EXAMINATION OF PROPOSED MANUFACTURING STANDARDS USING LOW TEMPLATE DNA

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Forensic DNA laboratories rely on reagent and plastics manufacturers to supply high-quality products with minimal interference from contaminating DNA. With the increasing sensitivity of short tandem repeat (STR) amplification systems, levels of DNA that were previously undetected may now generate partial profiles. To address the concern of laboratories worldwide, accrediting bodies in the United Kingdom and Australia proposed guidelines, PAS377 and ISO 18385, respectively, for minimizing the risk of human DNA contamination events during the manufacturing process. As a manufacturer, we need to understand the limit of detection for the analysis methods currently being used and what level of contaminating DNA would interfere with analysis in customer labs.

This poster compares the sensitivity of qPCR to STR analysis and discusses the suitability of each method in the manufacturing process for the purpose of certifying a product as Forensic Grade.

To determine the sensitivity of STR analysis, we analyzed the sensitivity of its two major components: the capillary electrophoresis (CE) instrument and the STR reagents. To determine the sensitivity of the CE instrument, we amplified a high amount of DNA (500pg) to eliminate the stochastic effect of amplification of low template DNA amount. This ensured that any dropouts at low input amount are due to CE limitation and not PCR variability. We tested instrument sensitivity using default and enhanced conditions as recommended by the UK guideline: longer injection and lower peak calling threshold. Under enhanced conditions, the limit of detection (LOD) for the CE instrument is 0.5pg.

In contrast to STR analysis, qPCR analysis is sensitive down to 0.25pg input DNA. In addition to increased sensitivity, qPCR analysis is more suitable for testing a higher number of samples: more cost effective and simpler data interpretation. Testing a large sample number is necessary for increased statistical confidence in a destructive test where a representative sample from each batch is tested and destroyed.

Therefore, we propose that qPCR analysis is used for testing plastic consumables. For STR reagents, we propose using STR analysis as it will simultaneously test all components of the kit for presence of contaminating DNA. While this paper discusses the LOD for the test methods, the limit that is acceptable to the forensic laboratories still needs to be determined.