IMPLEMENTATION OF MESSENGER RNA BODY FLUID TESTING IN FORENSIC CASE WORK.

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INTRODUCTION

The identification of body fluids in forensic casework has traditionally involved protein based tests that indicate, though cannot confirm, the presence or absence of a specific body fluid and for some body fluids such as menstrual blood and vaginal material, there are no protein based tests to detect the presence or absence of these body fluids. For these reasons a new method for identifying body fluids was required. At ESR we have developed a multiplex PCR system known as CellTyper that utilises messenger RNA (mRNA) and can identify blood, saliva, semen, menstrual blood and vaginal material in individual stains or in mixtures of body fluids. Messenger RNA transcripts specific to each type of body fluid have been identified and a multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) system developed to identify these body fluids along with three housekeeping genes. This multiplex can detect semen and seminal fluid (semen without spermatozoa present).

We have targeted the co-isolation of RNA and DNA from the same sample and, using the RT-PCR CellTyper multiplex, we can determine the type of body fluid present while also generating a DNA profile from the same stain. We have undertaken an extensive validation exercise undertaken prior to casework implementation which included a further assessment of sensitivity, variability and specificity as well as more operational requirements such as quality control and quality assurance activities. Aspects of the limitations and reporting of such new technology will be described.

WHY MESSENGER RNA?

Messenger RNA translates the genetic code of DNA in the cell into what type of protein is made. Different cell types present in body fluids, for example the cells present in blood or saliva, have different functions and therefore require different proteins. Consequently different cell types within body fluids have different mRNA markers present. Using reverse transcriptase PCR (RT-PCR), the mRNA markers can be multiplexed together so that all body fluid(s) present can be detected in one reaction. Multiplexing also avoids confirming the expected results – minimising contextual effects.

CO-EXTRACTION OF DNA AND RNA

We have successfully modified the Promega DNA IQ™ to enable co-extraction of DNA and RNA from the same sample without compromising the potential DNA profile. This method means that integration into the standard laboratory procedure was achieved with no detrimental effect on the quality or quantity of DNA extracted.

LIMITATIONS

Over-amplification of some body fluid markers resulted in non-specific artefact peaks. This occurred when over-amplified peaks were greater than 5000rfu. When peaks are over-amplified (greater than 5000rfu) we recommend using less starting material or diluting the cDNA. While the RNA may also be diluted, this results in cDNA having to be re-synthesised.

We have also found that there were artefacts at the same position as body fluid markers. We removed this problem by redesigning primers to move the position of the body fluid marker away from the artefact.

QUALITY CONTROL

As mRNA profiling is a new method and uses different consumables from DNA profiling, we have defined the critical reagents and established a testing protocol of these reagents before use. We have also established the performance criteria expected of these reagents before use.

Proficiency testing is an important aspect in forensic science and we have established proficiency tests and reporting in a similar manner required for DNA profiling. We discovered that previously frozen body fluids are not suitable for developing proficiency tests, as results are not reliably obtained from frozen body fluids. This also is applicable to case samples where they should be stored dry rather than frozen. Subequently we made modifications to the extraction controls used at ESR to allow for mRNA testing from body fluids frozen only once.

Other controls include a reverse transcriptase control to detect undigested (contaminating) DNA. This also required the inclusion of a Quantifiler™ test of the RNA extracts to check for residual DNA and also for the presence of PCR inhibitors.

REPORTING

Reporting the presence or absence of a body fluid is relatively straightforward when only one mRNA marker is used and is detected. However, when more than one mRNA marker is used to detect a specific body fluid reporting becomes more difficult particularly in the situation when only one mRNA marker is detected. We have used more than one mRNA marker in our multiplex to detect some body fluids and our reporting of a body fluid takes into account whether one or both mRNA markers are detected and the results of any applicable presumptive testing.

CONCLUSION

We have developed, validated and implemented a mRNA multiplex into operational forensic casework that detects circulatory blood, menstrual blood, vaginal material, semen (with and without spermatozoa) and saliva.