A72 Validating the Use of a Human and Male Specific Quantitation Kit to Establish Minimum Amplifiable Quantities for Autosomal and Y-STRs

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After attending this presentation, attendees will understand how to establish and validate a minimum amplifiable quantity for terminating evidentiary sample processing. Termination at the quantification stage may be useful due to the expectation of results that will not lead to acceptable genotyping data for both autosomal and Y chromosome STRs. The audience will be led through the validation study and shown how to correlate quantification data to both detection and match interpretation thresholds (MIT). The goal is to establish quantitatively based cutoff values where evidentiary sample processing will be terminated.

This presentation will impact the forensic science community by demonstrating how the establishment of minimum amplifiable quantities for terminating evidentiary sample processing at the quantification stage will result in increased productivity and reduced costs for a laboratory. The termination of evidentiary processing of samples that are expected to yield no useful genotyping information will save a laboratory time at two different stages. First, the laboratory will save time in sample processing, as fewer samples will need to move onto subsequent and more expensive stages. Second, and more importantly, this will allow the laboratory to save time during the interpretation/report writing stage. In addition, this will prevent needless consumption of evidence. Validated early termination of evidentiary sample processing will have the added benefit of cost savings for the lab in analyst time, equipment usage, and reagent cost. Decreased analyst time per case will mean that a single analyst, as well as the laboratory as a whole, can process more cases overall. Increased productivity, reduced cost, and improved efficiency will benefit the individual laboratory, the criminal justice community, and the public that a laboratory serves.

A forensic biology laboratory should try to derive the maximum amount of information from each sample tested. However, it is inefficient, wasteful and not cost effective for a laboratory to process a sample that will lead to negative or uninterpretable results. The question becomes: is there a minimum amplifiable DNA quantity for effective evidentiary sample processing?

The FBI Quality Assurance Standards for Forensic DNA Testing Laboratories that went into effect July 1st, 2009 state: "In order for a laboratory to determine that evidentiary sample processing is to be terminated after DNA quantitation, the laboratory shall have a validation study to support that determination." A laboratory that chooses to establish a minimum quantity for amplification must perform an internal validation study in order to set the maximum quantity of DNA in an evidentiary sample that will not be amplified.

Here we describe a validation study using a human and male specific quantitation kit which establishes a maximum DNA quantity for the termination of evidentiary sample processing after the quantification stage. We performed amplification using four different STR typing systems (three autosomal and one male specific). All amplification was done according to the manufacturer's specifications on 96-well thermal cyclers. Samples were run on a 16-capillary electrophoresis genetic analyzer and analyzed using available genotyping software.

Initially, two different male samples were quantified in quadruplicate and the average quantity was used. Dilutions ranging from
0.001 ng/µL – 0.007 ng/µL were prepared and amplified in triplicate for both male samples using the maximum allowable sample volume. The amplified target amount ranged from 10 pg – 70 pg; based on previous work, this range was shown to produce genotyping data in the stochastic range for the different STR typing systems. To determine a stochastic range, the Match Interpretation Threshold (MIT) and peak amplitude threshold (PAT) must be defined. The PAT is the threshold above which a peak can be considered a true allele. The MIT is the threshold at which the taller of two heterozygous alleles must surpass for the sister allele to be reliably detected above the PAT. The stochastic range resides between the MIT and PAT as this is the range where allelic drop-out is expected to occur.

Sample data was analyzed with consideration to the PAT and MIT, in regards to the profile as a whole and at individual loci. A minimum amplifiable quantity was established as the amount of DNA that must be amplified to produce alleles above the MIT, also taking into account the variability of a human and male specific quantitation kit. Of 1,979 casework samples quantified between June and July of 2009, 18% of the samples could have terminated after the quantification stage assuming a quantification threshold for human DNA of 0.005 ng/µL. Of these, 25% percent are estimated to contain enough DNA in order to continue with processing if the same amount of sample was extracted and combined with the original extraction. Assuming a male quantification threshold of 0.003 ng/µL, 41% of the samples could have terminated after the quantification stage. Nine percent of these could be salvaged by performing a second extraction and combining both extractions together.

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Establishing a minimum amplifiable quantity for amplification is expected to reduce the number of fully analyzed samples by 20%. We estimate that one amplification kit per month would be conserved in addition to the DNA processing and interpretation time that would be saved by implementing a quantification threshold.

Minimum Quant, Interpretation Thresholds, Quality Standards

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