## B80 The Evaluation of the Qubit® 2.0 Fluorometer Quantitation System and Comparison to Real-Time Quantitative PCR

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After attending this presentation, attendees will understand the basic functionality of the Qubit® 2.0 Fluorometer and how the instrument compares to current real-time Polymerase Chain Reaction (PCR) technology in terms of cost, time required, and accuracy of the results.

This presentation will impact the forensic science community by providing a comparison between quantitation methods currently employed by forensic laboratories and a newer fluorescence-based method, the Qubit® 2.0 Fluorometer, which is currently being used in the next generation sequencing workflow.

Real-Time quantitative PCR (RT-qPCR) is the current forensic method to determine the concentration of human DNA and is accurate, reliable, human-specific, and has a high degree of sensitivity; however, the method is time consuming, typically requiring two hours, and relies heavily on the accuracy of the serially diluted standards. As the field of DNA quantification advances, new methods that can accurately quantify the DNA more efficiently and without using a large amount of the sample could potentially replace RT-qPCR in forensic laboratories. The Qubit® 2.0 Fluorometer is a fluorescence-based quantitation system that measures the concentration of double stranded DNA (dsDNA) with a detection range between 10pg/µL and 1,000ng/µL; however, the assays are currently not human-specific and measure the total amount of dsDNA that is present in a sample.[1]

This study compares the quantitation values of a sample when measured by the Qubit® and the Plexor® HY kit with an Applied Biosystems® 7500 Real-Time PCR System. Fifteen anonymous donors provided buccal swabs for use in the study. DNA from each sample was extracted using the "tip dance" protocol of the EZ1 Advanced robot and an initial quantitation value of the samples was determined using the Qubit® instrumentation. A dilution was made based on the resulting concentration to target 5ng/µL or 2ng/µL, depending on the starting concentration of the extracted DNA sample. This was followed by a serial dilution to produce a range of concentrations in order to test the limit of detection of the two Qubit® Assay kits: High Sensitivity (HS) and Broad Range (BR). The HS Assay kit is accurate for initial concentrations of 10pg/µL to 100ng/µL, while the BR Assay kit is accurate for 100pg/µL to 1,000ng/µL. The same dilutions were used with both the HS and BR kits. For comparison, the same samples were quantified using the Plexor® HY system. The results indicated that the Qubit® BR Assay kit is not ideal for forensic samples as it is unable to read samples with concentrations below 1ng/µL when using 2µL of sample to quantify the sample. The HS kit was much more consistent reading the samples that had concentrations below 1ng/µL. When the quantitation values generated by the Qubit® were compared to those of RT-qPCR, it was evident that RT-qPCR was more sensitive when small amounts of DNA were present in the sample. After capillary electrophoresis, the peak heights on the electropherograms were analyzed for the amplification reactions targeting 1ng (or 1,500 Relative Fluorescence Units (RFU) per allele) based on the Qubit® and RT-qPCR quantitation values. The average RFU value for the samples quantified using the Qubit® was 1,588±313 and the average RFU value for the RT-qPCR was 1,080±434 RFU per allele and Qubit® variance was 20% while the RT-qPCR variance was 40%, thus indicating that the Qubit® yielded results as consistently as RT-qPCR-derived profiles and more closely to the desired 1,500 RFU per allele.

The short amount of time needed to quantify a sample, along with the low cost, would make the Qubit® useful in forensic laboratories. The Qubit® takes less than five seconds to determine the concentration of a sample, thereby allowing analysts to quantify a large number of samples in a relatively short amount of time. It costs less than $1.00 to run a sample on the instrument and is cheaper than the reagents currently required for use with the Applied Biosystems® 7500 (~$3.00 per reaction); however, based on the results of this study, RT-qPCR is still more sensitive when directly compared to the Qubit®, and specificity for human DNA is required for implementation in forensic laboratories according to the Quality Assurance Standards for Forensic DNA Testing Laboratories published by the Federal Bureau of Investigation.

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**Reference:**

1. *Qubit® 2.0 Fluorometer User Manual*. Calsbad: Invitrogen™, 2010.

**Qubit®, DNA Quantitation, Comparison**