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**VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**

**VALIDATION OF THE APPLIED BIOSYSTEMS® QUANTSTUDIO™ 5 REAL-TIME  
PCR SYSTEM: PERFORMANCE CHECK OF QUANTSTUDIO™ DESIGN &  
ANALYSIS SOFTWARE UPDATE V1.5.1**

**Prepared in July, 2024**

This summary amends the previous summary (prepared in June, 2021) to specifically delineate which software testing studies were deemed unnecessary during the evaluation of the update of the Design & Analysis Software to ver. 1.5.1.

**PURPOSE**

This performance check was conducted to test the function of the Applied Biosystems® QuantStudio™ 5 Real-Time PCR Instrument for quantifying DNA samples, following the update of the Design & Analysis Software to ver. 1.5.1.

**MATERIALS AND METHODS**

The Applied Biosystems® QuantStudio™ Design & Analysis Software (Thermo Fisher Scientific, Waltham, MA) is used on the Applied Biosystems® QuantStudio™ 5 Real-Time PCR Instrument (Thermo Fisher Scientific) to set up and run experiments using the Promega PowerQuant® DNA Quantification System assay (Promega Corp., Madison, WI). Data are collected and analyzed using this software. The software update from the previously installed ver. 1.4.3 to ver. 1.5.1 consisted of expanding compatibility with additional QuantStudio™ models, and the following fixes:

Fixed missing show threshold option on Plot Settings dialog

Fixed flicking effect on well table during reanalysis

Fixed error when opening file

Fixed software unable to open experiment file extension containing upper case

Fixed incorrect warning message when user open SAE file in SAE environment

Fixed error when print audit report

Fixed analysis settings reset after importing plate setup file

Fixed unable to open non-SAE file despite of the allowed settings

Fixed Java error when launch software application

Fixed DA software hang when user esig (Review and Approve Result) on run file

Fixed SAE password policy validation did not use the value set in SAE server settings

Fixed target list location on Amplification plot for better usability

The fixes and features described above from the software developer's release notes were evaluated and determined to result in minor revisions to the software, such as fixing error messages and improved usability. Since these revisions were minor in nature, as opposed to impacting the analytical process, a functional test of the software was performed on both QuantStudio™ instruments following the software update. Two female and one male buccal DNA sample, previously extracted and purified according to the Virginia Department of Forensic Science Procedures Manual<sup>1</sup>, were used. Additional regression and reliability testing, required for major revisions to instrumentation software, were not applicable.

A manual PowerQuant® reaction plate setup was performed for each instrument following the manufacturer's recommendations, with the exception that the PQ Dilution Buffer (used to prepare the standards) was also used for the no-template control.<sup>2</sup> The plates were prepared on the same day using the same male gDNA standard, dilution buffer and manually prepared master mix. The samples were quantified once on each plate using 2 µL of DNA extract added to 18 µL of PowerQuant® amplification cocktail. Amplification and detection were performed using the QuantStudio™, which was calibrated for the following dyes: FAM for the autosomal target, CAL Fluor® Gold 540 (CFG540) for the male target, TMR for the internal positive control (IPC), Quasar® 670 for the degradation target, and CXR for the passive reference dye. The raw data was collected with QuantStudio™ Design and Analysis Software ver. 1.5.1, and analyzed using Promega's PowerQuant® Analysis Tool software, ver. 1.0.0.0.

## RESULTS

Estimated DNA concentrations for the autosomal, degradation and male targets were similar for both instruments using the version 1.5.1 of the software. A small amount of male DNA was detected in each of the female buccal cell extracts; however, this was consistent with the values previously measured in these extracts using the PowerQuant® assay in conjunction with the QuantStudio™ prior to the software update (data not shown). The same male/female mixture and degradation flags were observed on both instruments. The male buccal extract showed some degradation, with approximate [Auto]/[D] ratios between 7 and 8. The software did not flag the internal PCR control (IPC) in any samples, with the exception of one of the female buccal samples, where the IPC measured in one assay was 0.35, which is slightly above the 0.3 threshold set in the software to indicate potential inhibition. The controls also performed as expected, based on the standard curve R-squared values and no DNA being detected in the no-template controls.

## CONCLUSION

A functional check of the QuantStudio™ instrument was performed, following a minor revision to the QuantStudio™ Design & Analysis Software (to ver. 1.5.1). It showed the system

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<sup>1</sup> Forensic Biology Extraction of DNA, Procedures Manual. Virginia Department of Forensic Science. December 28, 2018.

<sup>2</sup> PowerQuant® System Technical Manual. Promega. Revised 1/2020.

works as expected when used in conjunction with the PowerQuant<sup>®</sup> assay to estimate the amount of human DNA present in forensic samples.