G80 The Use of Forensic Messenger RNA (mRNA) Analysis to Determine Stain Age

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After attending this presentation, attendees will understand the principles of forensic mRNA analysis and learn how it may be used as a method to determine the age of biological stains found at crime scenes.

This presentation will impact the forensic science community by proposing a novel method for determining the age of biological stains. The proposed method could prove crucial for forensic casework and could run parallel to the predominant DNA analysis method. Where DNA analysis is used to identify a suspect, mRNA analysis could be used to determine the age of biological stains, that in turn could place the suspect at the scene of a crime at a specific time.

RNA has been presumed to be very unstable, as one of its features is that it degrades rapidly as it is broken down by ribonucleases. In previous literature however; it has been shown that RNA may be extracted from biological stains up to 16-years-old. For stain age determination, it has been suggested that using a decay rate ratio, derived from two endogenous controls differentially expressed within the body fluid stain, should eliminate the effect of any external decomposition factors. This should then be expressed as a linear change in mRNA expression over time.

The majority of previous research concentrates on determining the age of bloodstains. In this study, blood and saliva stains were used. The goal of the study was to try to determine the age of these biological stains by isolating mRNA and quantifying the expression of housekeeping genes 18s, GAPDH, and ACTB also known as β-Actin. This study aims to demonstrate proof of principle and explore any limitations to a decay rate ratio, to see if there is any correlation between the age of a stain and the amount of genetic material present.

Blood and saliva samples have been collected on a regular basis over the past two years. Bloodstains were prepared using the finger prick method and depositing blood on to a sterile filter paper. Saliva stains were prepared by swabbing the inside of the cheek with a buccal swab. All samples were taken from healthy individuals. The samples were stored at room temperature and protected from sunlight. Both blood and saliva samples were extracted using Qiagen RNeasy Mini Kit with the appropriate modifications for each body fluid. The extracted samples then underwent RNA quantitation, DNase digestion, and reverse transcription using M-MLV reverse transcriptase and random hexamers. The resulting complimentary DNA (cDNA) was then quantified using absolute quantitation on a 7500 Fast Real-Time PCR System, using pre-designed Taqman Gene Expression Assays for human housekeeping genes 18s, GAPDH, and ACTB (β-Actin). Only two housekeeping genes were run together at one time, this was to compare the differences between all three, for example the decay rate ratio between 18s and GAPDH as well as 18s with ACTB and then GAPDH with ACTB. The two values from quantifying the two housekeeping genes were then expressed as a ratio. The proof of principle would be demonstrated by a linear downward expression of the two genes over time.

The results from initial experiments show that mRNA can be extracted from stains up to two years old. Further experiments are being carried out to obtain enough data to determine the proof of principle.

Future work will involve optimising the protocols and identifying the shortest stain age per body fluid as well as exploring the use of more housekeeping genes. With bloodstains being the current focus in most of the literature, experiments will be carried out to determine the expression levels of human housekeeping genes in semen to see if it is possible to identify the time of deposition. Identifying the time a semen stain was deposited in a sexual offence case could prove to be vital when trying to convict or exonerate an individual.

mRNA, Stain Age Determination, Housekeeping Genes

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