

## B57 Developmental Validation of the Quantifiler™ Trio Assay for the QuantStudio™ 7 Flex System

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**Learning Overview:** After attending this presentation, attendees will understand how to adapt standard Quantifiler™ Trio run and data analysis methods for use with an alternative real-time Polymerase Chain Reaction (PCR) system.

**Impact on the Forensic Science Community:** This developmental validation will enable laboratories to internally validate and implement the Quantifiler™ Trio assay on the QuantStudio™ 7 Flex system. Additionally, this presentation will impact the forensic science community by providing it with an alternative Quantifiler™ Trio run and data analysis workflow that can be used in place of the Human Identification (HID) Real-Time PCR Analysis Software.

The Quantifiler™ Trio DNA Quantification Kit is a commonly used assay for the detection, quantitation, and quality assessment of amplifiable human nucleic acid in forensic samples. The multiplexed, TaqMan™-based assay utilizes multi-copy loci for simultaneous detection of three human-specific genomic targets plus an internal PCR control. In combination, these components are used to: measure the total quantity of human DNA present in a sample, measure the quantity of male DNA present in a sample, evaluate human DNA degradation, indicate the presence of male-female mixture, and assess inhibition.

The Quantifiler™ Trio assay was designed and developmentally validated for the 7500 Real-Time PCR Instrument.<sup>1</sup> The newer QuantStudio™ 5 Real-Time PCR Instrument is also supported by the vendor for the assay, and can run the HID Real-Time PCR Analysis Software v1.3 that is marketed to simplify use of the Quantifiler™ quantitation assays for human identification applications. While this software is sold separately from the assay and instruments, the Quantifiler™ Trio assay user guide, support, and reference materials assume use of the software for run setup and data analysis.<sup>2</sup> The software cannot be installed on or run with alternate real-time PCR instruments.

This presentation will describe a developmental validation of the Quantifiler™ Trio assay with an alternate instrument, the QuantStudio™ 7 Flex Real-Time PCR System and a run and data analysis workflow that does not require use of the HID Real-Time PCR Analysis Software. The validation studies were performed in accordance with Scientific Working Group on DNA Analysis Methods (SWGDM) Validation Guidelines for DNA Analysis Methods and ISO 17025 method validation standards.<sup>3</sup> The workflow utilizes the instrument-standard QuantStudio™ Real-Time PCR Software v1.3 for run setup and raw data analysis, in combination with a custom Python® script to produce reports detailing run and sample quality metrics, plus the sample quantitation results. The Python® script is freely available and can be used or modified for use by any laboratory interested in performing Quantifiler™ Trio quantitation on the vendor-supported instruments or an alternative real-time PCR system.

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### Reference(s):

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2. Applied Biosystems by Life Technologies, Thermo Fisher Scientific. *Quantifiler HP and Trio DNA Quantification Kits User Guide*. Pub. No. 4485354, Rev. H (2018).
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### Quantitation, Validation, Software