mRNA profiling for the identification of sperm and seminal plasma

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Abstract

mRNA profiling is a promising new method for the identification of body fluids from biological stains. In this study we aimed to establish a multiplex RT-PCR protocol for the detection and differentiation of sperm and seminal plasma. The Agilent Bioanalyzer and Nanodrop spectrophotometer were shown not to be suitable for assessing RNA quality and quantity of forensic stains. Semen specificity of the mRNA markers was successfully confirmed with singleplex PCR. Our data indicated that semen samples down to 0.1 µl and up to 20-year-old could be identified with mRNA profiling. With the semen multiplex, including 2 sperm markers (PRM1, PRM2) and 2 novel seminal plasma markers (SEMG1, PSA), samples from azoospermic men (absence of sperm in semen) are clearly distinguishable from those of normozoospermic men (having a normal sperm production). We think that our multiplex RT-PCR protocol is a reliable and sensitive method for the identification of semen in forensic samples.

Key words

Forensic science, mRNA, semen, PRM1, PRM2, SEMG1, PSA
1. Introduction

Currently, the identification of semen is sample- and time-consuming due to the requirement of separate presumptive tests for sperm and seminal plasma, both of them being necessary to identify azoospermic men. We recently showed that multiplex reverse transcription-polymerase chain reaction (RT-PCR) is a suitable tool to profile mRNA of several body fluids from biological stains [1]. In the present study we thus aimed to establish a multiplex RT-PCR protocol for the concurrent and sensitive detection of sperm and seminal plasma markers. The following genes were analyzed: protamine 1 and 2 (PRM1, PRM2) for the identification of sperm; semenogelin 1 (SEMG1) and prostate specific antigen (PSA) for the identification of seminal plasma.

2. Materials and methods

Samples: Semen from 6 normozoospermic and 1 azoospermic men and blood, saliva, buccal swabs, vaginal swabs, menstrual blood swabs from 2-3 different individuals were collected on cotton swabs and dried at room temperature (10-50 µl body fluid or 5 µl of a semen dilution series). The human squamous carcinoma cell line UMB-SCC-745 was cultured under standard conditions and served as control. RNA-extraction, reverse transcription, endpoint PCR were described previously [1]. RNA quality and quantity were assessed with an Agilent 2100 Bioanalyzer using a RNA pico chip (Agilent, Basel, Switzerland) and a Nanodrop 1000 spectrophotometer (Witec, Littau, Switzerland), respectively. Primers for PRM1 and PRM2 were adopted from the literature [2], primers for SEMG1 and
PSA were designed using the online softwares Primer3 and Roche Applied Science-Universal Probe library (SEMG1 forward: 5’-FAM-TCGGTAACCATGTGAAAGGA, reverse: 5’-GTGTCATCCATGGACCAAGA, PSA forward: 5’-NED-TGTCCGTGACGTGGATTG, reverse: 5’-GGTTGGGAATGCTTCTCG). Amplicon sizes are 82 bp for PSA, 120 bp for SEMG1, 153 bp for PRM1 and 294 bp for PRM2. Enzymatic/immunologic tests included acid phosphatase test (standard protocol), RSID semen test (Galantos Genetics, Mainz, Germany) and PSA Semiquant (Seratec, Göttingen, Germany).

3. Results and discussion

The quantity of the extracted RNA was below the detection limit of the Nanodrop spectrophotometer. The Agilent Bioanalyzer uses the RNA Integrity Number (RIN) for quality assessment, which takes not only the ratio of the 18S to 28S ribosomal subunits into account but the entire electrophoretic trace. The RIN classification is based on a numbering system with 1 being the most degraded profile and 10 the most intact. The RNA quality of the human cancer cell line UMB-SSC-745 was good with RIN 9.4, the RNA quality of the semen samples was poor, showing complete RNA degradation (RIN n/a). Nevertheless, after reverse transcription and marker-specific amplification, electropherograms reliably showed fragments of the expected sizes (Fig. 1). We therefore conclude that the Nanodrop Spectrophotometer and the Agilent Bioanalyzer are not suitable for assessing RNA quality and quantity of forensic samples.
Semen specificity of all mRNA markers was successfully confirmed with singleplex PCR, in that mRNA for the candidate genes were present in the semen samples but absent in all others. All RT minus controls (RT reaction without reverse transcriptase) were negative. Also high input amounts of genomic DNA (50 ng) didn’t show extraneous peaks at marker specific positions. The sensitivity of the assays was tested with semen dilution series on cotton swabs (5, 1, 0.5, 0.1, 0.05, 0.01 µl semen) from 5 different individuals. Our singleplex data indicated that semen samples down to 0.1 µl could be identified with mRNA profiling, slightly inferior to conventional enzymatic/immunologic tests, which identified semen down to 0.05 µl (Table 1). However, the achieved sensitivity of the mRNA method is suitable for forensic stains.

With the semen multiplex, including the 2 markers for sperm cells (PRM1, PRM2) and the 2 markers for seminal fluid (SEMG1, PSA), samples from azoospermic men can clearly be distinguished from those of normozoospermic men (Fig. 1). The semen samples used in this study were up to 20-year-old, revealing as good results as fresh semen samples.

4. Conclusion

Although the RNA quality of forensic samples is poor, mRNA profiling proved to be a reliable and sensitive method for the identification of semen. Major advantages of mRNA profiling are the possibility to co-extract DNA from the same piece of stain and to analyze a whole set of body fluid specific markers in a multiplex PCR reaction. We believe that mRNA profiling will be the body fluid
identification method of the future, replacing sample- and time-consuming presumptive tests.

References


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Conflict of interest

none
Table 1: Sensitivity of the mRNA profiling method compared to conventional enzymatic/immunologic tests. Semen dilution series of 5 different individuals were analyzed with the acid phosphatase test, the RSID semen test, the PSA semiquant test and mRNA profiling (singleplex PCR). The dark-colored squares represent 100% positive results, the light-colored squares represent positive results that were obtained only in 2-4 out of 5 samples, white squares represent 0-1 positive results out of 5 samples.

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Fig. 1: Multiplex PCR of a semen sample from a normozoospermic man (a) and an azoospermic man (b). The sperm-specific markers PRM1 and PRM2 are missing in sample b.