EVALUATION OF HALF REACTION VOLUMES OF THE AMPFLSTR® IDENTIFILERPLUS® FORENSIC AMPLIFICATION KIT IN STR ANALYSIS.

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Many forensic laboratories have transitioned to using the AMPFlSTR® IdentifilerPlus® Forensic Amplification Kit for STR analysis to streamline evidence processing by increasing sensitivity, increasing resistance PCR inhibitors and reducing the time for DNA amplification. Each IdentifilerPlus kit contains the reagents to amplify 16 STR loci, including the 13 core CODIS markers, for approximately 200 samples. At a cost of over $17 per sample, using full reaction volumes for every sample may be unnecessarily expensive. This study was performed to evaluate the effectiveness of DNA amplification when the total volume of IdentifilerPlus reactions is reduced by half.

Purified DNA samples containing heterozygous alleles at all loci with the exception of amelogenin (female) were amplified in duplicate on an ABI 9700 thermocycler using the recommended 28 cycle protocol. Samples were amplified with the IdentifilerPlus kit using half the volume of reagents and template DNA as the required by the procedure recommended by ABI, for a total reaction volume of 12.5 µl. They were then injected on an Applied Biosystems™ 3130xl Genetic Analyzer for 2, 4, or 6 seconds, and the resulting data were analyzed with GeneMapper ID. A DNA target range of 0.001-1.25 ng was assessed to determine the effects of half reaction volumes on the sensitivity of amplification, locus detection, stochastic threshold, and peak height ratios (PHR). The results of these studies were also compared to the values obtained from the previous internal validation of the IdentifilerPlus system at the full reaction volume of 25 µl.

For the amplification sensitivity study, the high DNA input samples in half reactions produced similar peak height values when compared to full reaction volumes. The target DNA input for half reaction volumes of the IdentifilerPlus kit was determined to be 0.5 – 1.0 ng, the same quantities recommended and validated for full reaction volumes. Importantly, the stochastic threshold increased from 145 RFU for full reactions to 325 RFU for half reaction volumes, making half reactions much less useful for evidence samples.

The PHR for half reactions were similar to those seen in our previous study of the IdentifilerPlus kit using full reaction volumes. These data suggest that PHR is not affected by using half reaction volumes with greater DNA input amounts, i.e., more than 0.2 ng. However, with lesser DNA target amounts, i.e., less than 0.2 ng the percentage of heterozygous peaks with PHR >60% was consistently greater than 80%, while at half reaction volumes, the percentage of heterozygous peaks >60% PHR was lower, in the 60-80% range.

The increase in stochastic threshold and PHR variability at lower DNA quantities suggest that input amounts of 0.75ng or higher, are more useful and more reliable for half reactions. Results of tests for reproducibility, accuracy, and precision will be presented at this meeting. The Harris County Institute of Forensic Sciences processed 1560 known DNA samples from January through May 2012. The use of half reaction volumes for IdentifilerPlus would provide a savings of over $13,000 within this time period alone.

In conclusion, the use of half reaction volumes of the IdentifilerPlus amplification kit may be a viable option for processing known forensic DNA samples where an unmixed sample and a high

quantity of DNA is expected. This would result in a significant cost savings for the laboratory. For unknown samples, the doubling of the stochastic threshold makes the use of half-reaction volumes unsuitable. ⌘