H11 Investigation of the Utility of Five Messenger RNA (mRNA) Markers (SEM1, KLK3, PRM1, PRM2, and TGM4) in the Identification of Semen

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After attending this presentation, attendees will better understand the effect of mRNA profiling in the identification of semen.

This presentation will impact the forensic science community by illustrating that five semen mRNA markers could be used as an alternative to the traditional method in the identification of semen.

mRNA profiling is a promising method studied for nearly ten years in identifying biological fluids.[1-5] In this study, the utility of five mRNA markers (Protamine 1 (PRM1), Protamine 2 (PRM2), Semenogelin 1 (SEM), Kallikrein 3 (KLK3), and Transglutaminase 4 (TGM4)) in identification of semen was investigated. 18SRNA, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Transcription Elongation Factor (TEF), and Ubiquitin-Conjugating Enzyme (UCE) mRNA markers were used as endogenous control. RNAs were analyzed by reverse transcriptase and multiplex Polymerase Chain Reaction (PCR) method following combined isolation of DNA/RNA/micro RNA (miRNA) in order to investigate the rate of presence in semen, specificity and sensitivity of the selected markers, and if they were appropriate in defining stains kept under different environmental conditions. The products were run in capillary electrophoresis.

The five mRNA markers selected were identified in all of the 25 fresh semen samples and the rate of presence in semen was 100%. All markers could be detected in 0.1µl semen by multiplex PCR method. The most sensitive one among these markers was found to be SEM1 (the sensitivity was defined to be 0.0025µl). None of the five semen markers could be identified in samples of blood, menstrual blood, saliva, and vaginal fluid. It was found that the stabilities of the selected semen mRNA markers were proportional with their sensitivities. It was observed that the stability of TGM4, KLK3, and PRM2 in stains was lower compared to SEM1 and PRM1.

Fifteen semen stains which were kept for five years at 4°C, room temperature (22-25°C, humidity ~60%), and 37°C in paper envelopes were used for the goal of testing time-dependent stability. It was found that the RNA integrity of the samples kept for the same period at a lower temperature, but in a damp environment (room temperature), disrupted much faster compared to the ones which were kept in dry, hot weather (37°C in incubator).

A full DNA profile could be achieved in 0.04µl of semen in which DNA/RNA were isolated in combination and a partial DNA profile could be achieved in 0.01µl of semen by using a commercial 15 Short Tandem Repeat (STR) PCR amplification kit.

The results showed that both multiplex DNA profile and semen-specific multiplex RNA profile could be demonstrated in a single sample by isolation of DNA/RNA in combination in a very small amount of semen and the selected five semen markers could be used as an alternative to the traditional method in identification of semen because of their sensitivity, specificity, and stability in stains. Although the sensitivities of SEM1 and PRM1 were higher, it was thought that all five semen markers selected could be used in forensic semen identification by using multiplex PCR protocols.

Reference(s):

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Semen Identification, mRNA Profiling, Body Fluid Identification