Laboratory Management

Corrective Measures Taken in Response to DNA Extraction Failures Using a Newly Validated Method

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Abstract: The Centre of Forensic Sciences (CFS) recently moved from organic DNA extraction protocols to a semi-automated magnetic bead purification protocol. Following an internal validation (per ASCLD/FBI QAS standards), methods were implemented using Qiagen Investigator extraction chemistry (buffer G2) in conjunction with the Qiagen EZ1 Advanced XL instruments. Shortly after that implementation, a subset of forensic substrates was identified where lower than expected yields of DNA were recovered. In addition to low yield, some of these samples also exhibited DNA profiles with peak height discordance or imbalance (using Applied Biosystems Identifiler-Plus). The materials that were resistant to extraction were primarily dark coloured material and were not restricted to a particular body fluid. This led to a suspension of DNA extractions using the Qiagen chemistry and instruments pending the outcome of an investigation.

A systematic review of approximately 1000 casework samples processed since implementation (some of which had already been reported to clients) was undertaken to determine those which may have been affected by the observed phenomenon. Based on specific criteria approximately 200 of these were re-purified or re-sampled from the original items in an attempt to recover more DNA and generate better quality profiles.

The subsequent root cause analysis determined that the Qiagen G2 buffer was not removing the cellular material from the problematic substrates, while a re-extraction of the original substrate with the CFS organic extraction protocol did yield expected DNA quantities. A re-validation of the EZ1 instruments was undertaken using CFS in-house extraction chemistry. This approach was successful and the methodology was brought back online. However, shortly thereafter new issues related to the formation of precipitates with this buffer, thought to be due to the concentration of SDS, were experienced. Those samples that were affected presented high autosomal to male quantification ratios (using Promega Plexor-HY) as well as peak height imbalance (notably at D13S317). The methodology was suspended again, and further studies were undertaken to optimize the buffer composition as well as determine the impact on any affected samples.

This presentation will focus on the investigative steps taken to determine the root cause of the incidents; the retrospective assessment of extraction results; the steps taken to mitigate the impact on individual cases; the communication of the issue to our clients; and the measures taken to re-validate and re-implement the silica bead technology purification. Our experience demonstrates the benefit for redundancy with multiple extraction platforms, and the value of a systematic approach to managing quality issues which impact a large sample set. Furthermore the need for continuous monitoring and critical assessment of newly implemented procedures, even those widely accepted within the forensic community, is highlighted.