B53 The Limitations of Real Time Quantitative PCR (QPCR) in Forensic DNA Analysis

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After attending this presentation, attendees will understand the limita- tions of QPCR methodology for DNA quantitation. As well as address the potential for protocol change to include BSA in reaction.

This presentation will impact the forensic community and/or humanity by demonstrating the limitations of QPCR methodology fol- lowing 2 years of laboratory experience. Protocols may be altered in light of this experience to allow more accurate determination of the amount of DNA in samples and more efficient interpretation of results.

This paper will present to the forensic community the findings after more than 2 years of casework experience performing QPCR with a variety of forensic DNA samples. The protocols used at the Centre of Forensic Sciences for DNA quantification by QPCR are described and interpretation of results while considering the following relevant information; sample type, purity of the DNA extract, concordance of replicate analyses and mor- phology of the QPCR amplification plot is discuss.

The Centre of Forensic Sciences (CFS) has been performing QPCR analysis in forensic casework since August 2003. The laboratory employs the in-house developed CFS-HumRT QPCR assay[1], which utilizes a custom designed TaqMan®-MGB sequence specific probe and the ABD 7900HT Sequence Detection System. The forensic community's STR- PCR experience, has shown that samples encountered in forensic biology, such as bloodstains on clothing, can be affected by PCR inhibitors. Such inhibitors also have an impact on QPCR, typically causing inaccurate quan- tification by recording less DNA than is actually present or by yielding a false negative result. The potential of the QPCR system to detect ampli- fiable DNA rather than total human DNA is considered valuable. Identifying problematic samples prior to STR amplification not only saves the laboratory time and resources but also offers an opportunity to consider how a sample might best be treated in order to secure a DNA result. Nevertheless accurate determination of the quantity of human DNA in a sample is a must, and thus additional safeguards must be incorporated into the QPCR process in order to identify and resolve the issue of inhibitors.

At the CFS, reference bloodstains and buccal swabs are quantified using 1µl of neat sample extract. However, for all unknown forensic samples, an additional QPCR test of a 1/5 dilution of sample with 3.2µg BSA is performed, in order to address the potential issue of a PCR inhibitor. Sample data are reviewed for concordance between the neat and diluted test results, in addition to examining the amplification plot morphology. Where non-concordance is observed or the QPCR amplification plot morphology is atypical, additional testing is conducted. This may include further dilu- tions of the DNA extract or additional purification of the DNA extract.

Based on QPCR analysis of over 25,000 forensic DNA samples, a small proportion of samples yielded quantification results that indicated a PCR inhibitor is present in the sample. In approximately 90-95% of cases, STR-PCR analysis proceeds immediately following DNA quantification, with no need for further sample purification or additional QPCR testing of dilutions. Of the small percentage of DNA samples that needed further analysis, it was noted that many exhibited colored extracts. As a routine practice for all samples with colored extracts, an additional test of a 1/10 dilution is incorporated, to be performed simultaneously with the neat and 1/5 dilution tests. Cigarette butts, swabs of drinking containers, swabs of knife blades/handles, swabs of condoms, clothing such as jeans, hats, ban- dannas containing dark dyes, are some of the sample types that may demonstrate QPCR inhibition. Also of great importance is the choice of the DNA extraction method utilized. Techniques that yield the highest quality/purity of DNA extract are preferred, since carryover of contami- nants (*i.e.*, proteins, dyes, phenol) can severely impact QPCR analysis by inhibiting the reaction.

The inclusion of a DNA dilution with BSA into the QPCR process has proven to be an effective method of resolving most quantification inaccu- racies associated with inhibition. However, this approach does have its lim-itations, specifically when working with DNA samples of low concen- tration (<100pg/µl). Inhibitors associated with these samples might effec- tively be reduced through sample dilution, but the concentration of DNA in these samples is also reduced, possibly beyond the lower limits of reliable detection. Other assays that utilize an Internal Positive Control (IPC) to identify inhibited samples, rather than a dilution with BSA, are also prone to the same limitation because the true quantity of DNA in the sample is still undetermined. Being aware of these limitations and considering all rel- evant sample information when interpreting results is a necessary com- ponent of any QPCR system particularly when assessing the possibility of a false negative.

References:

1. Developmental Validation of a Real-Time Quantitative QPCR Assay for Automated Quantification of Human DNA, *J. Forensic Sci*, September 2003, vol. 48, No. 5.

QPCR, Limitations, Forensic Science

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