

EVALUATION OF POWERQUANT® SYSTEM DEGRADATION STANDARD CURVE AND ITS ACCURACY

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The PowerQuant® System is a tool to quantify the human DNA extracted from a sample for downstream DNA analysis in forensic science. The Cuyahoga County Regional Forensic Science Laboratory (CCRFSL) utilizes the PowerQuant® System for quantifying the total amount of amplifiable DNA in forensic evidence and reference samples. This system is a 5-color, 4-target probe-based qPCR assay, and our laboratory utilizes a 4-point curve. The PowerQuant® System contains an internal PCR control (IPC) to test for false-negative results, as well as a degradation target to evaluate DNA degradation prior to STR amplification. The standard curve is a measure to evaluate the overall accuracy of a PowerQuant® experiment. This study highlights the troubleshooting sequence of a failed standard curve, especially the degradation curve.

The CCRFSL has been utilizing the PowerQuant® System in our laboratory on two different 7500 Real-Time PCR Systems from Applied Biosystems since 2017. In recent months, issues began to arise with many of the runs conducted on one of the 7500 Real-Time PCR instruments. These issues included the failing of the IPC in the 50-ng standard, the failing of the degradation curve with the 3.2-pg standard, and an overall inconsistency of the run results. Based on our protocol, these issues required that the quantitation assay be repeated, which would consume additional sample, resources and time.

A service call conducted by our Applied Biosystems Service Engineer detected a variation in temperatures across the block while ramping between 98° C and 68° C. The block was replaced, the appropriate performance checks were conducted, and the consistency in run results resumed.