S-rRNA.
All samples showed positive results for all three blood specific mRNA markers.

Keywords: *Body fluid identification; mRNA profiling; STR*

1. Introduction

Body fluids are one of the main evidence recovered from a crime scene, and increasingly in low quantities and with poor quality [1]. Identify a particular body fluid is a crucial step since the nature of the fluid is itself highly informative to the investigation [2]. Current and commercial tests use chemoluminescence and the detection of specific proteins (immunochromatography). These commercial kits commonly used in forensic laboratories are mostly presumptive, are carried out for only one body fluid at a time, and generally are limited in specificity or sensitivity [3]. The main limit showed is represented by the destruction of the sample tested. Many times a case can be solved only by using a limited amount of biological evidence, so these must be examined as possible by non-destructive methods. The most relevant reason for these tests to be non-destructive is represented by the preservation of DNA [4].

Bloodstains, as well as other biological traces, could benefit from the development of novel and more sensitive methods for their identification.

As an emerging technique for body fluid identification, mRNA typing has seen remarkable progress and wide application in forensic genetics in recent years [5].

Research showed that messenger RNA is much more persistent than it was previously thought on the grounds of inevitable RNA enzyme-catalyzed degradation.

Despite the degradation, caused by high temperature and/or humidity, our previous work suggests that under ideal preservation conditions, mRNA can last for long and be detected in 50 plus-year-old samples [6].

Due to this novel method, our work shows the application of the mRNA typing to an old murder occurred in Ferrara in 1998. The victim died from numerous stab wounds, after a prolonged struggle and with a large amount of blood had been spattered in the room in which corpse was found.

Some witnesses claimed to have seen the husband leave home with a light-colored sweater, spotted on the front, and walking toward his mother's home.

Investigators inspected the house of the suspect's mother finding a white sweater inside a washbasin located in the laundry. During the environmental examination, no bloodstains were found. After the luminol application, three areas of luminescence were identified on the front side.

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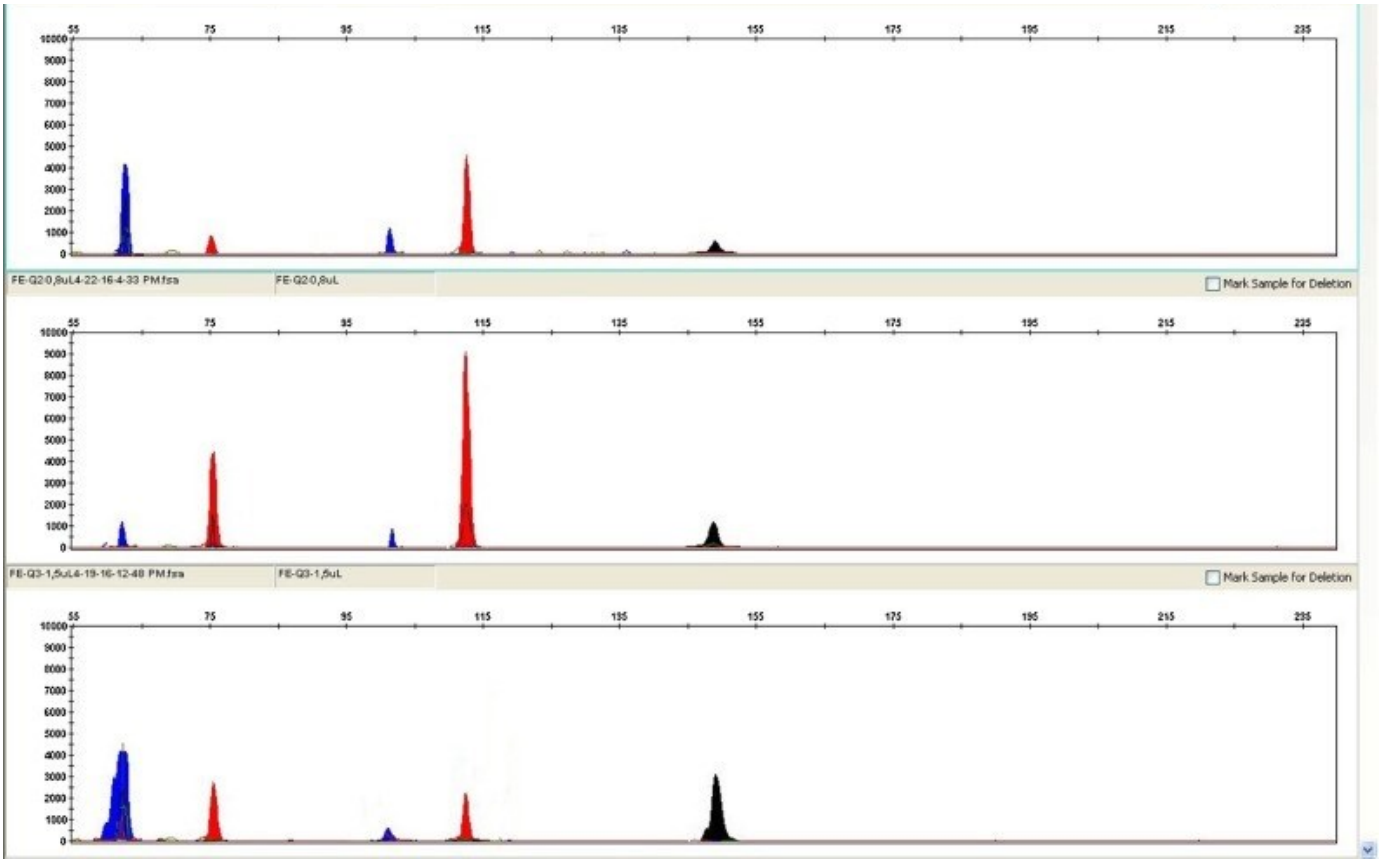
Figure 1 | mRNA profiles achieved from the analysed samples



To confirm the presence of human blood, these spots were sampled using 4N6FLOQSwabs® and tested with the HemDirect Hemoglobin test (SERATEC®). All samples showed negative results. Genetic analysis, accomplished with a conventional STRs kit (AmpFlSTR® NGM amplification kit (Thermo Scientific®)), revealed the presence of the victim's profile.

Although the negative results achieved after body fluid identification, it has been still possible to convict the suspect only by one witness evidence collected by investigators.

2. Material and Methods

Samples were collected from the luminescent areas previously isolated and labeled using luminol by three distinct 4N6FLOQSwabs®.

A DNA/RNA co-isolation protocol was developed incorporating the widely used AllPrep DNA/RNA Mini Kit (QIAGEN®), following a modified protocol developed in the laboratory.

cDNA was synthesized using the RETROscript (Ambion®). After cDNA quantification, samples were amplified using Multiplex PCR Mastermix (QIAGEN®) according to the manufacturer's instructions, in a total volume of 25 µL.

Markers, primer sequences, and concentrations were adopted from Van den Berge et al. [7]. This molecular system was previously tested in an ISFG Italian Working Group - GEFI collaborative work [8]. All thermal cycling steps were accomplished in a Veriti® 96-Well Thermal Cycler (Thermo Scientific®). DNA isolate from the co-extraction process was typed using a conventional STR kit, represented by the AmpFlSTR® NGM PCR Amplification kit (Thermo Scientific®). Detection of all amplified fragments was performed using an ABI PRISM 310 Genetic Analyzer (Thermo Scientific®) and allele calling was accomplished using GeneMapper ID-X V1.0 software (Thermo Scientific®). Allele designation was carried out in comparison to control DNA 007 and allelic ladder provided by the manufacturer. The detection threshold chosen for both DNA and mRNA profiling was set at 70RFU.

3. Results

As reported in figure 1, all samples collected revealed all three blood specific mRNA markers ALAS2, CD93, and HBB, specific for identification of blood, together with the simultaneous presence of the two housekeeping genes (ACTB; 18S-rRNA) used as internal reaction control.

Typing of co-extracted genomic DNA provided the same full STR profile from all the samples tested. As previously achieved, the profile was fully compatible with the victim's profile.

4. Discussion

Our results prove how this merging and novel

methodology can be helpful in forensic science for the identification of body fluid stains, such as bloodstains.

RNA and DNA co-extraction also represents the most important goal since the amount of sample is highly limited. The quantity and quality of DNA isolated using the co-extraction process seem to be enough in environmentally exposed samples, even though the results were slightly poorer than other conventional and commercial DNA isolation methods.

5. Concluding Remarks:

This study showed how without mRNA profiling, it would not have been possible to confirm the presence of human blood. Due to this lack, crucial evidence would be lost, endangering the entire investigation.

Gene expression analysis represents therefore a robust and alternative approach to conventional protein-based methods applied to body fluid identification, particularly when only low starting material or micro traces are available for laboratory analysis.

In conclusion, mRNA profiling is likely to play a major role in the future of forensic genetics, not only for the identification of body fluids and tissues, which was the topic of the proposed work, but also in the prediction and determination of the age of an individual, and as well as the age of a stain [9-10].

As suggested from our work, mRNA profiling will be the body fluid identification method of the future, supporting or replacing existing immunochromatographic methods.

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