CHARACTERIZATION OF THE SOURCES OF PEAK HEIGHT UNCERTAINTY RESULTING FROM ORDINARY ALTERATIONS DURING FORENSIC DNA PROCESSING: EXAMINGING VALIDATION SCHEMES FOR THE CALIBRATION OF NOCIt

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Forensic DNA mixtures may be complex and comprised of any number of contributors combined in any proportion. In these cases, classical DNA interpretation schemes may not allow for comparison between standard and evidence. In response to the issues associated with complex, low-template DNA analysis, a number of methods and/or recommendations to determine the likelihood ratio, which requires an assumption regarding the number of contributors (NOC), have been published. Classical approaches to determine the NOC have relied on counting methods. However, as the complexity of the DNA profile increases, the minimum NOC may not be equivalent to the actual number. To accurately assess the probability that a certain NOC gave rise to an evidentiary item, the probability of drop-out (Pr(DO)), baseline noise and stutter proportion must be considered. The Pr(DO), noise and stutter must be previously characterized via validation and are typically measured with respect to some value such as average peak height or mass/concentration of input DNA. Since proper interpretation relies heavily on validation and calibration results, it is of interest to identify and characterize any sources of peak height uncertainty that results from natural changes of laboratory factors affecting the result. It is also necessary to confirm that these natural laboratory alterations do not significantly impact the thresholds or probabilities, which are in turn utilized to establish the results obtained from NOCIt, a computational method we have developed for determining the NOC in complex, low-template DNA samples.

This work represents a detailed study into the characterization of uncertainty associated with injection, capillary lot, amplification, kit lot and sample. Briefly, 3 samples were amplified utilizing six targets and run in quadruplicate. To test injection variation the same sample preparation was injected four times on one capillary. An additional sample preparation was also injected 4 times, but in this instance the capillary lot was changed before each injection. The amplification variation was measured by amplifying the samples in quadruplicate utilizing one kit lot and effects of amplification kit lots were assessed by then amplifying the samples 3 additional times with 3 different kit lots. The peak height results were summarized by examining height concordance for every allele at different target amounts. Specifically, the data was separated by target, the parameter being tested (i.e. injection, capillary lot, amplification and kit lot), sample number, locus, and allele. Once organized, each of the observed peak heights for a given allele was divided by the largest observed peak height for that allele (PH_{max}). This resulted in a ratio between 0 and 1. For parameters with high levels of reproducibility, all four peak height ratios are expected to be close to 1.

Data indicate that uncertainty in RFU signal was heavily affected by the uncertainty associated with the amplification, followed by capillary lot, then kit lot and injection. For example, the signal originating from a 0.25 ng target resulted in concordance ratios of 0.97 ± 0.02, 0.89 ± 0.07, 0.72 ± 0.15 and 0.71 ± 0.19 for injection, capillary, amplification and kit lot changes respectively. Similar results were observed for all 6 targets tested. As a result, when calibrating NOCIt, or

when attempting to elucidate stochastic thresholds and/or rates of drop-out, the validation dataset should include, at a minimum, amplifications of multiple different samples run such that several capillary lots are incorporated into the calibration scheme. Studies which characterize changes in baseline noise with respect to laboratory alterations will also be examined and final recommendations regarding validation strategies will be presented.