



### A123 Do You Know How Much DNA You Really Have?

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The goal of this presentation is to provide attendees with insights including expectations of the consistency of DNA standards over long term use, data on the performance of different standards using the same kit chemistry, information that will show analysts how differences in standards affect quantitation results, and techniques for checking quantitation results and making corrections to them.

The presentation will impact the forensic science community by demonstrating the inaccuracies of current DNA standards being used to determine quantitation results and providing methods to correct for these inaccuracies.

Quantitation of DNA is a quality assurance step that: (1) ensures there is enough DNA in a forensic sample to provide the best possible results; and, (2) ensures the analyst has data to select the most appropriate amplification method. In fact, quantitation is such a crucial step that is mandated by the FBI. The Quality Assurance Standards for Forensic DNA Testing Laboratories, standard 9.4, states "The laboratory shall quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification." Apart from this requirement, more recently, rules on what is considered low copy number analysis have been defined based on the quantity of DNA present in a sample.

However, testing has shown that the quantitation kits used do not always provide accurate results. Furthermore, these tests have found the

DNA standard used in these quantitation kits can over- or underestimate the quantity of DNA in a sample by two to three fold.

To assess commercially available DNA quantitation standards, a study was designed to explore the variance in performance between several different quantitation kits. The study also examined if there was any difference between lot numbers of kits from the same manufacturer. To achieve this, only one kit chemistry was used but the standards were changed in the course of the experiment and results evaluated by slope,  $R^2$  value, Y intercept and resulting quantities of a serial dilution of blood samples. The results show a direct correlation between Y intercept value and the quantity of DNA in the sample.

This presentation will provide forensic scientists with insights including:

- Expectations of the consistency of DNA standards over long-term use.
- Data on the performance of different standards using the same kit chemistry.
- Information that will show analysts how differences in standards affect quantitation results.
- Techniques for checking quantitation results and making corrections to them.

Accurate and reliable quantitation results affect all aspects of the DNA process. Quantitation has a definite impact on all DNA validation studies conducted by laboratories especially when attempting to evaluate the sensitivity of a method. Accurate quantitation results will also ensure laboratories do not waste time and resources repeating processes because the results were not what were expected based on the DNA quantitation. As laboratories implement measures that provide for more accurate quantitation results of the samples they are processing, they can correct any inconsistencies and optimize their methods.

#### **Quantitation, DNA, Standards**