AN ASSESSMENT OF HUMAN NUCLEAR AND MITOCHONDRIAL DNA qPCR ASSAYS FOR DNA CONCENTRATION AND DEGRADATION

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Degraded, damaged, or low template DNA contained in evidentiary material from mass disasters, unidentified human remains, or cold cases presents a major challenge for forensic DNA examiners when nuclear DNA (nDNA) fails to amplify. In these cases, mitochondrial DNA (mtDNA) may be an alternative template for analysis. A real-time quantitative polymerase chain reaction (qPCR) assay can help determine whether the mtDNA is of sufficient quantity and robust quality to move forward with downstream sequencing and analysis. One fundamental issue with qPCR is that the nominal quantity of the nDNA calibrated with the commercial standards used for quantification can vary depending on the supplier and lot number(s). In 2018, the National Institute of Standards and Technology (NIST) released a commercially available human DNA standard, Standard Reference Material (SRM) 2372a, which consists of nDNA ***and*** mtDNA data on three well-characterized human genomic DNA samples. We used SRM 2372a to compare three qPCR assays: (1) a non-commercial FBI in-house triplex assay for mtDNA quantification, (2) Quantifiler Trio (Thermo Fisher Scientific) a commercially available assay for nDNA quantification, and (3) NovaQUANT (EMD Millipore Corporation) a commercially available assay for nDNA and relative mtDNA quantification. Additionally, extracts from eighteen skeletal remains were tested with the three assays for concordance of DNA concentration and the degree of degradation. Our assessment revealed that a robust, reproducible, accurate, and efficient qPCR assay is dependent on (1) the quality and reliability of the DNA standard, (2) the qPCR chemistry, and (3) the specific primers and probes used in the assay, when applicable. Our findings indicate qPCR assays do not always perform as expected and should be verified using a well-characterized DNA standard such as the NIST SRM 2372a.