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mRNA profiling: Application to an old casework

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ABSTRACT

Present work showed the application of mRNA profiling to an old casework where routinely methods used for specific blood identification revealed negative results.

Analysis involved a sweater worn by the suspect as claimed by two witnesses. No blood stains were found during routine examination. When luminol was applied, three areas of luminescence developed. In order to confirm the presence of human blood, traces were collected using sterile cotton-tipped swabs and tested using HemDirect Hemoglobin test (SERATEC®). All specimens tested were negative.

Relating to the novel and sensitive mRNA blood-specific markers profiling, material previously collected was sampled again and tested on three blood specific markers HBB, ALAS2 and CD93, together with two house-keeping genes represented by ACTB and 18S-rRNA.

All samples were positive for all three blood specific mRNA markers.

1. Introduction

Body fluid traces recovered at crime scenes are among the most important evidence to forensic investigators. The first step of identifying a particular body fluid is highly important since the nature of the fluid is itself very informative to the investigation [1]. Driven by the importance for forensic applications, body fluid identification methods have been extensively developed.

As an emerging technique, mRNA profiling has seen remarkable progress and wide application in forensic genetics in recent years [2].

Due to this novel and sensitive technique, this work showed the application of the method 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 throughout the living room. The victim was married and so authorities questioned her husband regarding his wife's life. They were known to have had a stormy marital relationship. The husband stated that he had seen his wife leave their home in the morning with her sister and that was the last time that he had seen her alive.

Some witnesses questioned by the police claim to have seen the husband leave home with a light-colored sweater and go to her mother's home. Two of them claim that the sweater was spotted on the front. From this moment, the husband became the suspect. Police inspected the house of the suspect's mother finding a white sweater inside a wash basin located in the laundry. Sweater corresponded to the one worn by the suspect. No blood stains were found during the routine examination. When luminol was applied, three areas of luminescence developed in the front side. Fig. 1 shows the evidence collected by Police and luminol reaction.

In order to confirm the presence of human blood, traces were sampled using sterile cotton-tipped swabs and tested using HemDirect Hemoglobin test (SERATEC®). All samples were negative. Genetic analysis, accomplished with AmpF*l*STR® NGM™ amplification kit (Thermo Scientific®), revealed the presence of the victim's profile. Fortunately, although these negative results in the confirmatory test, it has been still possible to convict the suspect only by one witness evidence collected by investigators.

2. Materials and methods

For DNA/RNA co-extraction, a 1 cm × 1 cm piece was cut from each trace previously identified on the evidence after luminol application.

After a preliminary eluition step, DNA/RNA co-extraction was performed using AllPrep DNA/RNA Mini Kit (QIAGEN®), adopting a modified protocol developed in the laboratory.

For the reverse transcription reaction, random primers and RETROscript (Ambion®) were used. After cDNA quantification, samples were amplified using Multiplex PCR Mastermix (Qiagen®) according to the manufacturer's instructions, in a final volume of 25 μL.

Markers, primer sequences and concentrations were adopted from Van den Berge et al. [3] and previously tested in an ISFG Italian

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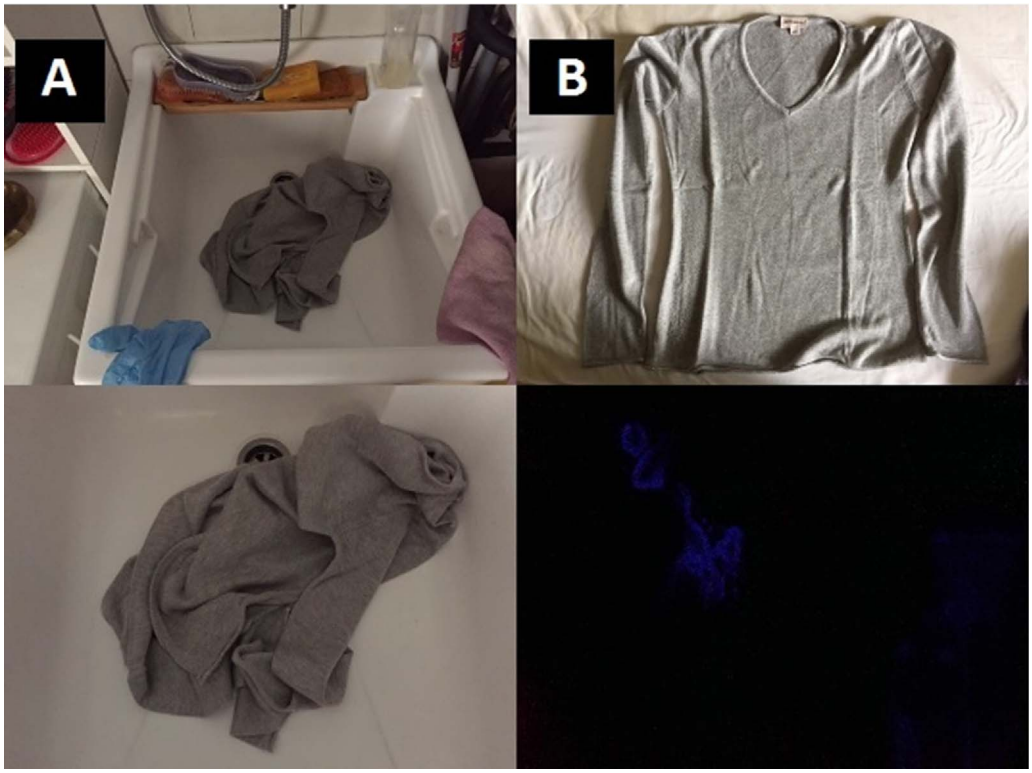

Fig. 1. sweater collected from police (A); areas of luminescence developed after luminol application (B).

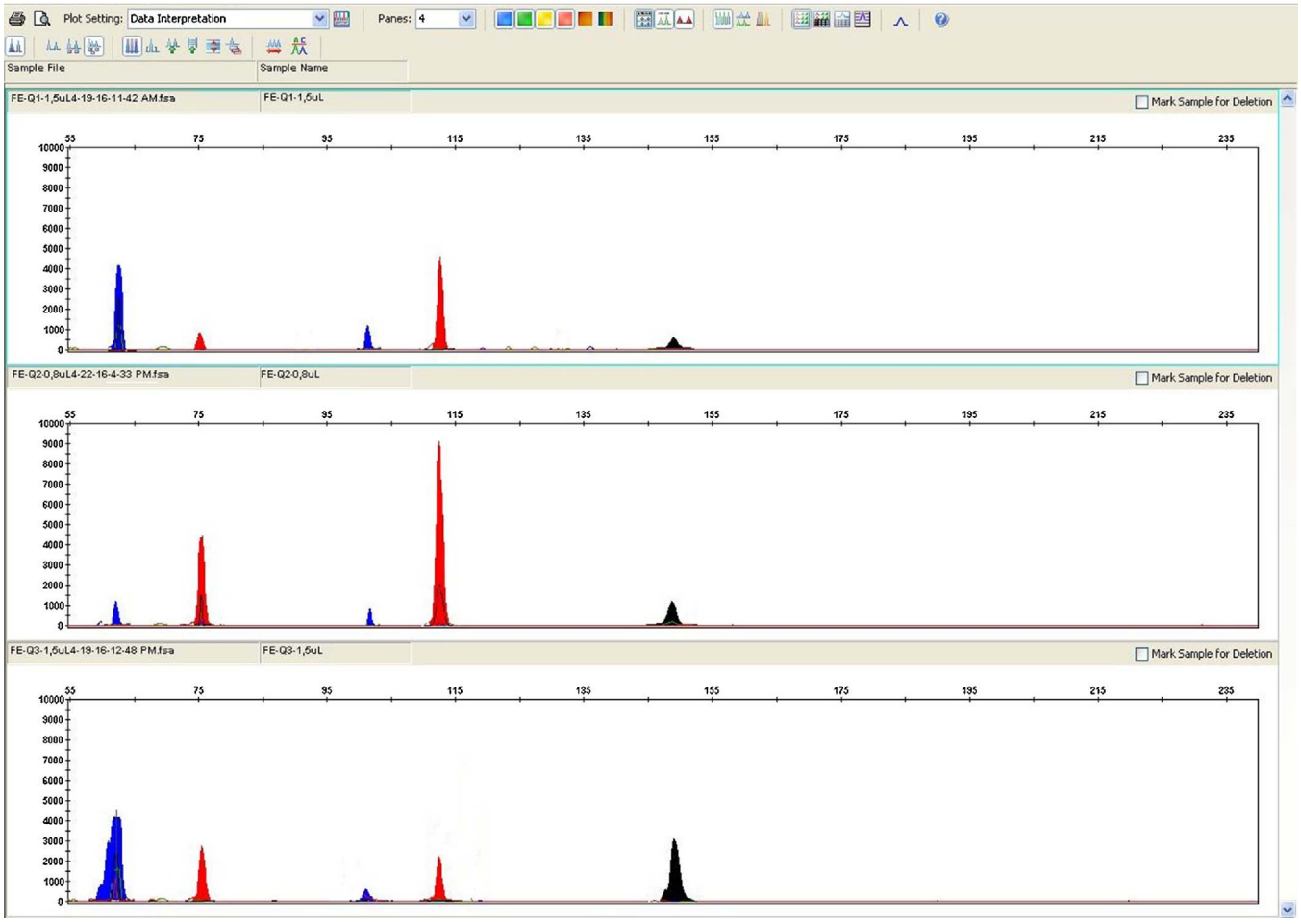

Fig. 2. mRNA profiling achieved from analyzed traces.

Working Group – GEFI collaborative exercise.

All thermal cycling steps were accomplished in a Veriti® 96-Well Thermal Cycler (Thermo Scientific®).

DNA was STR typed using the AmpF*l*STR® NGM amplification kit (Thermo Scientific®).

Detection of all amplified fragments was performed using an ABI PRISM 310 Genetic Analyzer (Thermo Scientific®) and allele calling was performed using GeneMapper ID-X V1.0 software (Thermo Scientific®).

NGM® allele designation was carried out in comparison to control DNA 007 and allelic ladder provided by the manufacturer. The detection threshold for both DNA and mRNA profiling was set at 70RFU.

3. Results

As illustrated in Fig. 2, all samples were positive for all three blood specific mRNA markers ALAS2, CD93, and HBB, together with two housekeeping genes (ACTB; 18S-rRNA) used for identification of blood.

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 victim's profile.

4. Discussion

Results emphasize how this novel technology can play a critical role in forensic science for the identification of body fluid stains, such as blood stains.

The possibility of co-extracting RNA and DNA from the same stain sample is one of the most important goal since the amount of sample is often limited in forensic casework. The quantity and quality of DNA from co-extracted samples seemed to be sufficient also for casework and environmentally exposed samples, even though the results was slightly poorer than others conventional DNA extraction methods.

5. Conclusion

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

Gene expression profiling analysis represent therefore a robust and alternative approach to conventional protein-based body fluid identification.

In summary, 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 focus of the present work, but also in the determination of the age of an individual, as well as the age of a stain.

As suggested from this work, mRNA profiling will be the body fluid identification method of the future, supporting or replacing conventional protein-based body fluid identification.

Conflict of interest statement

None.

Acknowledgments

None.

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