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mRNA profiling in ancient blood stains

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ABSTRACT

In order to test mRNA profiling as a method to identify aged blood stains, this work tested two sets of blood stain samples, 50 and 60 plus-year-old respectively, on three blood specific markers HBB, ALAS2 and CD93, together with two housekeeping genes represented by ACTB and 18S-rRNA.

Blood traces were also tested using HemDirect Hemoglobin test (SERATEC®) to compare results.

Results: showed HBB as a very stable mRNA molecule highly suitable for the detection of old blood stains. HBB give positive results in over 80% of the 60-year-old samples and over 90% of the 50- year-old samples.

All membranes were all weakly positive for blood, except for five 60 years back samples. Full STR profiles were accomplished for all blood samples. This finding proves mRNA profiling an effective and alternative way of identifying aged bloodstains.

1. Introduction

Human body fluids, especially blood, are often recovered from a variety of crime scenes, sometimes in extremely low quantities [1]. Blood stains, as well as all biological evidences, could benefit from the development of novel and more sensitive methods for their identification.

As an emerging technique for body fluid identification, messenger RNA profiling has seen remarkable progress and wide application in forensic genetics in recent years.

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

Despite degradation, caused by high temperature and/or humidity, some Authors suggests that under ideal preservation conditions, mRNA can last for long and be detected in 20 plus-year-old samples.

Inspired by the long-lasting feature of mRNA, the aim of this study was to detect the durability of three blood specific markers ALAS2, CD93, and HBB, together with two housekeeping genes (ACTB; 18S-rRNA) in old blood stains dated 50 years and 60 years back respectively.

2. Materials and methods

A total of 20 aged blood stains stored in the Institute of Legal Medicine of Ferrara, Italy, were evaluated. Samples were collected in the form of blood-soaked cotton tissue. Stains were divided into two sets: 10 samples identifying 50-year-old group and 10 samples identifying 60-year-old group. Fig. 1 shows some of the blood stains used in the present work.

Each stain was cut into two equal parts in order to perform a DNA/RNA co-extraction and at the same time to detect human hemoglobin using HemDirect Hemoglobin test (SERATEC®) in order to compare results. DNA/RNA were extracted 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.

Primer sequences and concentrations were adopted from Van den Berge et al. [2] and previously tested in an ISFG Italian 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.

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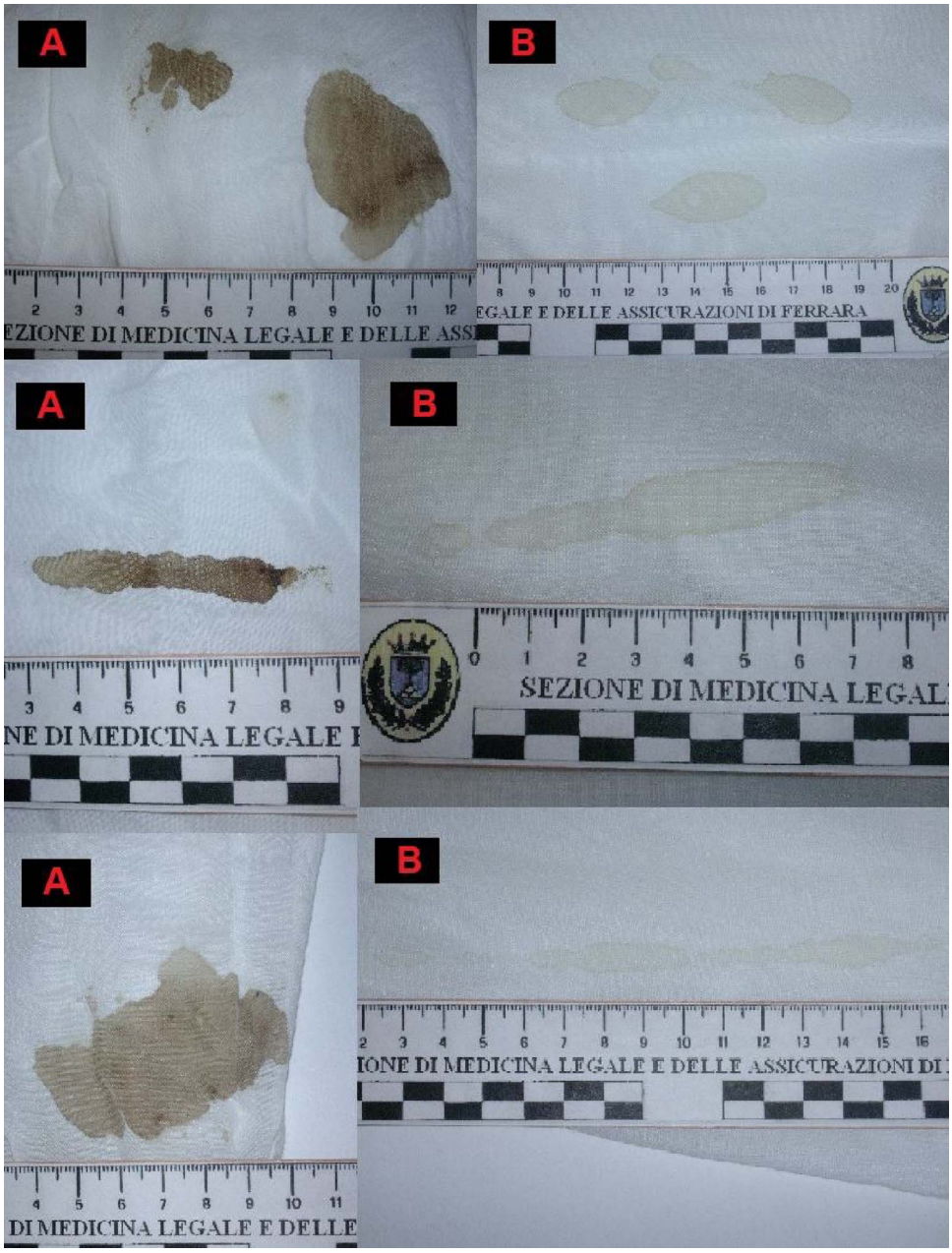

Fig. 1. 50-plus-years old (A)and 60-plus-years old (B) blood stains.

3. Results

Results showed all blood stains positive for mRNA identification. The most stable of the three blood markers selected was HBB, returning positive results in over 80% of the 60 years and over 90% of the 50 years back samples, even better than the housekeeping genes ACTB and 18S-rRNA.

At the contrary, CD93 and ALAS2 markers gave weaker signals in aged samples, particularly in 60 plus-year-old specimens.

The worst marker was CD93 returning negative results in over 60% of the 60 years and over 45% of the 50 years back samples.

All membranes were all weakly positive for blood, except for five 60 years back samples were negative. Fig. 2 shows the comparison between negative immunoassay and positive result of the same trace (60 plus-year-old samples) achieved using mRNA profiling.

DNA was also recovered from the RNA extraction process, using a DNA/RNA co-extraction.

DNA extracted from all the old blood stains used gave full STR profiles. Only one locus drop out at D2S1338 was observed for one 60-year-old sample.

4. Discussion

As expected, HBB, as a subunit of hemoglobin, was identified as a very stable mRNA molecule highly suitable for the detection of old blood stains.

Previous Authors have reported the detection of HBB in 23 years old blood stains [3]; this study extends that detection window to over 60 years.

Other markers (CD93 and ALAS2) give weaker signals in aged samples. Particularly, CD93 drop out was observed, both in 50 and 60 plus-year-old samples. As suggested by Van den Berge et al. [2] and as observed in GEFI (Italian working group of ISFG)collaborative exercise, CD93 is less stable marker. Based on the laboratory experience, that is a reasonable outcome as these markers did not respond as well in fresh samples where a high degree of locus drop out was previously observed.

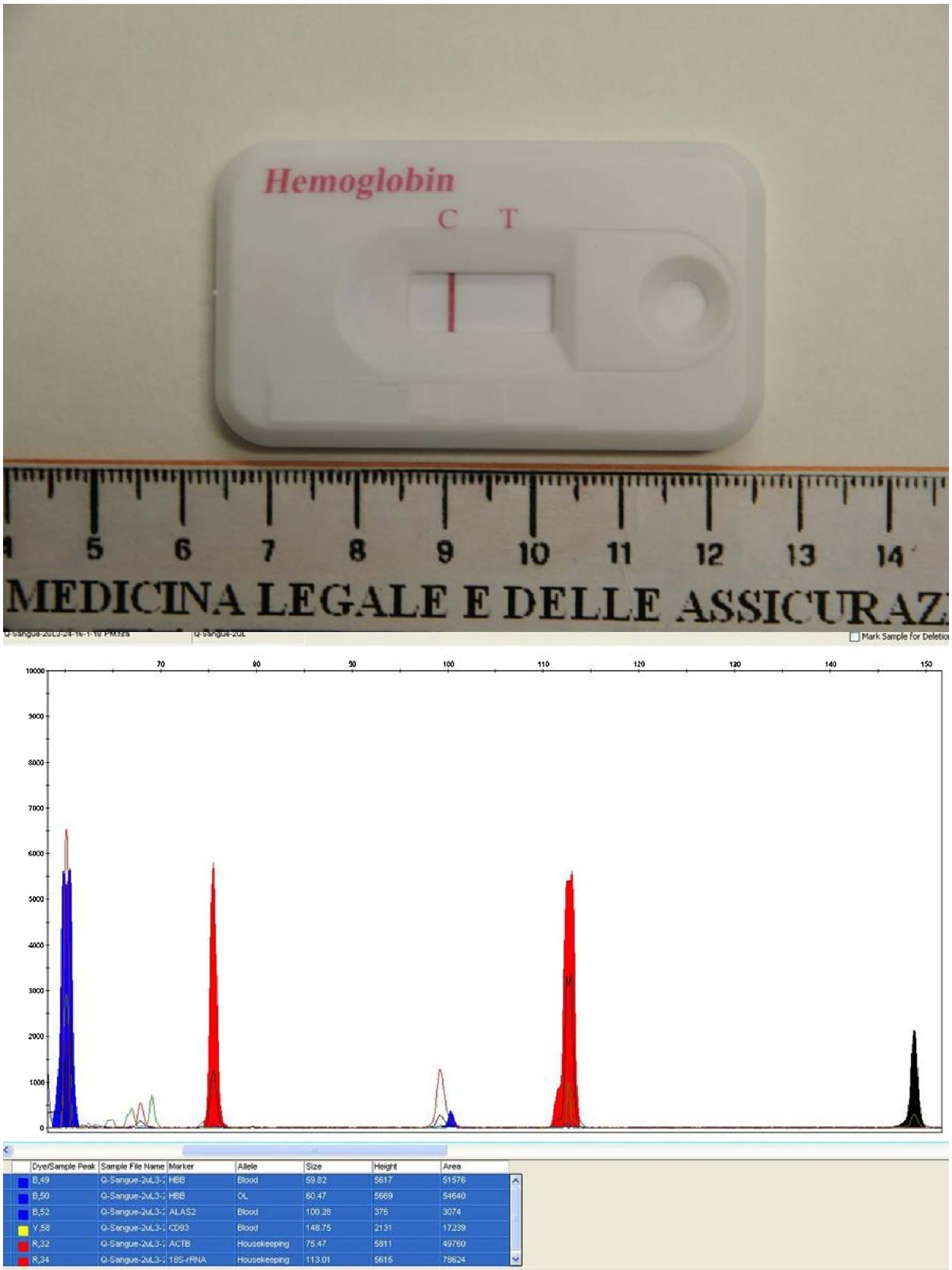

Fig. 2. Comparison between the confirmatory test and mRNA profiling achieved from the same 60 plus years old specimen.

5. Conclusion

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

Especially, our findings showed the crucial role of sample

preservation and storage. Specimens used for this study were stored at room temperature, isolated from light and moisture, which are important reasons why RNA can be extracted at high recovery.

Although evidence recover from a crime scene, generally, show a high degree of degradation, it's crucial to ensure good sample preservation and storage at the laboratory in order to achieve better mRNA profiling results. Blood stain samples used for the present work were collected in the form of blood-soaked cotton tissue that represents the better and ideal storage conditions.

Finally, further studies may provide more information on whether other aged body fluid stains can be detected in a similar way and also may be helpful to understand the effect of the fabric/surface of deposit.

This could be helpful to understand interactions developing between samples and surface of deposit and, finally, inhibition of molecular reaction caused by the same ones.

Conflict of interest statement

None.

Acknowledgments

None.

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