ACCEPTANCE CRITERIA FOR EVALUATING NEGATIVE CONTROL QUANTIFICATION DATA

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Since approving the Qiagen Investigator Quantiplex Pro quantification kit (Quant Pro) for casework use, the Signature Science Forensic DNA Laboratory has encountered no-template control (NTC) and reagent blank (RB) samples in which a C_T value is reported for one of the three test targets (Degradation, Human, Male) instead of being listed as "Undetermined." Per ABI 7500 data collection software specifications, an undetermined designation is applied to any sample target exhibiting a C_T value of 40 or greater. NTC and RB samples are expected to contain no quantifiable amount of DNA and, therefore, would be expected to have a C_T value reported as undetermined for all three targets tested in the Quant Pro kit. However, it has been documented by developmental validations (see User Guides for Quantifiler HP and Trio DNA Quantification Kits) that this is not always the case. Both Qiagen and Applied Biosystems explain this occurrence as possibly coming from exogenous DNA in a particular well of the PCR plate or from sporadic signal created by one of the targets in the assay. As a result, it is important to differentiate between this 'background' and other processing factors including plate setup errors and contamination introduced by carryover during plate setup. These types of analyst errors will not be detected in a DNA profile if the carryover contamination or plate setup error only affected the quantification plate and not the DNA extract; therefore, quantification results alone are not a clear indication of contamination and samples should be evaluated further to determine if true contamination is present. Given these findings, Signature Science gathered internal negative control data generated from 68 Quant Pro runs over time and used statistical analyses to establish a range of acceptable C_T values for negative controls run with the Quant Pro quantification kit. C_T values were evaluated for each test target for each NTC, RB, and Standard-4 run on all plates with the exception of known outlier RBs that were shown to have true contamination based on STR typing results. Standard-4 was included to represent low-level DNA concentrations and allowed for the differentiation between low-level DNA known to be present and false positive negative control results. We generated density plots to see 1) if there was a statistically significant difference between Standard-4 data and the negative control data, and 2) if the negative control data followed a normal distribution. The fact that both a) and b) were met allowed us to determine the C_T value associated with the Limit of Blanks such that 90% of the negative control data generated are expected to have C_T values above that Limit. We also evaluated electropherograms for all RBs that had a C_T value generated during quantification to determine if any detectable DNA profile was observed in those RBs. Results for all but one RB ranged from no allele calls up to several peaks observed below the analytical threshold (AT) that were not called. One RB had one peak above the AT and several below AT, and the run was rejected (that RB's C_T value was below [stronger than] the Limit established by this validation). The results of this validation show that if a reagent blank has a C_T value above the Limit of Blank, it is not statistically significantly different from the exogenous background (i.e., is not contaminated).