B54 Comparative Analysis of Human-Specific DNA Quantitation Techniques

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After attending this presentation, attendees will have information regarding how the reproducibility of three commonly used methods—real- time PCR (Quantifiler™), slot blot (QuantiBlot®), and AluQuant®—was compared by having a single analyst perform each technique with sample dilutions covering the dynamic range of each method.

This presentation will impact the forensic community and/or humanity by demonstrating the advantages and disadvantages of each DNA quantitation method, all of which may be used to highlight the best quantitation method available to suit an individual laboratory's needs.

In the forensic science community, quantitation of human DNA in a forensic sample is an important step in generating the short tandem repeat profile for that sample. Forensic laboratories employ many different technologies to perform quantitation analysis. Unfortunately, many of the methods used can result in variations over time or between analysts and also can have wide ranging costs and analysis times. Many laboratories are actively pursuing new methods and technologies to implement for more efficient, accurate, and reproducible quantitation of human DNA. In this study, the reproducibility of three commonly used methods—real-time PCR (Quantifiler™), slot blot (QuantiBlot®), and AluQuant®—was com- pared by having a single analyst perform each technique with sample dilu- tions covering the dynamic range of each method.

Reproducibility was evaluated in terms of standard deviation over four time points—within month, within week, within day, and within run. Although QuantiBlot® quantitation gave fairly reproducible results within a limited concentration range, the method consistently failed to produce quantifiable results for samples at the upper and lower ends of the stated dynamic range. Overall, Quantifiler™ and AluQuant® methods performed similarly with increasing reproducibility as the sample dilutions decreased across their dynamic ranges. While AluQuant® quantitation proved more reproducible when measured within month and within week, Quantifiler™ quantitation performed best when samples were repeatedly measured within a day. Consistency between analysts was also evaluated by having three technicians perform quantitation of the same set of samples using the Quantifiler™ and QuantiBlot® methods. Overall, QuantiBlot® produced more consistent results than manually performed Quantifiler™, demon- strating the increased susceptibility of the latter method to human error and subtle pipetting differences. However, it should be noted that QuantiBlot® quantitation is only useful for measuring DNA over a very narrow dynamic range and automation of the Quantifiler™ method would likely increase the consistency of this method beyond that of QuantiBlot® and other manual techniques.

Lastly, other factors that may be of concern to forensic laboratories regarding quantitation techniques, including time and cost per sample, were evaluated. As expected, QuantiBlot® took significantly more time to perform and analyze per sample. In addition, automated AluQuant® required less time for quantitation than manual Quantifiler™; however, automation of the latter method would likely make them comparable. Further, unlike automated AluQuant® quantitation, automated Quantifiler™ would require no analyst intervention until the review of the analyzed data that is generated, possibly making it more efficient when adapted to an automated platform. In the cost comparison, QuantiBlot® was the least expensive method of the three, while AluQuant® was cheaper per sample than Quantifiler™ due to the higher cost of reagents for Quantifiler™.

In conclusion, since AluQuant® and Quantifiler™ perform similarly in reproducibility studies, forensic laboratories should carefully prioritize and consider other pertinent factors in deciding which quantitation method to use, including throughput needs of the laboratory, and availability of per- sonnel, workspace, and funding for implementation, equipment purchase, and reagent acquisition. It is hoped that the results of this study may impact forensic DNA laboratories by displaying the advantages and disadvantages of each quantitation method, all of which may be used to highlight the best quantitation method available to suit an individual laboratory's needs.

DNA, Quantitation, Real-Time PCR

 ** Presenting Author*