RELATIVE HUMAN AND BACTERIAL CONTRIBUTION FROM TOTAL RNA EXTRACTS OF FIVE HUMAN FORENSIC-LIKE STAINS

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The use of mRNA profiling is being explored as an alternative to traditional serology to aid in body fluid identification in forensic casework. While each nucleated cell within an individual contains the same DNA, the RNA expression of each tissue is unique. Tissue-specific mRNA markers have been identified, and specific PCR primers were designed and utilized in an effort to conclusively identify forensically relevant body fluids. However, after total RNA isolation from human body fluid stains, determining the quantity of RNA obtained may be useful for downstream analysis. The presence of bacteria within the body and the environment may skew total RNA quantification. Thus, total RNA quantification may overestimate the amount of human RNA within the sample, defeating the purpose of the quantification step and making the optimal amount of input RNA for PCR more difficult to determine.

In an effort to optimize PCR conditions and, in turn, produce more uniform results by way of capillary electrophoresis, the relative human and bacterial contributions to total RNA were determined in five body fluids: menstrual blood, peripheral blood, saliva, semen, and vaginal secretions. Real-time PCR was performed with primers specific for human 18S rRNA and bacterial 16S rRNA genes. With regard to their bacterial contribution, preliminary results indicate that the investigated body fluids are essentially split into two groups. Total RNA from saliva, vaginal secretions, and menstrual blood contained considerable bacterial RNA, while total RNA from peripheral blood and semen did not. The human contribution was less variable across the five body fluids, however important differences were noted. The lowest amount of human RNA was obtained from saliva, which contained the greatest amount of bacterial RNA. These findings suggest that relying solely on traditional spectrometry prior to downstream gene expression analyses may result in difficulties in interpretation and reproducibility, particularly for sensitive techniques such as microarray gene expression analyses.