A82 Evaluation of Quantitation Methods for Implementation in Forensic Casework

Sarah C. Schultheis, MS, Dixie Peters, MS,and Arthur J. Eisenberg, PhD, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107; and Bruce Budowle, PhD, Forensic & Investigative Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, EAD 310, Fort Worth, TX 76107*

After attending this presentation, attendees will be familiarized with the use of Quantifiler® Duo and Plexor® HY and how they compare with each other in performance capabilities and salient features.

This presentation will impact the forensic science community by providing validation data and practical guidance to support use of a robust multiplex DNA quantitation kit.

The quantity of DNA added to an amplification impacts the ability to obtain a usable genetic profile. In forensic biological evidence, the total DNA recovered from a sample can be comprised of human and non-human DNA (e.g., comprised of human and bacterial DNA) and/or can be mixtures of human male and female contributors. The amount of female DNA can be present in excess in mixed samples such that no male DNA profile can be obtained. Therefore, determining the amount of total human and male DNA derived from a sample will enable an analyst to make an informed decision regarding autosomal and Y-STR amplifications. The amount of DNA is important for STR assays because there is a narrow optimal template range for DNA typing.

Quantifiler® Duo is a commercially available kit designed to quantify the concentration of total human DNA and human male DNA simultaneously. The system makes use of three 5' nuclease assays simultaneously in a real time PCR format: a target-specific human DNA assay (ribonuclease P RNA component H1, located on chromosome 14), a target-specific human male DNA assay (sex-determining region Y), and an internal PCR control assay (a synthetic sequence not found in nature). The internal PCR control can be used to assess the presence of inhibitors. The ability to determine optimal template addition and inhibition will enable greater success, potentially reduce labor, cost of supplies, and minimize consumption of evidentiary DNA samples.

Commercially available Plexor® HY is also designed to quantify the concentration of total human DNA and human male DNA simultaneously. The system measures the decrease in fluorescence by utilizing specific interactions between two modified nucleotides, isoC and isoG. The human autosomal DNA target is a multicopy, 99 base pair segment on chromosome 17, while the human male target is a 133 base pair Y-chromosome region. The internal PCR control is a novel 150 base pair sequence. A passive reference is added to each sample, which is used to normalize the data from the other three dyes to this signal.

Human male DNA (from the Quantifiler® Human kit) and K562 DNA (female) were used to assess the sensitivity of detection of the assay and of total human and human male mixtures. In addition, concordance studies were performed. For the sensitivity study concentrations of 50, 12.5, 3.13, 0.78, 0.2, 0.05, 0.012, and 0.003 ng/µl were prepared and analyzed. Duplicate samples were run on separate plates. Mixtures of male:female ratios included 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024. Duplicate samples were run for the mixture study as well. For the concordance study, selected casework samples from UNTCHI casework that had been quantified previously using Quantifiler® Human were compared with data from Quantifiler® Duo and Plexor® HY.

The results to be presented, in concert with current casework experience, form part of the validation foundation for properly implementing a robust methodology to quantify the amount of total human and male DNA derived from forensic samples.

Quantifiler® Duo, Validation, Casework

 ** Presenting Author*