HID Real-Time PCR Analysis Software v1.1

USER BULLETIN | NOVEMBER 2010

SUBJECT: Validation and new features

In this user bulletin

This user bulletin covers:

Overview

The HID Real-Time PCR Analysis Software v1.1 was designed specifically for the 7500 Real-Time PCR System and for the Quantifiler® Human, Quantifiler® Y Human Male, and Quantifiler® Duo DNA Quantification Kits. Quantifiler®-specific templates and quality flags, as well as STR reaction and dilution calculation tools, enable streamlined data and sample assessment within the software. New features of the HID Real-Time PCR Analysis Software v1.1 include streamlined sample entry, enhanced reporting and export capability, and improved data analysis tools. Validation consisted of the experiments listed below, comparing Quantifiler® Human, Y Human Male, and Duo kit data collected and analyzed with the HID Real-Time PCR Analysis Software v1.1 to data collected and analyzed using the HID Real-Time PCR Analysis Software v1.0.

The data generated by the validation experiments demonstrates that the HID Real-Time PCR Analysis Software v1.1 is reliable and reproducible in performing HID Quantifiler® kit assays. Our experiments with the kit demonstrated some difference between software versions C_T values and quantity values for the in Quantifiler Duo kit, and also in C_T values for the Quantifiler® Human and Y Human Male kit. However, this variability could be a result of factors such as instrument, assay setup, or time between assay runs.

Validation studies performed

- Precision and Accuracy
- Reproducibility and Sensitivity

Validation

1 Materials and methods

1.1 Reagents

To minimize variation due to hand pipetting and lot-to-lot differences, the following setup procedures were employed throughout the study.

One lot of the Quantifiler® Human, Y Human Male, and Duo kits was used for all validation studies. A master mix consisting of the primer set and PCR reaction buffer was made daily for all of the plates run on that day. A master mix of each sample dilution was prepared before pipetting into the plate wells.

Quantifiler® Duo kit

For the precision and accuracy study, 12 replicates of the eight serial dilutions of standards, ranging from 23 pg/µL to 50 ng/µL, were prepared and run with each software version and instrument for the Quantifiler® Duo kit only. The reproducibility and sensitivity study used standard DNA sample 007 in these concentrations: 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 ng/µL.

Quantifiler® Human and Male kits

A sensitivity study compared results of the new Quantifiler® Human and Y Human Male (hybrid) plate assay using both versions of the software. The samples on the hybrid plate consisted of replicate standard curve dilution series for each kit as well as four replicate serial dilutions of standard control DNA 007 in these concentrations: 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 ng/µL.

Note: For all validation studies, identical sample dilutions were prepared and analyzed with each software version.

1.2 Instruments

Two Applied Biosystems 7500 Real-Time PCR System instruments with thermocycler firmware G2.10 were used for the validation. Each 7500 instrument used for the validation was connected to two computers, one with each version of the software (v 1.0 and v1.1). The setup of the computer with v1.1 tested both the upgrade pathway and fresh installation for HID Real-Time PCR Analysis Software v1.1.

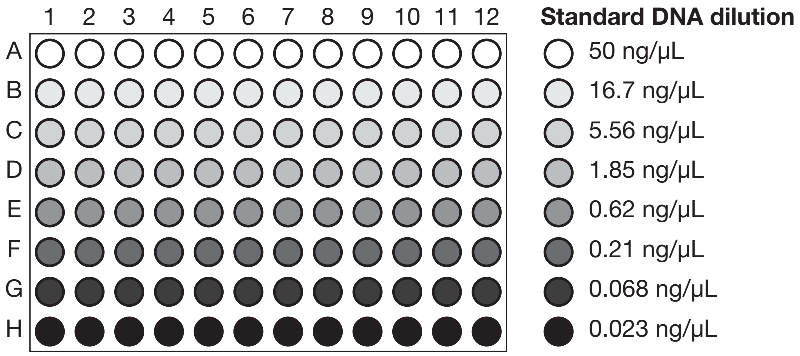
After software installation, each instrument was calibrated using standard protocols to run the Regions of Interest (ROI), background, optical, and pure dye calibrations, in addition to the TaqMan® RNase P Instrument Verification Plate with both HID Real-Time PCR Analysis Software v1.0 and v1.1.

2 Experimental setup

2.1 Quantifiler® Duo kit precision and accuracy study

Eight serially diluted samples prepared from the Quantifiler® Duo kit stock standard were analyzed. Figure 1 shows the plate setup for the Quantifiler® Duo kit. Each of the eight standard dilutions was run 12 times on each plate, then C_T values were compared. In addition, six standard curves were generated per plate for slope, Y-intercept, and R^2 comparisons.

Figure 1 Precision and accuracy plate layout for Quantifiler® Duo kit



2.2 Quantifiler® Duo, Human, and Y Human Male kit reproducibility and sensitivity study

Control DNA sample 007 was diluted and tested, using the Quantifiler® Duo, Human, and Y Human Male kits and each software version. A single standard curve dilution series was prepared and used to test each software version with the diluted control DNA samples. As Figure 2 and Figure 3 show, eight dilutions of sample DNA 007 were prepared in these concentrations: 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 ng/µL. C_T values of each sample concentration and each calculated quantity were compared between the two software versions tested (v1.0 and v1.1).

Figure 2 Reproducibility and sensitivity plate layout for Quantifiler® Duo samples

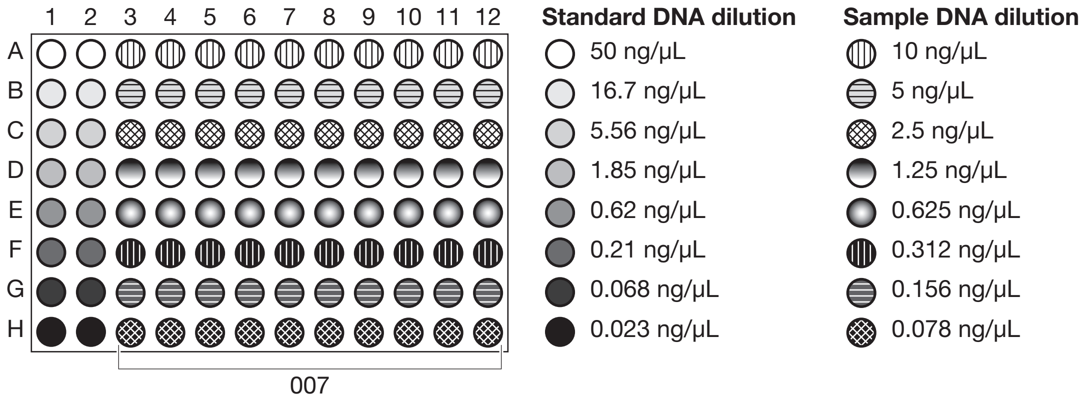
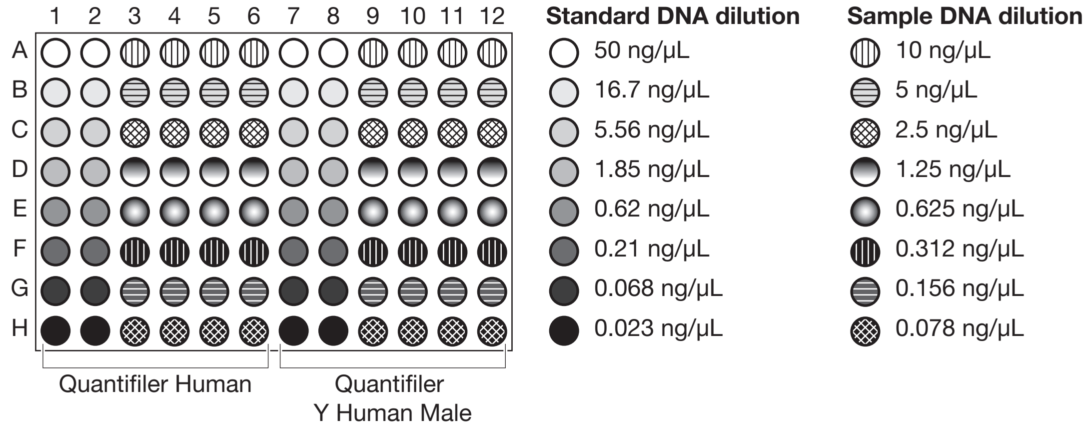


Figure 3 Reproducibility and sensitivity plate layout for Quantifiler® Human and Y Human Male samples



2.3 User interface evaluation

The HID Real-Time PCR Analysis Software v1.1 was tested to verify the accuracy of the HID-specific quality flags as well as the plate setup and run monitoring. The HID flags were analyzed to determine if samples were flagged correctly given the thresholds set. Plate setup and performance were evaluated during the course of data collection and setup.

3 Data collection

Run methods for each Quantifiler® kit were configured as outlined in the respective Quantifiler® kit user manuals. Although there is no 9600 Emulation mode checkbox in HID Real-Time PCR Analysis Software v1.1 or v1.0, a calculated ramp-up of 64% and ramp-down of 100% was used in all validation experiments to mimic the 9600 Emulation mode in SDS v1.2.3.

4 Data analysis

All runs were analyzed using the manual analysis mode in each software version, with the baseline set to start at cycle 3 and end at cycle 15. The manual C_T threshold was set to 0.2.

5 Results

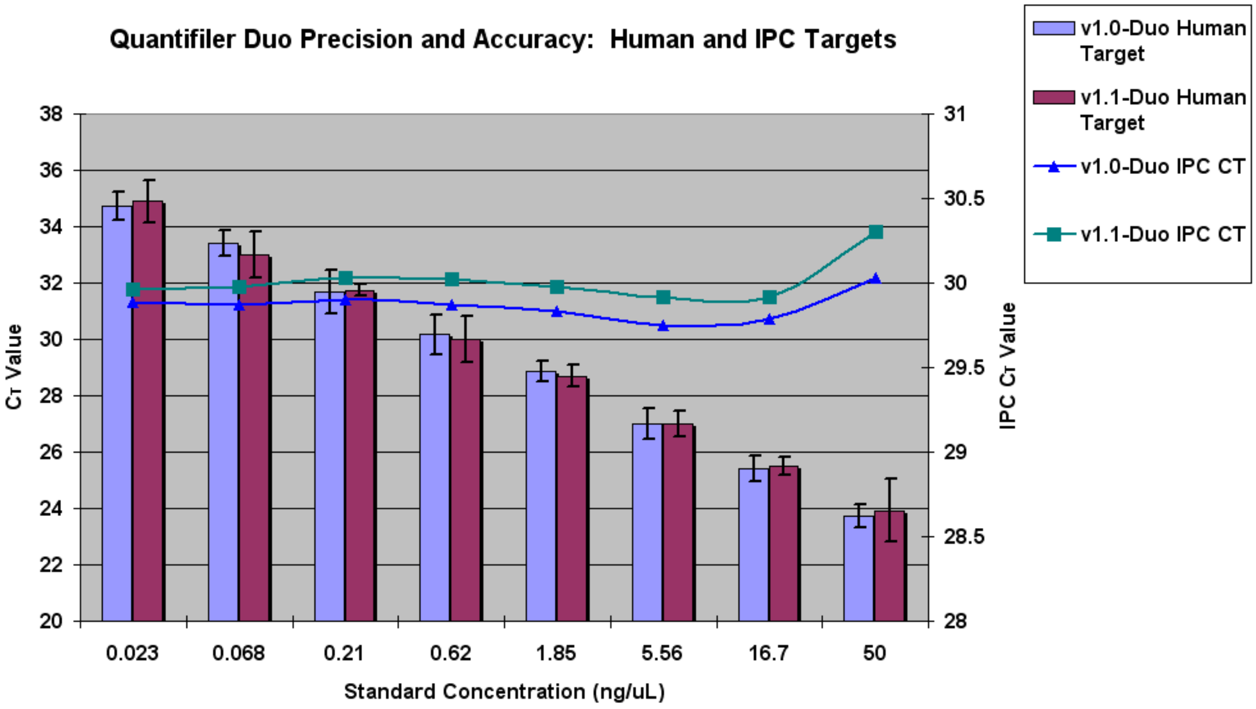
5.1 Precision and accuracy

For the precision and accuracy studies, the following values were determined:

- Average C_T
- IPC C_T values
- Slope
- Y-Intercept
- R^2

C_T Results

Figure 4 Mean C_T values for the Quantifiler® Duo kit with human and Internal PCR Control (IPC) targets generated by each software version at each DNA standard concentration. Error bars indicate standard deviations.

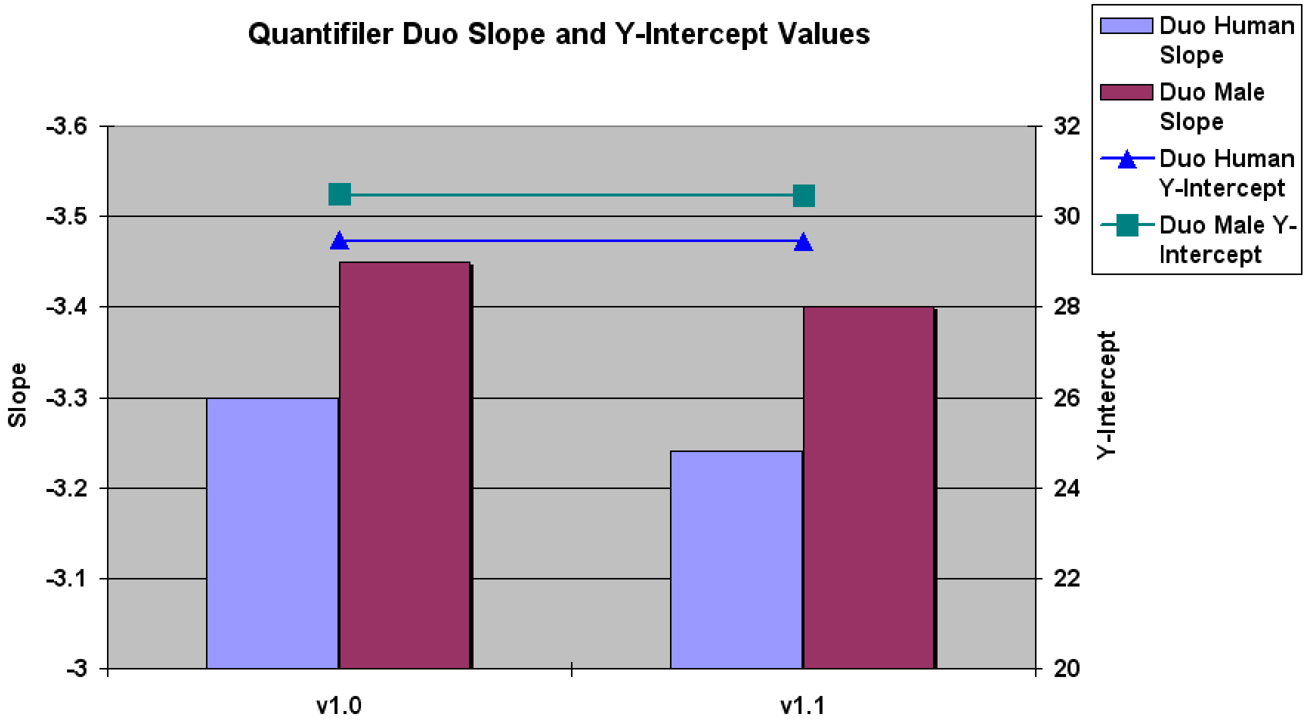


Overall, the two versions of the software produced data that was highly similar or identical. Statistical analysis showed no significant difference between the Duo Human and Duo Male targets (data not shown). Statistical analysis of the IPC C_T values demonstrated a statistically significant difference between data produced by the two versions of the software. However, the mean difference was 0.14 C_T between software versions, only slightly more than the mean difference of 0.12 C_T between instruments.

Standard curve metrics

Figure 5 shows the average slope and Y-intercept values for the Quantifiler® Duo kit generated by each software version. Differences in slope, Y-intercept, and R^2 standard curve metrics between the software versions were statistically insignificant (R^2 data not shown).

Figure 5 Mean slope and Y-intercept values for the Quantifiler® Duo kit generated by each software version



5.2 Reproducibility and sensitivity

Sensitivity differed slightly between the two software versions in studies using the 007 DNA standard with each of the Quantifiler® kits.

Quantifiler® Duo kit

C_T values for the human target in the 007 dilution series indicated a small though statistically significant mean difference of 0.05 C_T between the software versions in studies with the Quantifiler® Duo kit (data not shown). Mean C_T values for the male target did not differ significantly between the software versions. As shown in Figure 6 and Figure 7, human and male target quantities calculated with HID Real-Time PCR Analysis Software v1.1 differed slightly (0.49 ng/µL for the human target; 0.54 ng/µL for the male target). Because the mean C_T values were nearly identical, we attribute the difference in target quantities to the differences in the standard curves. No statistical difference was observed in the IPC C_T values of the 007 dilution series.

Quantifiler® Human and Male kits

We observed more variable sensitivity between the software versions in studies with the Quantifiler® Human and Y Human Male kit. As shown in Figure 8 and Figure 9, the HID Real-Time PCR Analysis Software v1.1 reported lower mean C_T values and higher calculated quantities with the Quantifiler® Human kit, and higher mean C_T values and lower calculated quantities with the Quantifiler® Y Human Male kit. In addition, v1.1 of the software reported a higher IPC C_T value than v1.0.

Figure 6 Mean quantities of human target DNA determined with the Quantifiler® Duo kit by each version of the software. Error bars indicate standard deviations.

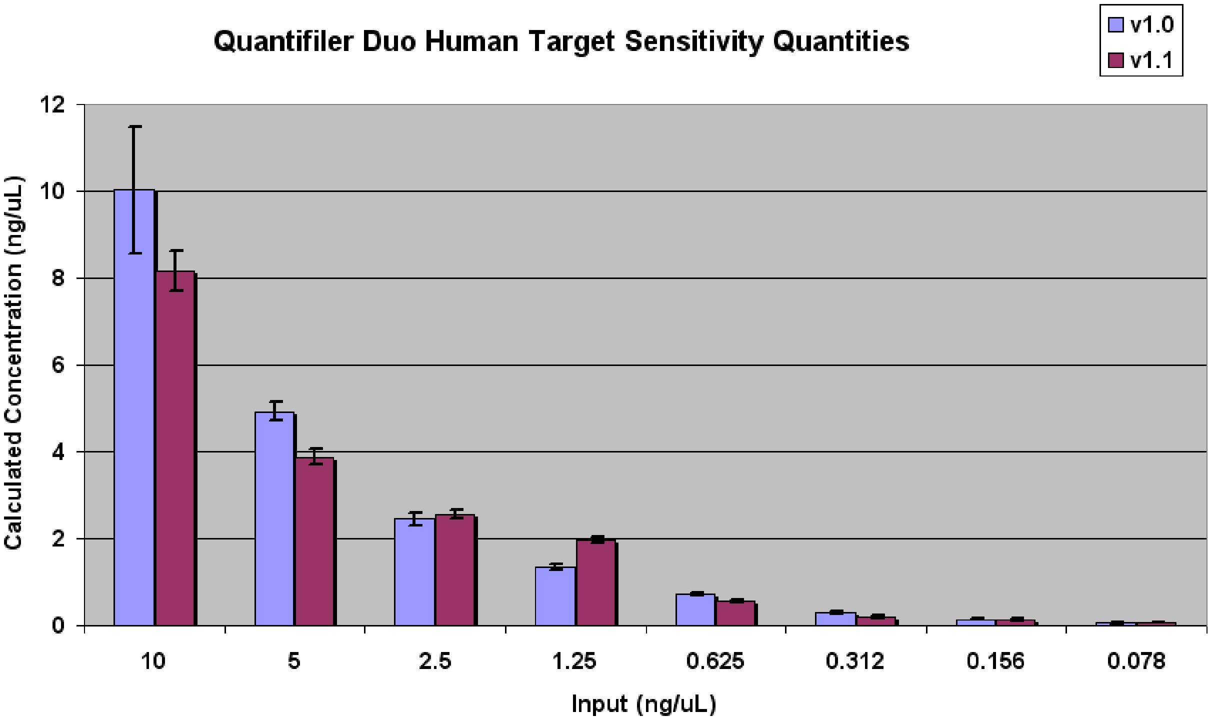


Figure 7 Mean quantities of male target DNA determined with the Quantifiler® Duo kit by each version of the software. Error bars indicate standard deviations.

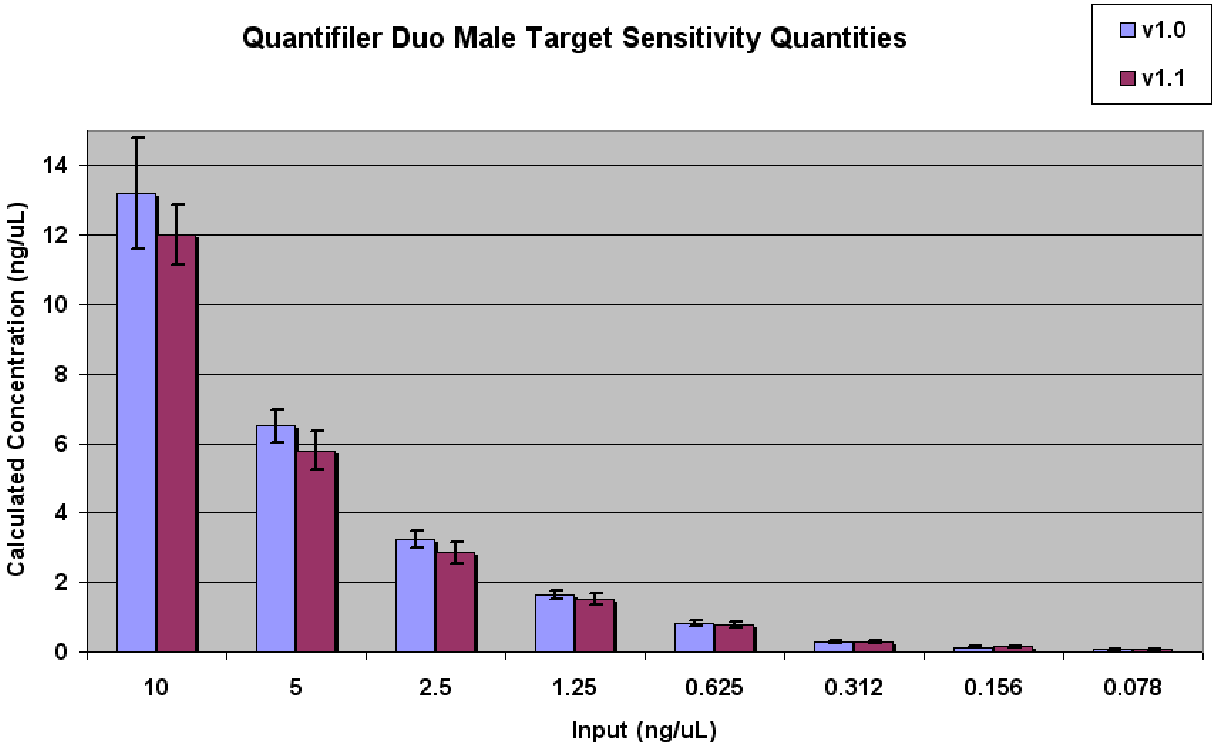


Figure 8 Mean C_T of human target DNA determined with the Quantifiler® Human kit by each version of the software. Error bars indicate standard deviations.

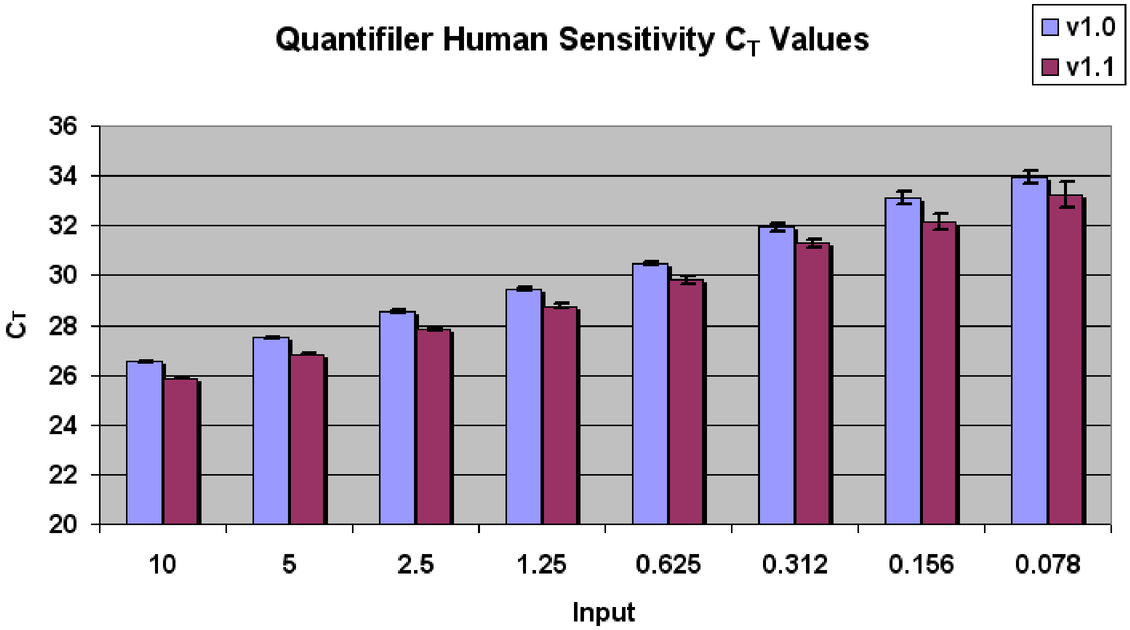
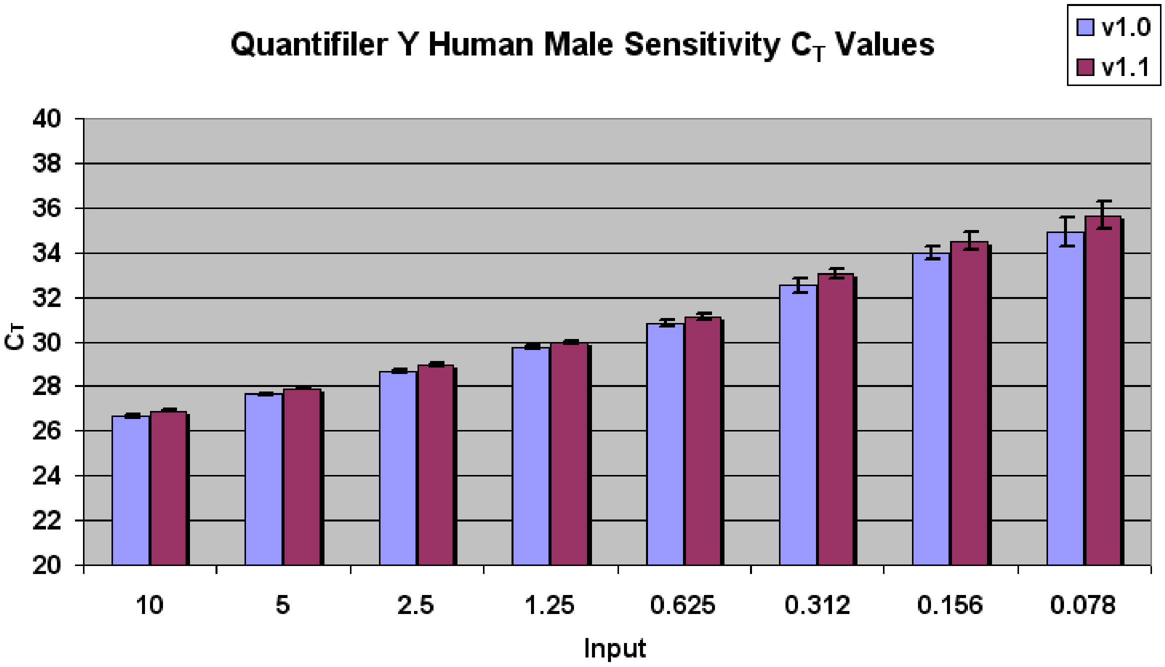


Figure 9 Mean C_T of human male target DNA determined with the Quantifiler® Y Human Male kit by each version of the software. Error bars indicate standard deviations.



6 Discussion

6.1 Quantifiler® Duo kit: precision and accuracy

The data generated with the Quantifiler® Duo kit did not demonstrate statistically significant differences between v1.0 and v1.1 for the human and the male targets. The maximum mean C_T difference observed was 0.21 C_T for both the human (50 ng/µL standard) and male (0.023 ng/µL standard) targets. The mean difference in IPC C_T values was 0.14 C_T, which is small but statistically significant. A similar difference between the two 7500 Real-Time PCR Systems used in the study suggests that the instruments might have introduced variability. Statistical analysis of the standard curve metrics (slope, Y-intercept, and R^2) did not demonstrate statistically significant differences between the two versions of the software.

6.2 Quantifiler® Duo, Human, and Male kits: sensitivity

Quantifiler® Duo kit

C_T values for the 007 dilution series showed a small but statistically significant difference for the human target only (0.05 C_T), while the male and IPC targets showed no significant difference in these C_T values. Calculated mean quantities showed more variability between versions of the software for both human (0.49 ng/µL) and male (0.54 ng/µL) targets. We observed a similar difference between instruments, suggesting that both the instruments and the individual standard curves introduced variability.

Quantifiler® Human and Y Human Male kits

Sensitivity differed more between software versions in studies using the Quantifiler® Human and Y Human Male kits. The HID Real-Time PCR Analysis Software v1.1 reported values that were 0.7 C_T less for the Human kit, and 0.4 C_T more for the Y Human Male kit, than the HID Real-Time PCR Analysis Software v1.0. As a result, mean differences in quantity calculations were 0.33 ng/µL for the Quantifiler® Human kit and 0.16 ng/µL for the Quantifiler® Y Human Male kit. Re-analysis of raw data from each run with each version of the software produced identical C_T values, demonstrating that there is no difference between the C_T algorithms used by the two versions of the software. The differences in data generated by v1.0 and v1.1 may be attributable to small differences in plate preparation and sample run during different experiments.

7 Conclusions

- This validation study demonstrated the reliability and reproducibility of the new HID Real-Time PCR Analysis Software v1.1 for performing quantitation using the Quantifiler® Human, Quantifiler® Y Human Male, and Quantifiler® Duo DNA quantification kits and the 7500 Real-Time PCR System.
- HID Real-Time PCR Software v1.1 generated C_T values and standard curve metrics (slope, Y-intercept, and R^2 values) with the Quantifiler® Duo kit that were nearly identical to data generated with HID Real-Time PCR Analysis Software v1.0. Quantity values demonstrate a statistically significant difference, but that difference is attributable to differences in the individual standard curves.
- Quantifiler® Human and Y Human Male kit assay results demonstrated some variability between the software versions (v1.0 and v1.1). Further re-analysis of the raw data with each software version produced identical results. Thus, the variability could be a result of factors such as instrument, assay setup, or time between assay runs.
- All workflows, run methods, HID quality flags, and dilution calculations were defined and utilized identically for each version of the software.

8 References

Federal Bureau of Investigation (2000) Quality assurance standards for forensic DNA testing laboratories, Forensic Science Communications [Online]. (July 2000). Available at: **http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm**

Scientific Working Group on DNA Analysis Methods (SWGDAM) Revised validation guidelines (approved July 2003). Available at:**http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm**

Applied Biosystems HID Real-Time PCR Analysis Software v1.1 Getting Started Guide (PN 4401644).

Applied Biosystems Quantifiler® Kits User Bulletin, Validation Using SDS Software Version 1.2.3 on the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System (PN 4374659).

Applied Biosystems Quantifiler® Kits User's Manual (PN 4344790).

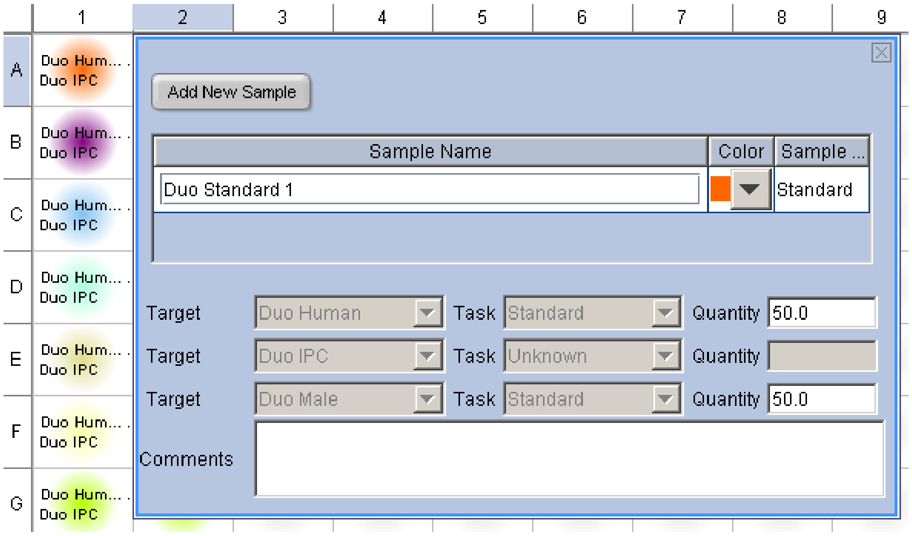
Applied Biosystems Quantifiler® Duo DNA Quantification Kit User's Manual (PN 4391294).

Applied Biosystems *HID Real-Time PCR Analysis Software v1.0 Validation User Bulletin* (PN 4401643).

New features of v1.1 software

Software function	New feature
User interface	
Hybrid template access	The Hybrid (Quantifiler® Human and Male) plate option is available as a template on the home screen.
Adding a sample to a well	Double-clicking the well in the plate layout view opens the Add Sample dialog box, so you can add a sample without returning to the Define Samples area (See Figure 10).
Start-up preferences	You can specify more start-up preferences.
Default settings • Plate layout view • Show in wells screen	Save settings as defaults in the plate layout view in addition to the well table view, and also in the Show in Wells screen. The new default settings, and column arrangement in the well table view, persist each time you open the software. Setup and analysis settings can be different.
Analysis tools	
Omitting targets	Omit either the human or male target from the standard curve during standard curve analysis in the Quantifiler® Duo assay by selecting the well of interest, then omitting the target (See Figure 11).
Selecting samples	Select all unknown samples for STR kit selection and dilution calculations with one click.
Sorting results	You can sort data by column or row in the Results table. The exported data file shows data sorted the way you chose for the Results table.
Exporting and reporting options	
Exporting .jpg of standard curve	Includes slope, Y-intercept, and R^2.
Standard curve	The standard curve includes data points for standard samples only.
Well selection	You can export and print results for specific wells, or for all wells.
Results table	Printed Results Table contains all report data (in v1.0, reports contained a Report Table and Report Summary, each with some of the data).
Long sample names	A sample name in a report can be up to 100 characters long.

Figure 10 One-step sample addition and well assignment



Duo Standard 1 | Standard

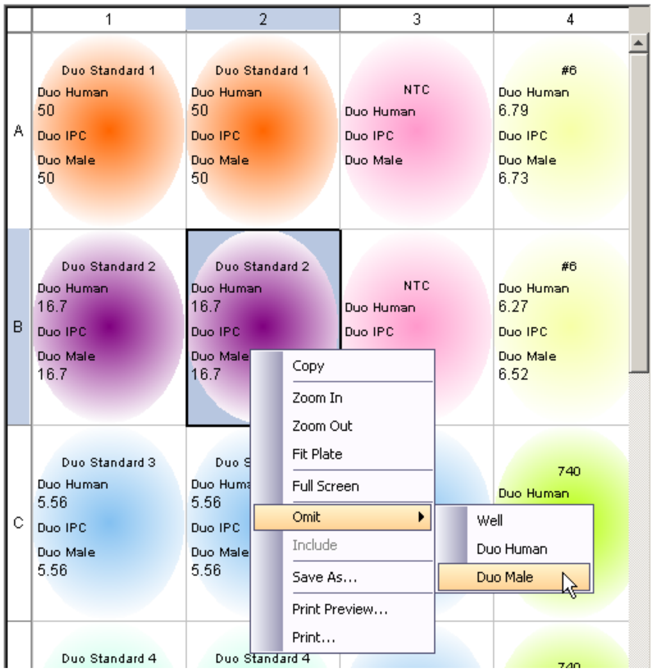
Target Duo Human Task Standard Quantity 50.0

Target Duo IPC Task Unknown Quantity

Target Duo Male Task Standard Quantity 50.0

Comments

Figure 11 Omitting a single target of the Quantifiler® Duo kit assay



	1	2	3	4
A	Duo Standard 1 Duo Human 50 Duo IPC Duo Male 50	Duo Standard 1 Duo Human 50 Duo IPC Duo Male 50	NTC Duo Human Duo IPC Duo Male	#6 Duo Human 6.79 Duo IPC Duo Male 6.73
B	Duo Standard 2 Duo Human 16.7 Duo IPC Duo Male 16.7	Duo Standard 2 Duo Human 16.7 Duo IPC Duo Male 16.7	NTC Duo Human Duo IPC	#6 Duo Human 6.27 Duo IPC Duo Male 6.52
C	Duo Standard 3 Duo Human 5.56 Duo IPC Duo Male 5.56	Duo S Duo Huma 5.56 Duo IPC Duo Male 5.56		740 Duo Human
	Duo Standard 4	Duo Standard 4		

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Information in this document is subject to change without notice.

Life Technologies Corporation assumes no responsibility for any errors that may appear in this document.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Limited Use Label License: Product Disclaimer

The products in this Bulletin may be covered by one or more Limited Use Label License(s). Please refer to the respective product documentation or the Applied Biosystems website under www.appliedbiosystems.com for the comprehensive license information. By use of these products, the purchaser accepts the terms and conditions of all applicable Limited Use Label Licenses. These products are sold for research use only, and are not intended for human or animal diagnostic or therapeutic uses unless otherwise specifically indicated in the applicable product documentation or the respective Limited Use Label License(s). For information on obtaining additional rights, please contact outlicensing@lifetech.com outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Purchase of this software product alone does not imply any license under any process, instrument or other apparatus, system, composition, reagent or kit rights under owned or otherwise controlled by Life Technologies, either expressly, or by estoppel.

© 2010 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. TaqMan is a registered trademark of Roche Molecular Systems, Inc.

4460964$A

Part Number 4460964 Rev. A 11/2010

applied biosystems™
by life technologies™

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone 760.603.7200
www.lifetechnologies.com

Technical Resources and Support
For the latest technical resources and support information
for all locations, please refer to our Web site at
www.appliedbiosystems.com