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Forensic Science International: Genetics Supplement Series 1 (2008) 37–38
www.elsevier.com/locate/FSIGSS

Research article

mRNA profiling for body fluid identification

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Received 17 August 2007; received in revised form 10 October 2007; accepted 11 October 2007

Abstract

mRNA profiling is a promising new method for the identification of body fluids from biological stains. A multiplex reverse transcription endpoint PCR method was adapted for the identification of blood, saliva, semen, vaginal secretions and menstrual blood. For specificity, sensitivity and suitability to biological stains the endpoint PCR method was satisfying. Up to 15 months old stains were shown to be suitable for mRNA profiling. In addition a realtime PCR assay was tested for sensitivity with blood and semen samples, providing comparable results to endpoint PCR or enzymatic tests.

Keywords: mRNA profiling; Body fluid identification

1. Introduction

Analysis of cell-specific mRNA expression is a new technique for the identification of body fluids from biological stains. In a number of studies mRNA markers for the forensically most relevant body fluids blood, saliva, semen, vaginal secretions and menstrual blood have been identified [1–3]. A multiplex reverse transcription endpoint PCR method was adapted using the following genes which are expressed in a tissue-specific manner: porphobilinogen deaminase (PBGD) and beta-spectrin (SPTB) for blood, statherin (STATH) and histatin 3 (HTN3) for saliva, protamin 1 and 2 (PRM1, PRM2) for semen, human beta-defensin 1 (HBD-1) and mucin 4 (MUC4) for vaginal secretions and matrix metalloproteinases 7 and 11 (MMP-7, MMP-11) for menstrual blood. Specificity, sensitivity and suitability to biological stains were tested and mRNA stability was followed over time. In addition a realtime PCR assay was tested for sensitivity with the mRNA markers SPTB for blood, PRM2 for semen and kallikrein 3 (PSA), semenogelin 1 (SEMG1) for seminal plasma.

2. Materials and methods

Samples: Body fluids were collected on cotton swabs and dried at room temperature. *RNA-extraction*: Qiagen Micro/Mini Kit or DNA/RNA Mini Kit. *Reverse transcription*: random primers, Superscript III from Invitrogen. *Endpoint PCR*: Most primers were adopted from Juusola and Ballantyne [1]. The primers for MMP-11, STATH and HBD-1 were self-designed using online softwares. ABI 10× Buffer I and Taq Polymerase were used for the singleplex and the Qiagen multiplex PCR kit for the multiplex. PCR products were detected with an ABI 310 Genetic Analyzer. *Realtime PCR*: cDNA was amplified using ABI TaqMan Gene expression assays and detected with an ABI PRISM 7000 sequence detection system. *Enzymatic tests*: Tetramethylbenzidine for blood, α-Amylase-Ecoline S+ for saliva, microscopy for the detection of spermatozoids and acid phosphatase for seminal plasma.

3. Results and discussion

Body fluid specificity and cross-reactivity of the mRNA markers were tested by singleplex PCR. It could be demonstrated that mRNA for the candidate gene was present in the corresponding body fluid but absent in all others. The optimization of the multiplex was challenging, but in the end a satisfactory result was obtained (Fig. 1). The sensitivity of the assays was tested with different amounts of dried body fluids (5, 1, 0.1, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} μl blood, saliva, semen) on cotton swabs (Fig. 2). The endpoint PCR and the realtime PCR methods were as sensitive as conventional enzymatic tests, except for blood samples. Up to 15 months old dried stains were

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doi:10.1016/j.fsigss.2007.10.064

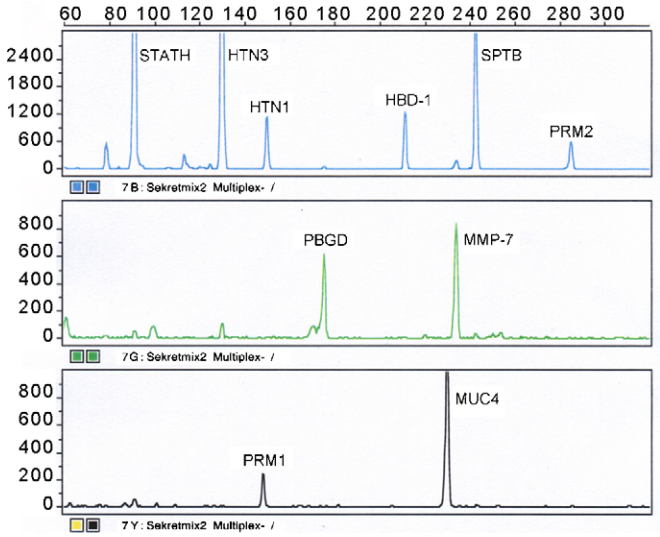

Fig. 1. Multiplex endpoint PCR result using RNA extracted from a blood–saliva–semen–vaginal secretion–menstrual blood mixture on a single swab. There are two peaks for every body fluid except for menstrual blood, because the MMP-11 marker was not yet integrated into the multiplex.

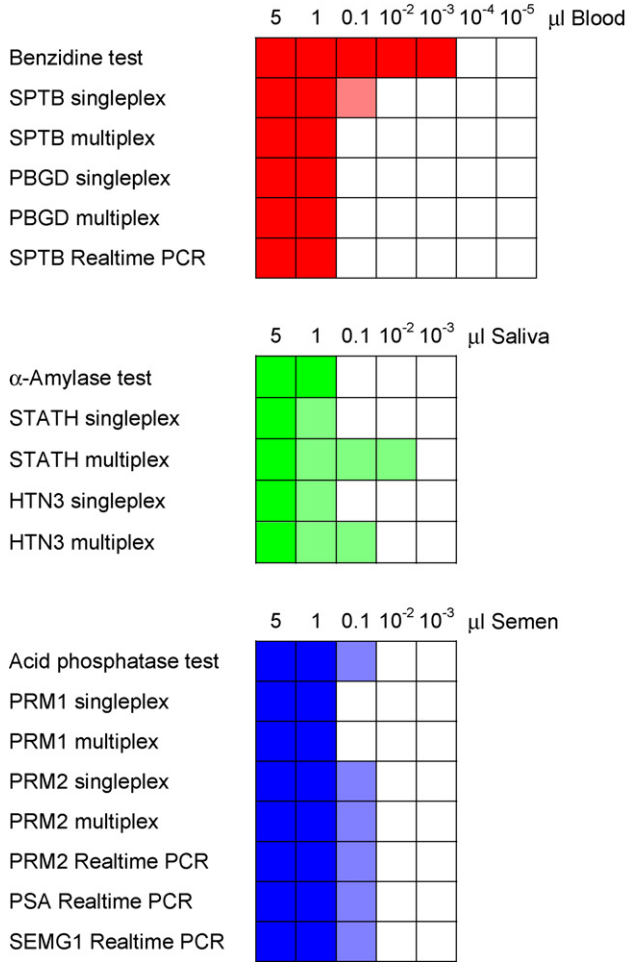

Fig. 2. Sensitivity of the RNA profiling methods (endpoint PCR and realtime PCR) compared to conventional enzymatic tests for the identification of blood, saliva and semen. Each experiment was carried out three times. The light-colored squares represent results that were obtained only in 1–2 out of 3 experiments.

shown to be suitable for mRNA profiling. There was only a slight tendency for decreasing peakheights in old samples. The multiplex endpoint PCR method was applied to casework samples. Thirteen artificial stains were prepared on different media. Most of them were identified correctly; there was one negative result with a chewing gum and one false positive result with a cigarette. Sixteen cases including 30 stains from routine casework were analyzed, for example sanitary towels, condoms, underpants, bed sheets. With most of the casework samples a reasonable result could be achieved, concordant with the enzymatic test results.

mRNA profiling is a promising alternative to conventional enzymatic methods for the identification of body fluids. Major advantages are the detection of all body fluids in a single multiplex reaction and the possibility for simultaneous DNA isolation without loss of material.

Acknowledgements

The authors thank C. Maake and A. Sulzer for scientific support and N. Giezendanner and T. Burtscher for technical assistance.

Conflict of interest

None.

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