User Bulletin

Quantifiler® Kits

April 2006

SUBJECT: 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

Note: The information in this User Bulletin is also contained in the *Quantifiler® Kits User's Manual* (PN 4344790 Rev. D, 4/2006), Sections 6.4 and 6.5.

In This User Bulletin

This user bulletin includes the following topics:

Applied Biosystems

1 Applied Biosystems 7500 Real-Time PCR System Validation (SDS Software v1.2.3)

Overview

The Quantifiler® Human DNA Quantification Kit and the Quantifiler® Y Human Male DNA Quantification Kits were tested (see the studies listed below) using the Applied Biosystems 7500 Real-Time PCR System with SDS Software v1.2.3, running on the Windows® XP operating system. The results were then compared to the previously validated ABI PRISM® 7000 Sequence Detection System with SDS Software v1.0.

The experimental data generated demonstrate that the 7500 System (SDS Software v1.2.3):

- Provides accurate results when used with the Quantifiler kits for the analysis of genomic DNA samples.
- Produced results that are statistically similar to the results produced on the previously validated 7000 System (SDS Software v1.0).

Validation Studies Performed

- Precision and Accuracy
- Reproducibility and Sensitivity
- Background
- Auto Baseline versus Manual Analysis

1.1 Materials and Methods

1.1.1 Reagents

To minimize variables from hand pipetting and lot-to-lot reagent differences, the following set up procedures were used throughout the study:

- Eight serial dilutions were made with one lot of standard DNA provided with the Quantifiler kits (first dilution prepared with 500 µL DNA and 1,000 µL 10 mM Tris-HCl (pH 8.0) and 0.1 mM Na_2EDTA ($T_{10}E_{0.1}$ buffer)).
- One manufactured lot of each kit was used for all validation studies:

Kit	Part Number	Lot Number
Quantifiler Human Kit	4343895	0501020
Quantifiler Y Human Male Kit	4343906	0501018

1.1.2 Instruments

Three 7500 systems (SDS Software v1.2.3) and three 7000 systems (SDS Software v1.0) were used for this study (six instruments total). Before the study, each instrument was calibrated by an Applied Biosystems service engineer (ROI calibration, background calibration, optical calibration, pure dye calibration, RNase P run).

The Biomek® FX Laboratory Automation Workstation was used to set up the real-time PCR reaction plates to minimize hand-pipetting variations:

- The PCR master mixes (PCR reagents with standard or sample DNA mixed together) were aliquoted into a 96-well plate (PCR master mix plate).
- Six empty 96-well plates and the PCR master mix plate were placed on the Biomek FX work surface.
- The Biomek FX aspirated 25 µL from the PCR master mix plate, then slowly dispensed it into the corresponding well in an empty 96-well plate. The plates were sealed, spun down, then quickly loaded onto a 7500 or 7000 system. This process ensured timely and precise replication of real-time PCR plates for six instruments at a time.

1.2 Experimental Setup

Precision and Accuracy Study

On each 96-well reaction plate, six sets of standard dilutions for each Quantifiler kit were set up for real-time PCR. Figure 1 shows the experimental plate layout.

For each instrument, six replicate plates were run consecutively. The cycle threshold (C_T), R^2, and slope values were compared statistically to determine precision and accuracy, which established 95% confidence intervals for each instrument type.

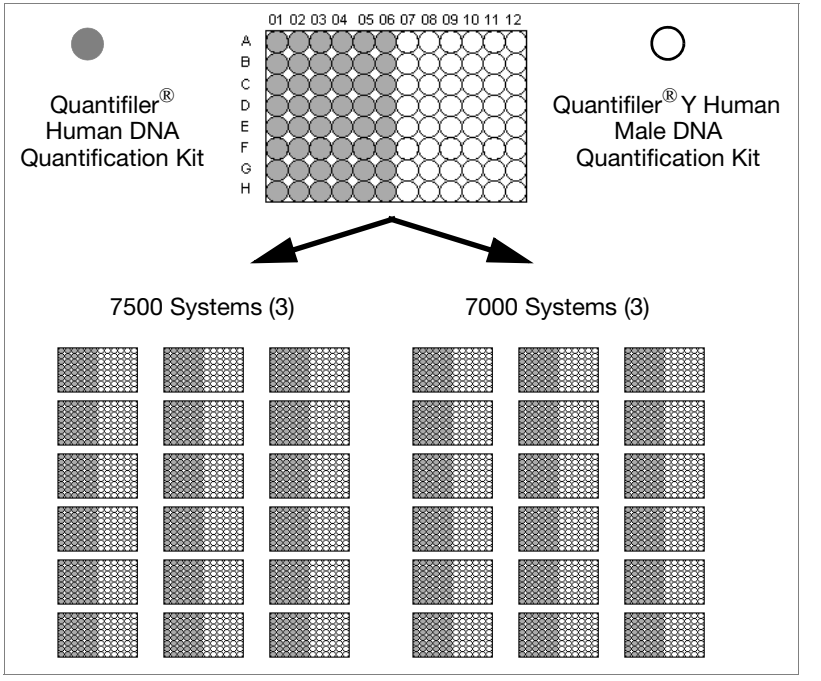


Figure 1 Plate layout – Precision and accuracy study on the 7500 System (SDS Software v1.2.3) and 7000 System (SDS Software v1.0)

Reproducibility and Sensitivity Study

On each 96-well reaction plate, the following were set up for real-time PCR:

- Standard dilution series (two replicates of each dilution point)
- Five replicate serial dilution sets of two sample DNAs (Raji and 9948B)

The experimental plate layout is shown in Figure 2.

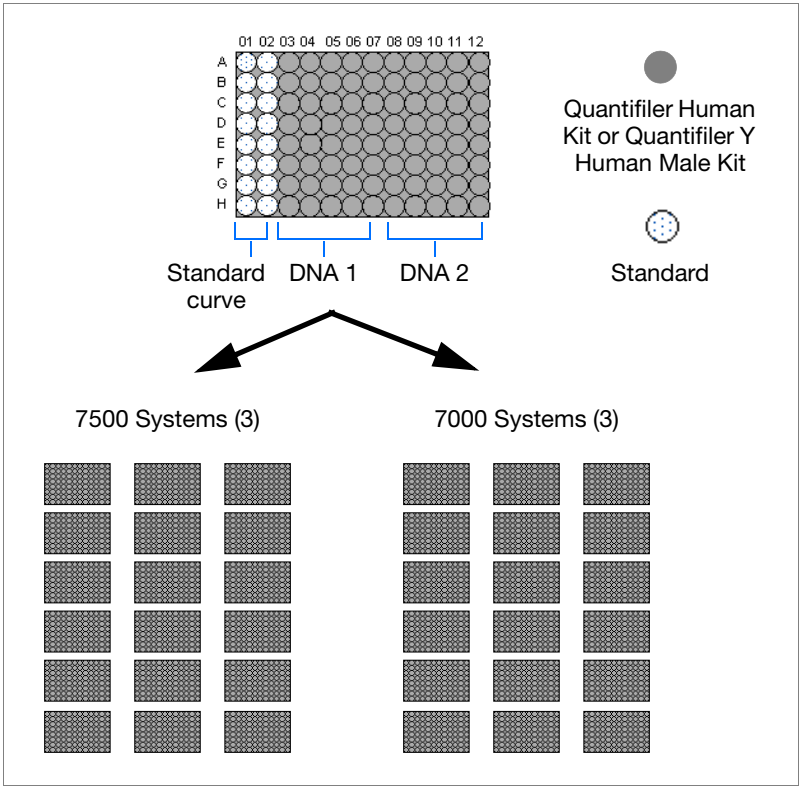


Figure 2 Plate Layout – Reproducibility and sensitivity study on the 7500 System (identical plate layout for both kits)

On each instrument, six replicate plates were run consecutively with each Quantifiler kit (for a total of 18 plates on 7500 systems and 18 plates on 7000 systems).

To demonstrate reproducibility and sensitivity, the replicate DNA samples were quantitated, and the results were compared statistically between instrument types.

Background Study

Ninety-five no template controls (NTCs) and one positive control (the 50 ng/µL standard DNA dilution sample) were set up on a 96-well plate. One plate from each Quantifiler kit was run on each instrument (for a total of 12 plates).

1.3 Data Collection

The standard thermal cycling protocol (9600 Emulation mode) described in the *Quantifiler® Kit User's Manual,* Chapter 3, was used for all instrument runs.

1.4 Data Analysis

Initial Data Compiling and Analysis

All runs were analyzed initially using Manual analysis mode, with the baseline set to 3 to 15 and the threshold set at 0.2.

Average values and standard deviations for C_T, slope, and R^2 were calculated for all replicate samples in a run.

For Auto-Baseline-to-Manual analysis comparisons, the run files from the 7500 System (SDS Software v1.2.3) were reanalyzed using Auto Baseline mode and a threshold of 0.2.

Statistical Data Analysis

For statistical analysis, the Stat-Ease Design-Expert® Software was used for all ANOVA (analysis of variance) calculations. For paired t-Tests analysis, MicroSoft® Excel Analysis ToolPak software was used.

1.4.1 Precision and Accuracy Studies

For the precision and accuracy studies between the two instrument types, the following values were determined:

- Average C_T
- Average Slope
- Average R^2
- 95% confidence intervals (CI) by ANOVA analysis

C_T Results

Table 1 shows the average C_T values (95% CI) for the 7500 System (SDS Software v1.2.3) and the 7000 System (SDS Software v1.0) at each standard curve dilution.

Table 1 C_T Values (95% CI)

Standard Curve Dilution (ng/µL)	7500 System		7000 System	
	Average C_T Value (95% CI)	C_T Value Range (95% CI)	Average C_T Value (95% CI)	C_T Value Range (95% CI)
50	23.29	23.21 to 23.37	23.05	22.97 to 23.13
16.7	24.98	24.90 to 25.06	24.56	24.48 to 24.64
5.56	26.53	26.44 to 26.61	26.08	26.00 to 26.16
1.85	28.05	27.97 to 28.14	27.53	27.45 to 27.61
0.62	29.44	29.36 to 29.53	29.00	28.92 to 29.09
0.21	30.86	30.78 to 30.94	30.33	30.25 to 30.41
0.068	32.40	32.32 to 32.48	31.61	31.53 to 31.70
0.023	33.98	33.88 to 34.05	33.03	32.95 to 33.11

Statistically, the two instrument types resulted in significantly different C_T values ($p < 0.0001$) when compared with the ANOVA analysis. No significant difference in C_T values was observed when comparing results from instruments of the same type.

Slope Results

Figure 3 shows the average slope values obtained for replicate standard curves run on each instrument. The slope values obtained for the 7500 System (SDS Software v1.2.3) are listed below and are within the ranges previously established on the 7000 System (SDS Software v1.0):

Kit	Slope	Established Slope Range
Quantifiler Human Kit	−2.93 to −3.18	−2.9 to −3.3
Quantifiler Y Human Male Kit	−3.05 to −3.36	−3.0 to −3.6

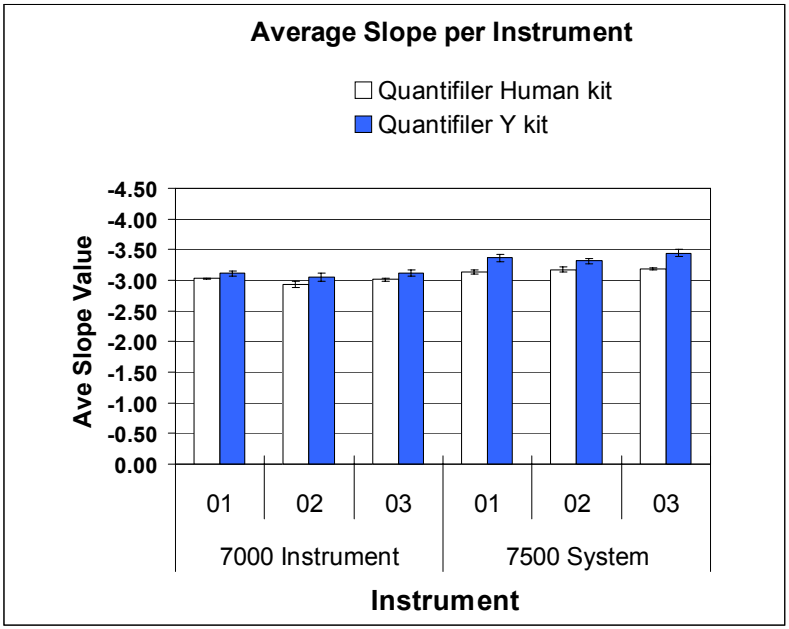


Figure 3 Average slope values – Replicate standard curves

R^2 Results

Figure 4 shows the average R^2 values obtained for replicate standard curves on each instrument. All R^2 values were greater than 0.98 and are within the established range.

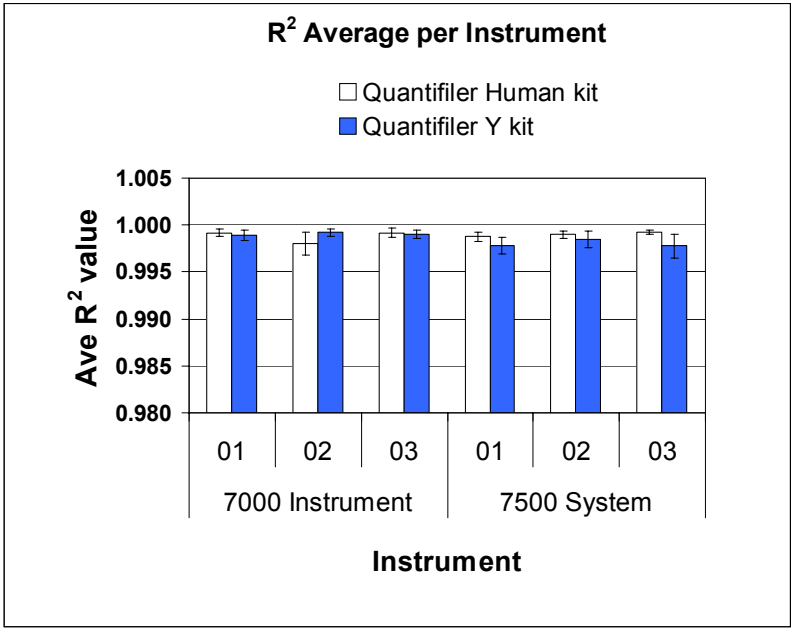


Figure 4 Average R^2 values – Replicate standard curves

1.4.2 Reproducibility and Sensitivity Studies

Two sample DNAs were quantitated for this study. Eight 3-fold serial dilutions for each sample were run (five replicates per dilution, 40 wells per sample). The C_T values were generated in Manual analysis mode, then the quantities were calculated using the standard curve on each plate.

Figure 5 shows average C_T values (each point n = 90 replicates) across a set of four serial dilutions (2 ng/µL to 0.5 ng/µL) with the Quantifiler Human Kit and the corresponding quantitated concentrations for one DNA sample. Similar results were obtained for the second DNA sample and the Quantifiler Y Human Male Kit (data not shown).

As the data show, differences in C_T values do not affect calculated quantities (calculated quantities were normalized resulting in comparable concentrations on both instrument types).

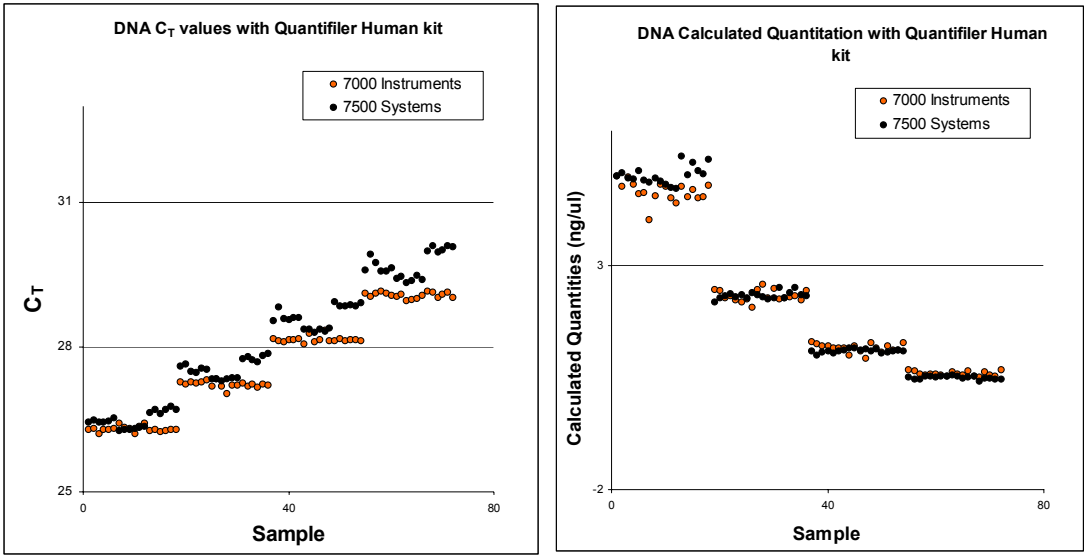


Figure 5 C_T values and quantitated concentrations – Quantifiler Human Kit (comparable data were obtained for the Quantifiler Y Human Male Kit)

Table 2 shows the average calculated quantities for each DNA sample obtained with the Quantifiler Human Kit. For sample concentrations between 2 ng/µL and 0.5 ng/µL, the percent difference between the quantitated values between instrument types did not exceed 16%. No statistically significant difference was observed for calculated quantities obtained using the Quantifiler Human Kit on the two instrument types.

Table 2 Average Calculated DNA Quantities – Quantifiler Human Kit

DNA Sample	7000 Avg Calculated Qty. (ng/µL)	7000 Std. Dev.	7500 Avg Calculated Qty. (ng/µL)	7500 Std. Dev.	Difference Between 7000 & 7500 Calculated Qty. (ng/µL)	% Difference of 7000 Qty. Value from 7500 Qty. Value
Raji	9.33	0.51	9.14	0.33	0.19	2.04
	4.58	0.15	4.24	0.12	0.34	7.72
	2.30	0.11	2.09	0.04	0.21	9.63
	1.16	0.05	1.07	0.03	0.09	8.01
	0.59	0.03	0.55	0.01	0.04	6.91
	0.27	0.01	0.26	0.01	0.01	3.43
	0.15	0.01	0.15	0.01	0.00	3.24
	0.08	0.00	0.07	0.00	0.01	8.04
9948	4.65	0.15	5.02	0.20	–0.37	7.58
	2.33	0.02	2.34	0.05	–0.01	0.36
	1.16	0.05	1.09	0.03	0.07	5.98
	0.59	0.02	0.50	0.03	0.08	15.52
	0.31	0.02	0.27	0.01	0.04	12.31
	0.17	0.01	0.15	0.01	0.02	10.80
	0.08	0.01	0.06	0.00	0.03	38.59
	0.05	0.01	0.04	0.00	0.01	18.14

Table 3 shows the average calculated quantities for each DNA sample obtained with the Quantifiler Y Human Male Kit. For sample concentrations of 2 ng/µL to 0.5 ng/µL, the percent difference between the quantitated values between instrument types did not exceed 18%. A minimal statistical difference was observed for calculated quantities obtained using the Quantifiler Y Human Male Kit on the two instrument types ($p = 0.0027$).

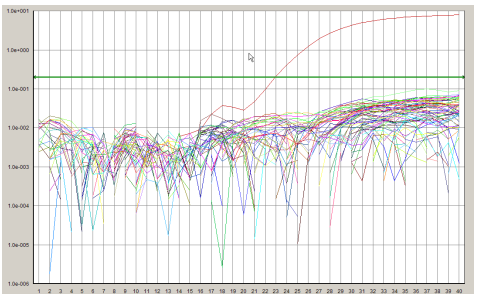
Table 3 Average Calculated DNA Quantities – Quantifiler Y Human Male Kit

DNA Sample	7000 Ave. Calculated Qty. (ng/µL)	7000 Std. Dev.	7500 Ave. Calculated Qty. (ng/µL)	7500 Std. Dev.	Difference Between 7000 & 7500 Calculated Qty. (ng/µL)	% Difference of 7000 Qty. Value from 7500 Qty. Value
Raji	9.12	0.40	9.09	0.07	0.03	0.34
	4.60	0.20	4.66	0.04	–0.06	1.29
	2.53	0.07	2.36	0.05	0.17	7.04
	1.29	0.09	1.19	0.03	0.10	8.12
	0.66	0.05	0.62	0.03	0.05	7.36
	0.33	0.02	0.30	0.02	0.02	7.89
	0.15	0.02	0.14	0.01	0.02	11.55
	0.070	0.02	0.057	0.01	0.01	19.85
9948	4.71	0.12	4.56	0.06	0.15	3.19
	2.43	0.14	2.30	0.06	0.12	5.14
	1.34	0.09	1.13	0.05	0.21	17.34
	0.68	0.03	0.62	0.03	0.06	9.93
	0.33	0.03	0.28	0.03	0.05	15.60
	0.18	0.01	0.14	0.01	0.04	24.87
	0.08	0.00	0.05	0.00	0.02	34.65
	0.04	0.00	0.03	0.00	0.01	38.29

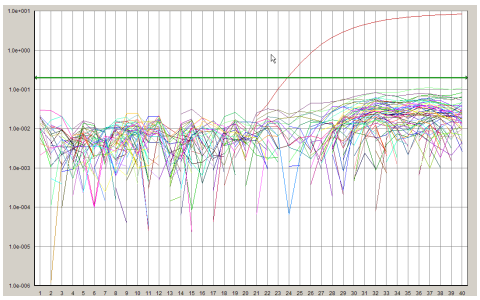
1.4.3 Background Study

Figure 6 shows background amplification plots for 95 NTCs and one positive control for both kits (one plate each) run on the 7000 System (SDS Software v1.0). Figure 7 shows background amplification plots for the 7500 System (SDS Software v1.2.3).

On all instruments, the 95 NTC samples yielded negative results (all C_T values >40) with both Quantifiler kits.

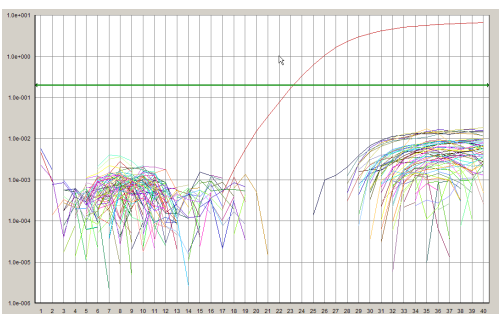


Quantifiler Human Kit

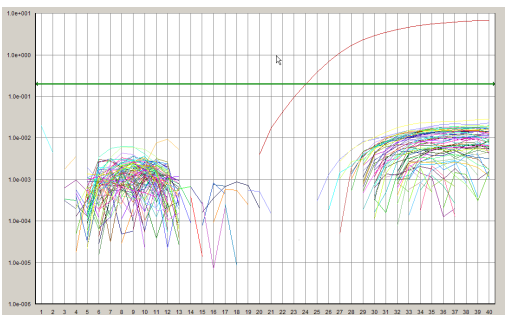


Quantifiler Y Human Male Kit

Figure 6 Background amplification plots – 7000 System (SDS Software v1.0)



Quantifiler Human Kit



Quantifiler Y Human Male Kit

Figure 7 Background amplification plots – 7500 System (SDS Software v1.2.3)

1.4.4 Auto Baseline Analysis Versus Manual Analysis Study

C_T Precision and Accuracy

For Auto-Baseline-to-Manual analysis comparisons:

- The SDS software v1.2.3 data from the studies described on the previous pages were reanalyzed in Auto Baseline mode (default threshold 0.2).
- The C_T values were compared to each other.

Figure 8 shows the C_T values obtained using the Auto Baseline and Manual analysis modes with the Quantifiler Human Kit. Similar data were obtained for the Quantifiler Y Human Male Kit.

No statistically significant differences were observed for C_T values generated using the Auto Baseline and Manual analysis modes with either Quantifiler kit.

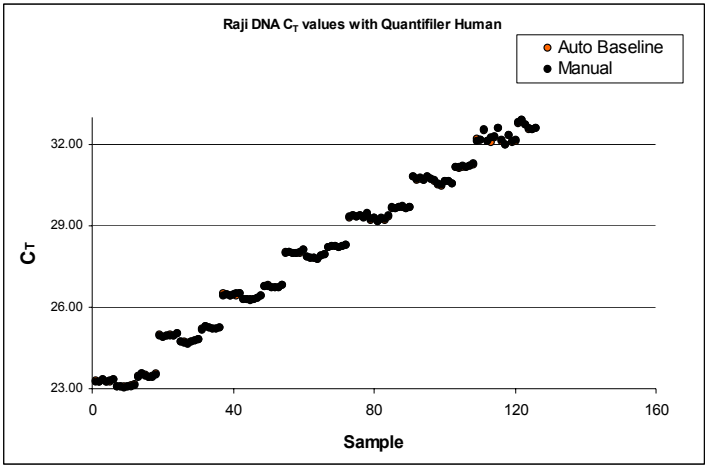


Figure 8 Comparison of C_T values between Auto Baseline and Manual analysis modes

C_T Reproducibility and Sensitivity

Figure 9 shows the C_T values and calculated quantities obtained using the Auto Baseline and Manual analysis modes with the Quantifiler Human Kit. Similar data were obtained for the Quantifiler Y Human Male Kit.

No statistically significant differences were observed for C_T values and calculated quantities derived using the Auto Baseline and Manual analysis modes with either Quantifiler kit.

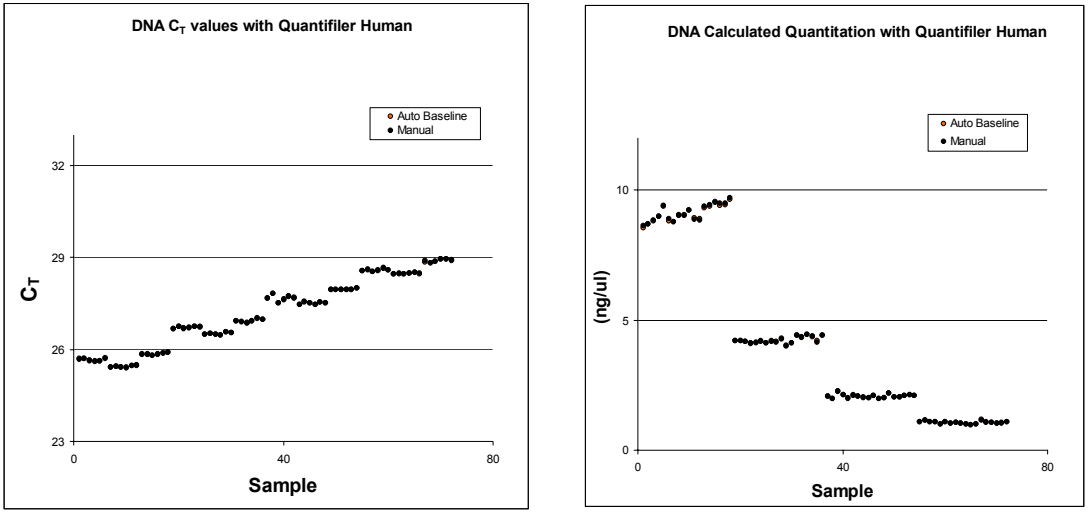


Figure 9 Comparison of C_T values and the corresponding calculated quantities – Auto Baseline and Manual analysis modes – Quantifiler Human Kit

1.5 Discussion

1.5.1 Precision and Accuracy Studies

7500 System Comparison: No statistically significant differences were observed in C_T, slope, and R^2 values between replicate samples run on the 7500 System (SDS Software v1.2.3) using both Quantifiler kits.

7500-to-7000 System Comparison: Statistically significant differences in C_T, slope, and R^2 values were observed in samples run on the 7500 System (SDS Software v1.2.3) versus the 7000 System (SDS Software v1.0) using both Quantifiler kits.

However, the data obtained from both instrument types are within the previously established parameter ranges published in the *Quantifiler® User's Manual,* Chapter 5, Table 5-1.

1.5.2 Reproducibility and Sensitivity Studies

Sensitivity: Similar C_T values and calculated DNA quantities were obtained at each of the standard curve concentrations, demonstrating similar sensitivity results between the 7000 System (SDS Software v1.0) and 7500 System (SDS Software v1.2.3).

Calculated Quantities: Data obtained using the Quantifiler Human Kit showed no statistically significant difference when the calculated quantities obtained from the 7000 and 7500 systems were compared ($p = 0.22$, with 95% confidence). However, minimally significant differences were observed between the two instrument types for calculated quantities using the Quantifiler Y Human Male Kit.

To further explore the extent of the difference between the two instrument types, the percent differences between the calculated quantities within the concentration range of 2 ng/µL to 0.5 ng/µL were determined. This range was selected because it represents the optimal input range for most STR kits. In this range, there was, at most, an 18% concentration difference between calculated quantities using the 7000 and the 7500 systems. The impact of the slight differences in calculated quantities should have minimal effect on results of STR analysis. However, laboratories should perform the appropriate studies to verify optimal input amounts for amplification.

1.5.3 Auto Baseline Analysis Versus Manual Analysis Studies

No statistically significant difference was observed for C_T values and calculated quantities derived using the Auto Baseline and Manual analysis modes on the 7500 System (SDS Software v1.2.3).

1.6 Conclusion

This validation study demonstrates that the Applied Biosystems 7500 Real-Time PCR System with SDS Software v1.2.3 is a robust, reliable, and reproducible system for performing DNA quantification using the Quantifiler kits.

When statistically comparing 7500 System (SDS Software v1.2.3) results (C_T, slope, and R^2 values) to results obtained using previously validated ABI PRISM® 7000 Sequence Detection System with SDS Software v1.0:

- Differences in calculated quantities are minimal (Quantifiler Y Human Male Kit) or insignificant (Quantifiler Human Kit) for unknown samples using the 7500 and 7000 systems.
- The differences observed should have little effect on resulting STR amplification based on calculated DNA quantities.
- No significant difference is observed between C_T values and calculated quantities derived by using Auto Baseline and Manual analysis modes.

2 ABI PRISM® 7000 Sequence Detection System Validation (SDS Software v1.2.3)

Overview

The Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit were tested (see the studies listed below) using the ABI PRISM® 7000 Sequence Detection System with SDS Software v1.2.3, running on the Windows® 2000 operating system., then compared to the previously validated ABI PRISM® 7000 Sequence Detection System with SDS Software v1.0.

The experimental data generated demonstrate that the 7000 System (SDS Software v1.2.3):

- Provides accurate results when used with the Quantifiler kits for the analysis of genomic DNA samples.
- Produced results that are similar to the results produced on the previously validated 7000 System (SDS Software v1.0).

Validation Studies Performed

- Precision and Accuracy
- Reproducibility and Sensitivity
- Background
- Auto Baseline versus Manual analysis

2.1 Materials and Methods

2.1.1 Reagents

To minimize variables from hand-pipetting and lot-to-lot reagent differences, the following set-up procedures were used throughout the study:

- Eight serial dilutions were made with one lot of standard DNA provided with the Quantifiler kits (first dilution prepared with 500 µL DNA and 1,000 µL 10mM Tris-HCl (pH 8.0) and 0.1 mM Na_2EDTA ($T_{10}E_{0.1}$ buffer)).
- One manufactured lot of each kit was used for all validation studies:

Kit	Part Number	Lot Number
Quantifiler Human Kit	4343895	0501022
Quantifiler Y Human Male Kit	4343906	0501020

2.1.2 Instruments

One ABI PRISM® 7000 Sequence Detection System was used for this study under the following conditions:

- All studies were run initially using SDS Software v1.0.
- The 7000 system computer was upgraded to SDS Software v1.2.3.
- The 7000 System (SDS Software v1.2.3) was calibrated by an Applied Biosystems service engineer (background calibration, pure dye calibration, RNase P run).
- For the following studies, v1.0 data was reanalyzed using SDS Software v1.2.3:
  - Precision and Accuracy
  - Reproducibility and Sensitivity
  - Background
- For Auto Baseline versus Manual analysis studies, new data were collected using SDS Software v1.2.3, analyzed in Auto Baseline mode, then reanalyzed in Manual mode.

2.2 Experimental Setup

Precision and Accuracy Study

On each 96-well reaction plate, six sets of standard dilutions for each Quantifiler kit were set up for real-time PCR. The experimental plate layout is shown in Figure 10.

Three replicate plates were run consecutively. The C_T, slope, and R^2 values were compared to determine precision and accuracy.

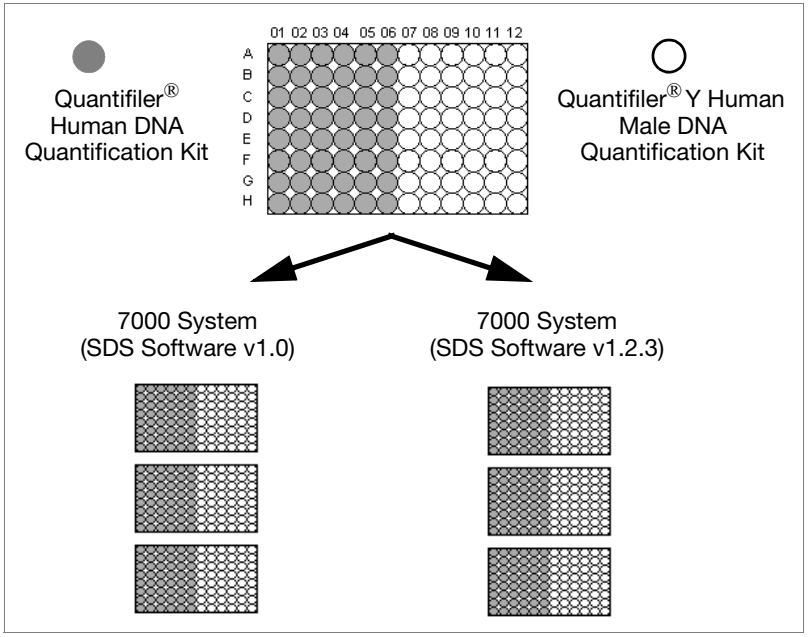


Figure 10 Plate Layout – Precision and accuracy study on the 7000 System

Reproducibility Sensitivity, and Background Study

On each 96-well reaction plate, the following were set up for real-time PCR:

- Standard dilution series (two replicates of each dilution point)
- Four replicate serial dilution sets of two sample DNAs (007 and 9948B)
- Sixteen no template controls (NTCs), which served as background samples

Figure 11 shows the experimental plate layout.

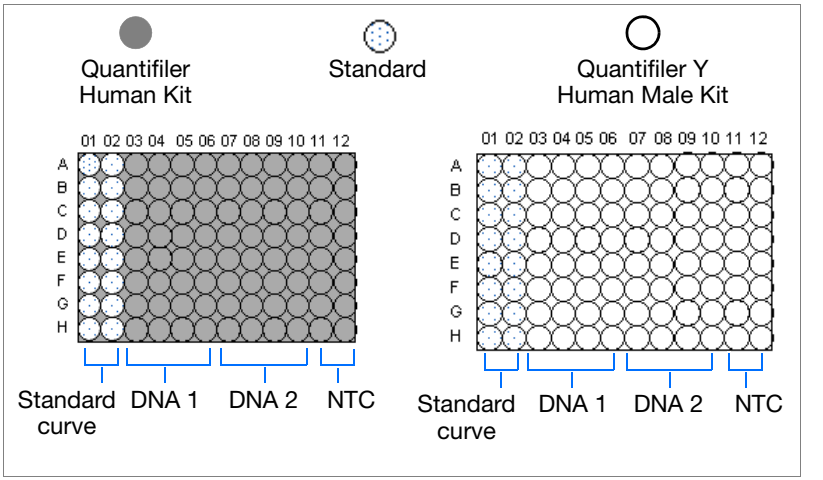


Figure 11 Plate Layout – Reproducibility and sensitivity studies – 7000 Systems

One plate was run with each type of Quantifiler kit.

To demonstrate reproducibility and sensitivity, the replicate DNA samples were quantitated and the results were compared between each software version.

2.3 Data Collection

The standard thermal cycling protocol (9600 Emulation mode) described in the *Quantifiler® Kit User's Manual*, Chapter 3, was used for both studies.

2.4 Data Analysis

Initial Data Compiling and Analysis

All runs were analyzed initially using Manual analysis mode, with the baseline set to 3 to 15 and the threshold set at 0.2.

Average values and standard deviations for C_T, slope, and R^2 were calculated for all replicate samples in a run.

The instrument was then upgraded to SDS Software v1.2.3, then the same run files were reanalyzed and exported with the same analysis settings.

For Manual-to-Auto-Baseline analysis comparisons, the run files from the 7000 System (SDS Software v1.2.3) were reanalyzed using the Auto Baseline mode and a threshold of 0.2.

2.4.1 Precision and Accuracy Studies

For the precision and accuracy studies between the two software versions, the average C_T, average slope, and average R^2 values were determined.

C_T Results

Figures 12 to 14 show C_T values obtained using the SDS Software v1.0 and v1.2.3. The data consistently show that SDS Software v1.2.3 yields lower C_T values (2% difference).

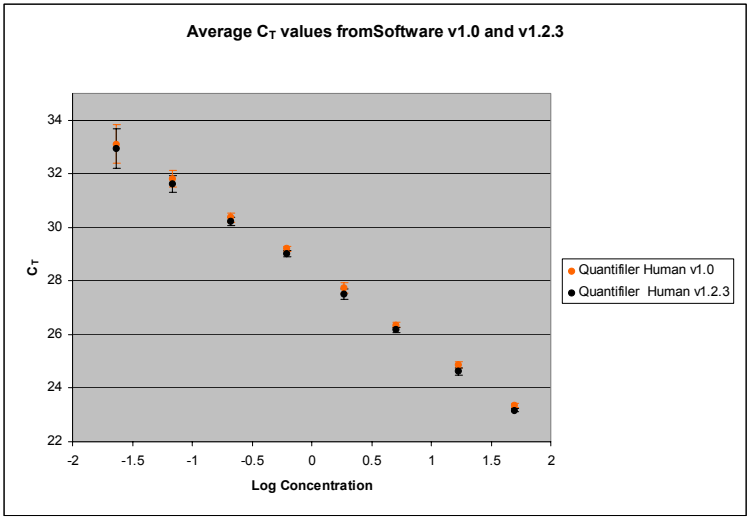


Figure 12 Average C_T values – Quantifiler Human Kit – SDS Software v1.0 and v1.2.3 (error bars indicate standard deviations)

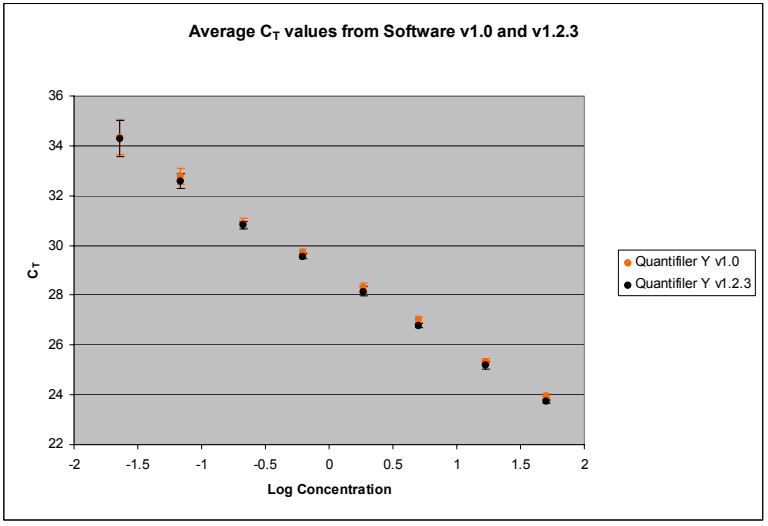


Figure 13 Average C_T values – Quantifiler Y Human Male Kit – SDS Software v1.0 and v1.2.3 (error bars indicate standard deviations)

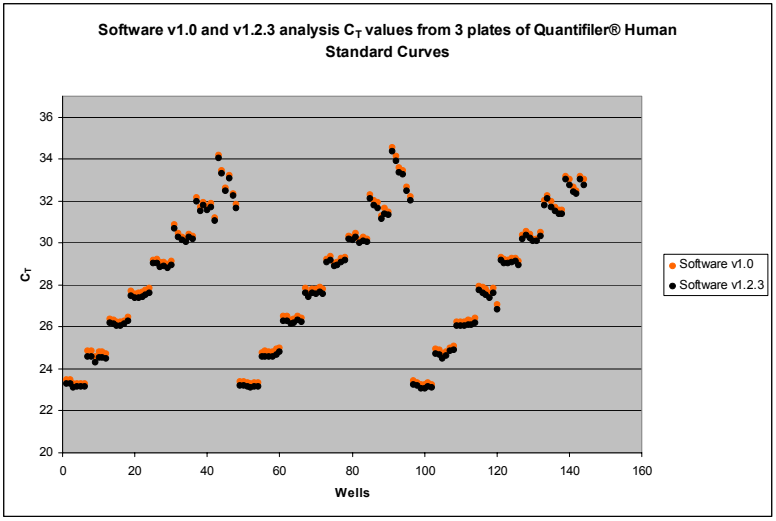


Figure 14 C_T Values per Sample – v1.0 compared to v1.2.3 – Quantifiler Human Kit

Slope Results

Figure 15 shows the average slope values obtained using the SDS software v1.2.3 compared to v1.0. The slope values obtained for the 7000 System (SDS Software v1.2.3) are within the established ranges.

Kit	Slope	Established Slope Range
Quantifiler Human Kit	−2.90 to −2.97	−2.9 to −3.3
Quantifiler Y Human Male Kit	−3.0 to −3.09	−3.0 to −3.6

A 1% slope difference is observed between the v1.2.3 and v1.0 software.

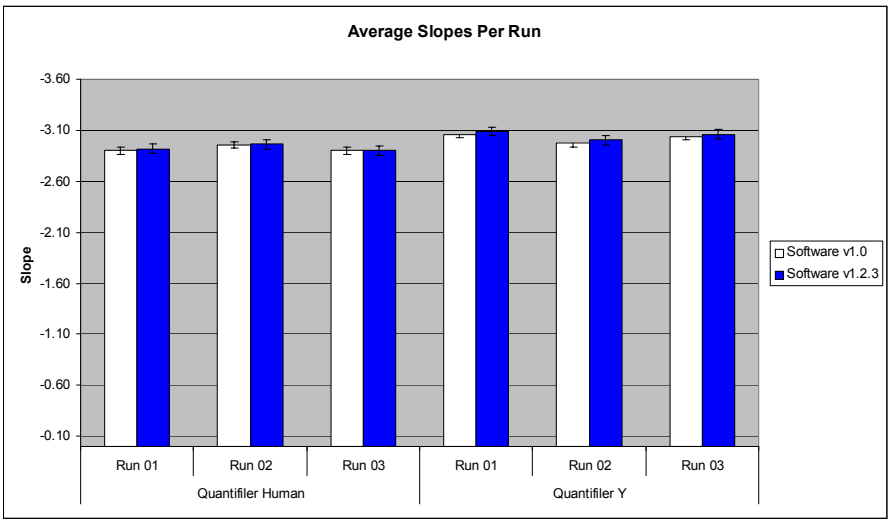


Figure 15 Average slope values – SDS Software v1.0 and v1.2.3

R^2 Results

Figure 16 shows that SDS software v1.2.3 yields data that are within the acceptable range of R^2 values: 0.98 to 1 for both kits (<0.5% difference).

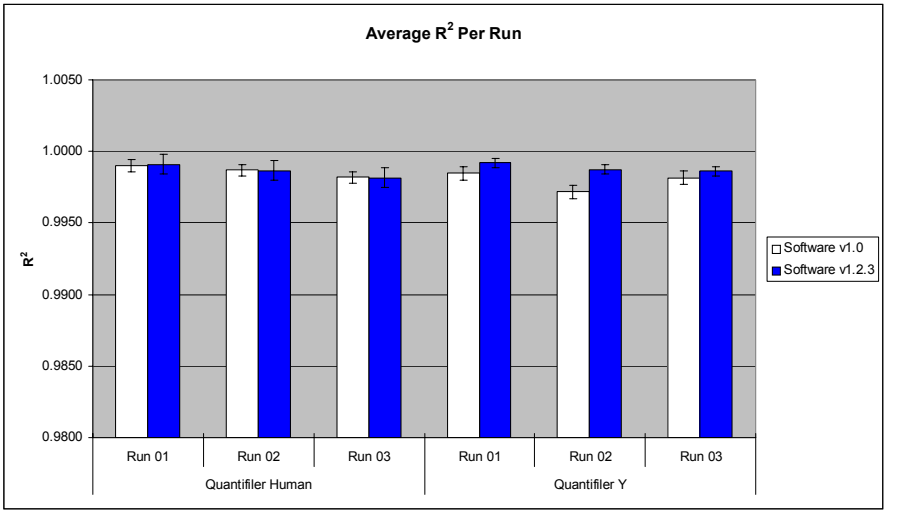


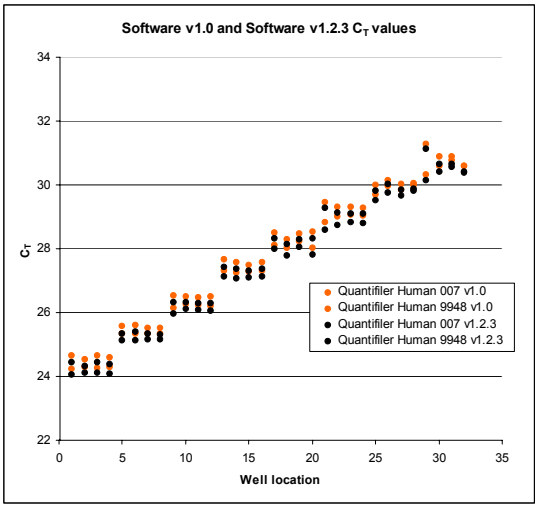
Figure 16 Average R^2 values – Quantifiler Human Kit and Quantifiler Y Human Male Kit – SDS Software v1.0 and v1.2.3

2.4.2 Reproducibility and Sensitivity Studies

Two sample DNAs were quantitated for this study. Eight 2-fold serial dilutions for each sample were run (four replicates per dilution, 32 wells per sample). The C_T values were generated in Manual analysis mode, then the quantities were calculated using the standard curve on each plate.

Figure 17 shows the C_T values for 007 and 9948B across a set of eight serial dilutions (~30 ng/µL to 0.1 ng/µL) with the Quantifiler Human Kit and the corresponding quantitated concentrations.

As the data show, differences in C_T values do not affect calculated quantities (calculated quantities were normalized, resulting in comparable concentrations from results generated with both software versions.)



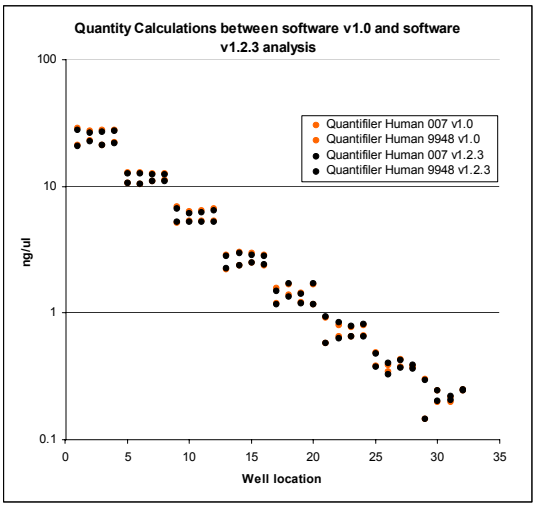
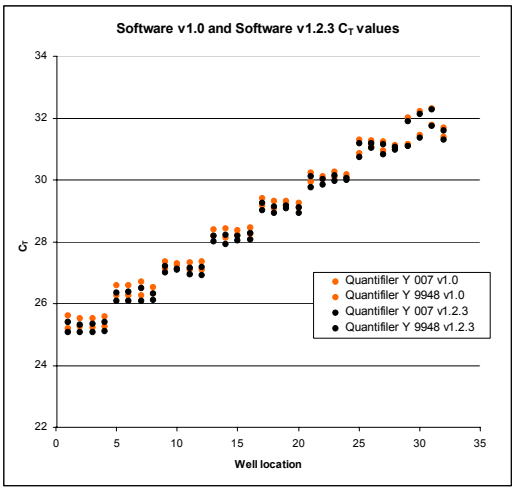


Figure 17 Average C_T values and quantitated DNA concentrations – 007 and 9948B – Quantifiler Human Kit

Figure 18 shows C_T results for the Quantifiler Y Human Male Kit that differ slightly between the v1.0 analysis and the v1.2.3 analysis. However, differences in C_T values do not affect calculated quantities (calculated quantities were normalized resulting in comparable concentrations from results generated with both software versions.)



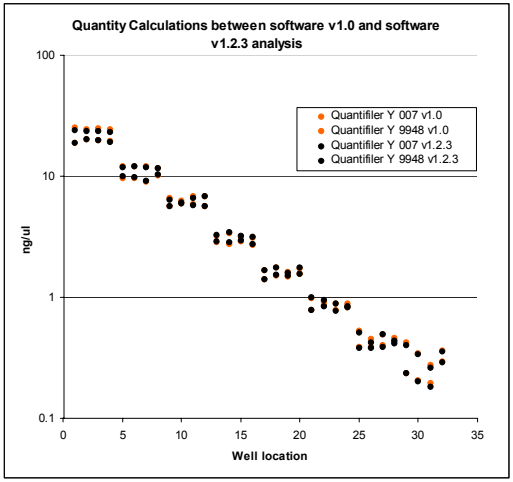


Figure 18 Average C_T values and quantitated DNA concentrations – 007 and 9948B – Quantifiler Y Human Male Kit

Figure 19 shows that there was a ≤6% quantity difference between results obtained with v1.0 and v1.2.3 software.

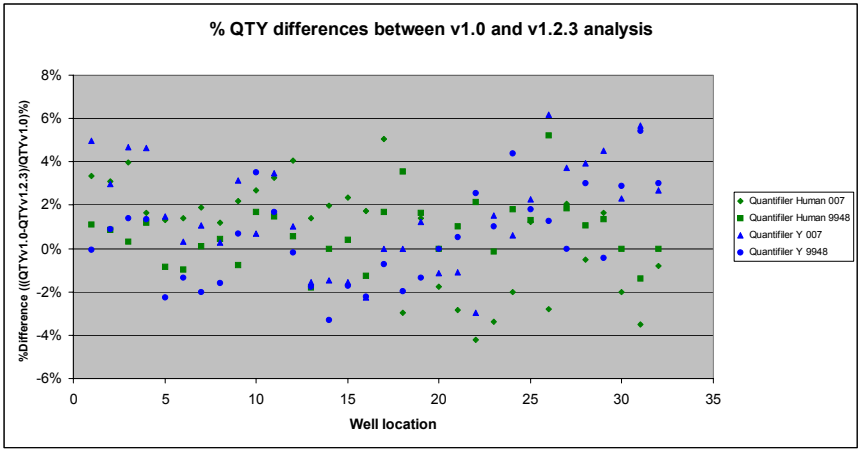
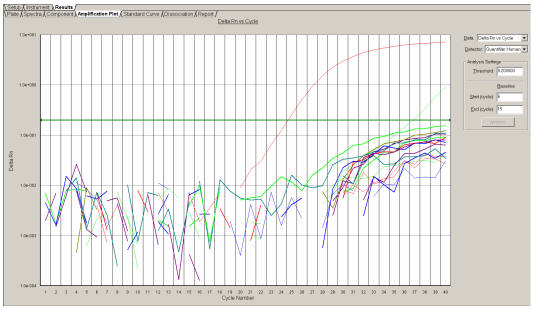


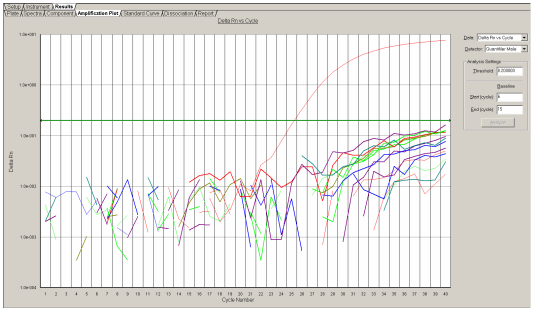
Figure 19 Percent DNA quantity differences – SDS Software v1.0 and v1.2.3

2.4.3 Background Studies

Figure 20 shows the background results for 16 NTCs and one positive control for both kits run on the 7000 System (SDS Software v1.0). One out of 16 NTCs for the Quantifiler Human Kit resulted in a <40 C_T result (36.81 C_T). Remaining NTCs resulted in >40 C_T values (negative results).



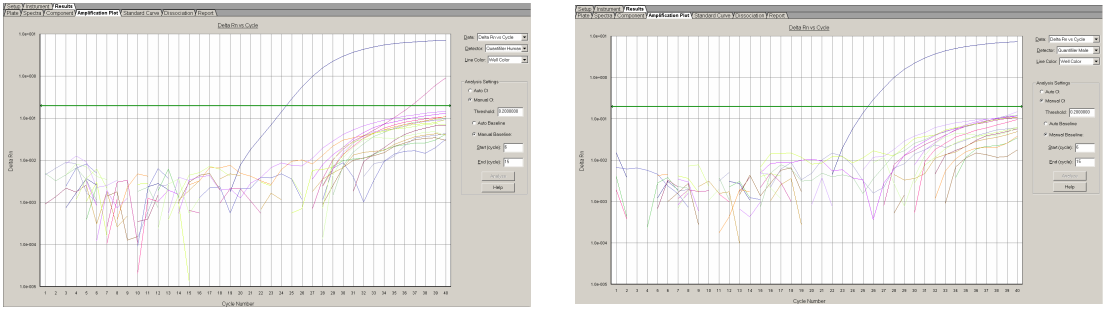
Quantifiler Human Kit



Quantifiler Y Human Male Kit

Figure 20 Background amplification plots – 7000 System (SDS Software v1.0)

Figure 21 shows the background results for 16 NTCs and one positive control for both kits reanalyzed on the 7000 System (SDS Software v1.2.3). One out of 16 NTCs for the Quantifiler Human Kit resulted in a <40 CT value (38.26 C_T). Overall, the NTC results do not change when analyzed with version 1.2.3.



Quantifiler Human Kit

Quantifiler Y Human Male Kit

Figure 21 Background amplification plots – 7000 System (SDS Software v1.2.3)

2.4.4 Auto Baseline Analysis Versus Manual Analysis Studies

C_T Precision and Accuracy

For Manual-to-Auto-Baseline analysis comparisons:

- Data from initial runs were collected with SDS Software v 1.2.3 and analyzed in Manual analysis mode, then reanalyzed in Auto Baseline analysis mode (default threshold 0.2).
- The C_T values were compared to each other.

Figures 22 and 23 show the average C_T values between Auto Baseline analysis and Manual analysis. There is a <2% difference between the two analysis methods for both kits.

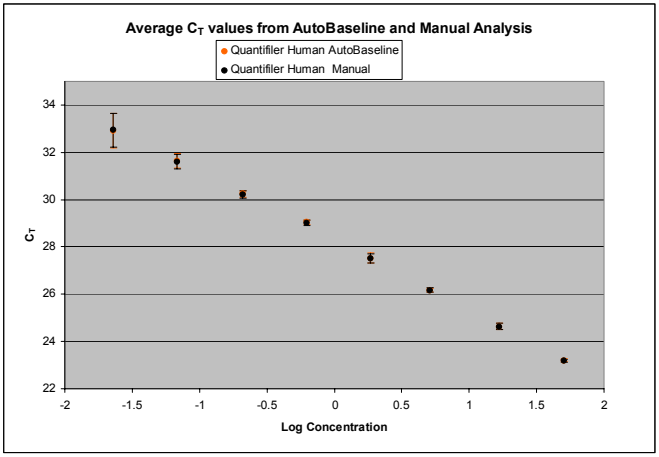


Figure 22 Comparison of C_T values between Auto Baseline and Manual analysis – Quantifiler Human Kit (error bars indicate standard deviations)

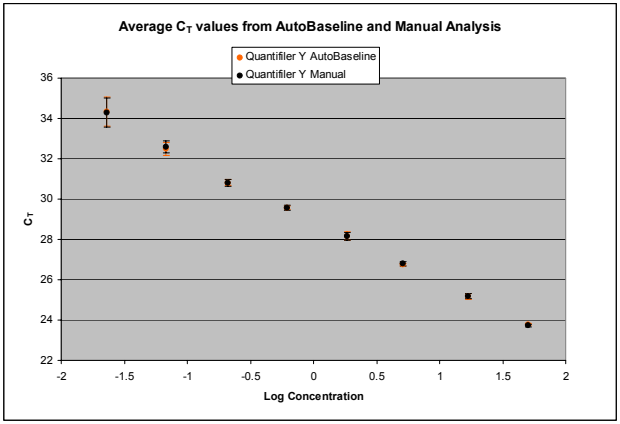


Figure 23 Comparison of C_T values between Auto Baseline and Manual analysis – Quantifiler Y Human Male Kit (error indicate standard deviations)

C_T Reproducibility and Sensitivity

Figure 24 shows the C_T values obtained using the Auto Baseline and Manual analysis modes with the Quantifiler Human Kit.

No significant differences were observed for C_T values generated using the Auto Baseline and Manual analysis modes with either Quantifiler kit.

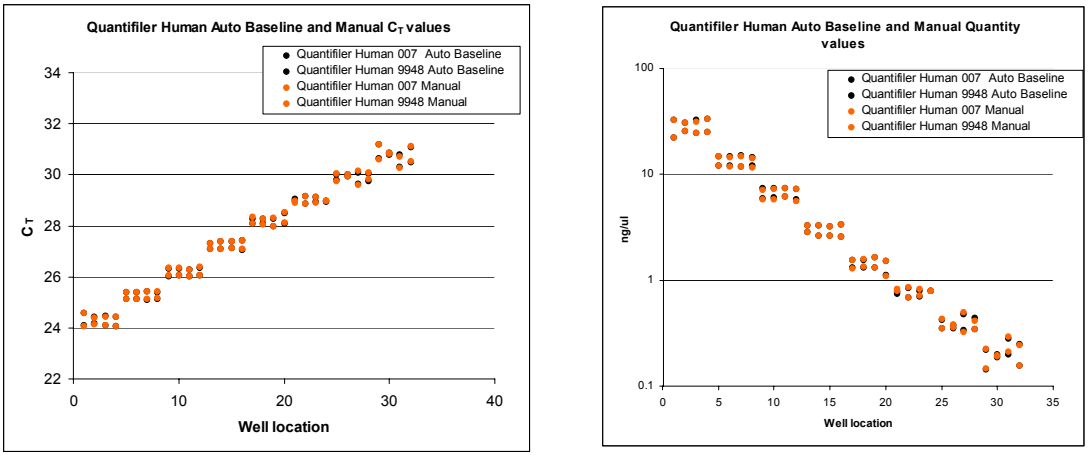


Figure 24 Average C_T values and average calculated quantities for 9948 and 007 – Quantifiler Human Kit (~30 ng/µL to 0.1 ng/µL)

Figure 25 shows the C_T values obtained using the Auto Baseline and Manual analysis modes with the Quantifiler Human Kit.

No significant differences were observed for C_T values generated using the Auto Baseline and Manual analysis modes with either Quantifiler kit. Auto Baseline C_T values overlap the manual C_T values. The corresponding quantities also overlap.

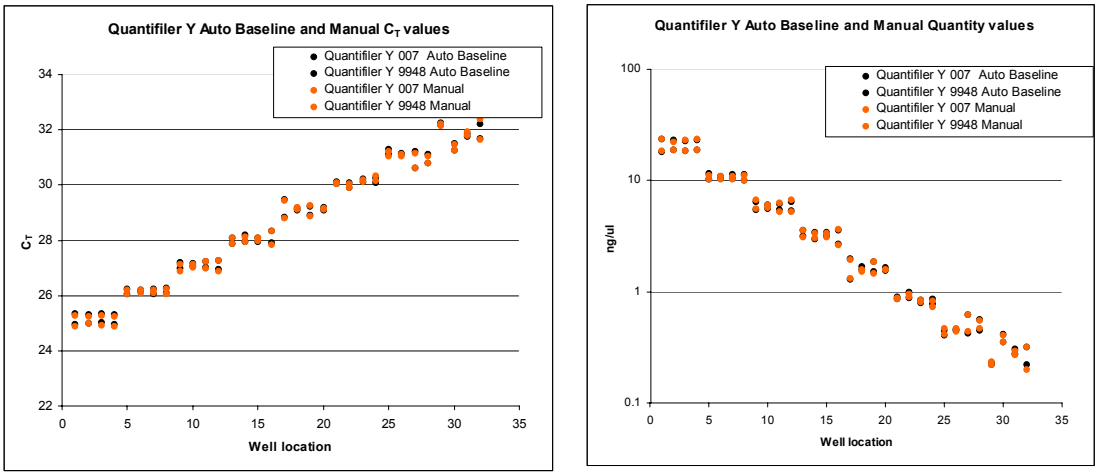


Figure 25 Average C_T values and average calculated quantities for 9948 and 007 – Quantifiler Y Human Male Kit

2.5 Discussion

2.5.1 Precision and Accuracy Studies

The results from SDS Software v1.0 and v1.2.3 on a 7000 System slightly differ in C_T value (2% difference), slope (1%), and R^2 (<0.5%) for both Quantifiler kits. All v1.0 data and v1.2.3 data are within the *Quantifiler® User's Manual* published parameter ranges.

2.5.2 Reproducibility and Sensitivity Studies

For both Quantifiler kits, there was a maximum difference of 6% when the calculated quantities using v1.0 and v1.2.3 were compared. Such minor differences in calculated quantities should not affect the ability to obtain interpretable STR profiles using the optimal input amount determined by individual laboratories during validation of the Quantifiler kits.

2.5.3 Manual Analysis Versus Auto Baseline Analysis Studies

C_T values and their corresponding calculated quantities showed a maximum 8% difference between Auto Baseline and Manual analysis modes on the 7000 System (SDS Software v1.2.3). However, the differences observed should have little effect on resulting STR amplification based on calculated DNA quantities.

2.6 Conclusion

This validation study demonstrates that the ABI PRISM® 7000 Real-Time PCR system with SDS Software v1.2.3 is a robust, reliable, and reproducible system for performing DNA quantification using the Quantifiler kits.

When comparing 7000 System (SDS Software v1.2.3) results (C_T, slope, and R^2 values) to results obtained using the previously validated 7000 System (SDS Software v1.0):

- Small percentage differences are observed in C_T, slope, and R^2 values.
- Differences in calculated quantities are minimal for unknown samples using the 7000 System (SDS Software v1.2.3) and 7000 System (SDS Software v1.0).
- The differences observed should have little effect on resulting STR amplification based on calculated DNA quantities.
- No significant difference is observed between C_T values and calculated quantities derived using Auto Baseline and Manual analysis modes.

3 References

Scientific Working Group on DNA Analysis Methods. Revised validation guidelines (approved July 2003). Available at: **http//www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm**.

DNA Advisory Board. Quality assurance standards for forensic DNA testing laboratories (approved October1998). Forensic Science Communications 2000;2(3). Available at: **http//www.fbi.gov/programs/lab/fsc/backissu/july2000/codispre.htm**.

Green, R., Roinestad, I., Boland, C., Hennessy, L., *Developmental Validation of the Quantifiler Real-Time PCR Kits for the Quantification of Human Nuclear DNA Samples*, Journal of Forensic Science, July, 2005, Vol 50, No. 4.

Applied Biosystems Quantifiler® Kit User's Manual, Part Number 4344790, Rev. B, Applied Biosystems, 11/2003.

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04/2006

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