A39 Comparison of Genetic Markers and Developmental Validation of the Multicopy LINE-1 Marker for Use in a Sensitive Real -Time Quantification Method

Jaclyn B. Kenline, BS, 44 Doe Run Drive, The Woodlands, TX 77380; Lauren G. Bouse, MSFS, 12934 East SH-21, Bryan, TX 77808; Pablo A. Noseda, BS, 14723 TC Jester Boulevard, Houston, TX 77068; and David A. Gangitano, PhD, 14723 TC Jester Boulevard, Apartment 1118, Houston, TX 77068*

After attending this presentation, attendees will understand how to apply the Scientific Working Group on DNA Analysis Methods (SWGDAM) criteria in a developmental validation of a new marker for forensic DNA quantitation of low copy number and degraded samples and the comparison with different genetic markers in use.

This presentation will impact the forensic science community by giving information on a new marker that can be used as an alternative tool for the quantification of degraded and low copy number samples.

Three different genetic markers were studied for use in a sensitive real-time PCR quantification method using a SYBR® Green detection system. The markers studied were: a long interspersed nuclear element (LINE-1), a multi-copy short interspersed nuclear element (*Alu*), and a reduced size short tandem repeat marker (mini TH01). The markers were compared on target specificity, sensitivity, linearity, accuracy, and precision. The LINE-1 and *Alu* methods were the most sensitive systems with the ability to detect down to approximately 1pg/µl. However, the LINE-1 method was able to remain linear up to 50ng/µl compared to *Alu* which experienced a loss in linearity at 10ng/µl. The LINE-1 method displayed more accuracy and precision than the other methods at three different concentrations of a known DNA standard (10, 5 and 1ng/µl). LINE-1 displayed the following values (mean ± standard deviation) for each sample: 8.6±0.23 for 10ng/µl, 2.7±0.06 for 5ng/µl, and 0.42±0.03 for 1ng/µl. Although the LINE-1 method consistently estimated values lower than expected, the system performed similar to Quantifiler® human DNA quantitation kit for the same samples. The LINE-1 and mini TH01 primers displayed better target specificity than *Alu* according to the melt curves generated by each.

In addition to these comparative studies, the LINE-1 method was tested on species specificity, population, stability, inhibition, and mock case work samples. The LINE-1 method performance met all the SWGDAM criteria. With the exception of primates, the LINE-1 primers do not amplify other species. The samples tested from individuals of known ethnic origin were all positive. The stability of the system was tested by analyzing DNA that was artificially degraded with the DNase I enzyme. As expected, the system indicated that the amount of quantifiable DNA present in the samples decreases as the amount of degradation increases. The system was also tested in the presence of common PCR inhibitors with and without the addition of bovine serum albumin (BSA) to the system. Finally, the system was tested on several common mock case work type samples including touch DNA samples and samples that are considered to be low copy number or degraded. Utilizing the LINE-1 marker appears to provide an adequate screening and quantification method for the analysis of forensic case work samples, specifically low copy number or degraded samples.

It is recommended that forensic DNA analysts become familiar with the developmental validation SWGDAM criteria and its application.

Real-Time PCR, Low Copy Number, LINE-1

 ** Presenting Author*