Alabama Department of Forensic Sciences
Birmingham DNA

ABI Prism® 7000 Validation

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The validation studies referenced above have been reviewed and provide the necessary documentation required by the FBI Director's "Quality Assurance Standards for Forensic DNA Testing Laboratories" for a quantitation method to be used in the forensic casework section of the Alabama Department of Forensic Sciences Birmingham DNA laboratory.

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Known and Non-Probative Samples

8.1.3.1(a) **Has the procedure been tested using known and non-probative evidence samples?**

Experiment:

Eleven (11) evidence samples comprised of various origins that are encountered in routine casework were analyzed with the Quantifiler Human Kit on the ABI 7000. These quantitation results were then compared to the previously obtained Quantiblot results. All samples were then amplified using the Identifiler Kit and the Quantifiler Human results in an effort to determine the preferred amount of sample template to add to the PCR process.

Additionally, this laboratory participated in a NIST Quantitation study aimed at evaluating shipping conditions of standard DNA samples. Each of the NIST samples was analyzed with the Quantifiler Human Kit as well as the Quantiblot Kit, with results compared and tabulated as well.

Results:

Non-Probative Samples				
Sample Name	Sample Type	Quantifiler Human Result	Quantiblot Result	Percent Difference 100-[(QF/QB)*100]
96MB84292-1D	vaginal swab	0.0914	0.12	23.83
00BH01157-1A	victim standard	6.03	2	-201.50
96MB84292-1A	victim standard	1.36	1	-36.00
98BH00578-3	blood from currency	0.0993	0.03	-231.00
96BH29999-1C	vaginal swab	0.303	0.24	-26.25
96BH29999-1A1	victim standard	0.684	1	31.6
96BH32136-2A	semen on comforter	0.103	0.12	14.17
96BH32136-3A	suspect standard	2.14	1	-114.00
96BH32136-1A	victim standard	4.14	2	-107.00
00BH01157-1B	vaginal swab	2.48	0.6	-313.33
98BH00578-1A	suspect standard	4.8	1	-380.00

NIST Quantitation Study							
	Quantifiler Human Results				Quantiblot Results		Average % Difference 100 – [(QF/QB)*100]
NIST Sample	Tray 1	Tray 2	Tray 3	Average	Blot 1	Blot 2	Average	
A	1.35	1.57	1.48	1.47	0.5	2	1.25	-17.33
B	0.342	0.551	0.377	0.42	0.24	0.5	0.37	-14.41
C	0.141	0.152	0.114	0.14	0.12	0.12	0.12	-13.06
D	0.022	0.0283	0.0239	0.02	0.03	0.03	0.03	17.56
E	0.535	0.479	0.462	0.49	0.5	0.5	0.5	1.6
F	0.1	0.0706	0.105	0.09	0.06	0.12	0.09	-2.07
G	0.0276	0.029	0.0158	0.02	0.03	0.06	0.045	46.37
H	0.0365	0.056	0.0396	0.04	0.03	0.03	0.03	-46.78

Note: All quantitation results are in units of ng/µl
Note: The red numbers indicate the lower quant value

Known and Non-Probative Samples

The experimental results demonstrate that the Quantiblot method of quantitating DNA typically underestimated the amount of DNA present in a sample. An accurate quantitation result is critical to obtaining an adequate DNA profile downstream with the Identifiler Kit. If DNA quantities greater than the optimal range are added to the PCR mix, the analyst will likely have a more imbalanced PCR product as well as possible saturation of the detection system causing pull-up and a greater likelihood of Stochastic effects. When utilizing the Quantifiler results to determine DNA template addition, the resulting peak heights on the ABI 310 from the Identifiler amplicon were acceptable and produced no excessive pull-up or stochastic related issues.

Reproducibility

8.1.3.1(b)   **Has the reproducibility and precision of the procedure been monitored and documented using human DNA controls.**

Experiment:
A sample of K562 (10ng/µl) was diluted to 2ng/µl, 1ng/µl, .5ng/µl, .25ng/µl, .125ng/µl, and .06ng/µl. These samples along with a sample of 9947A (.1ng/µl) were run on the ABI 7000 Real Time PCR instrument in replicates of at least four (4) and were setup and run by two (2) separate analysts for a total of three (3) runs that spanned over two (2) days.

Results:

Plate	Sample Name (ng/ul)	CT Average	Quantity Average
CJMVAL2	K562 (10)	24.81	17.850
CJMVAL1	K562 (10)	24.71	16.068
SAOVAL1	K562 (10)	24.81	17.850
Total Averages		24.77	17.256
Standard Deviation		0.04	0.840
95% Confidence (+/- percent)			9.74
CJMVAL2	K562 (2)	27.15	2.423
CJMVAL1	K562 (2)	27.05	2.770
SAOVAL1	K562 (2)	27.20	2.793
Total Averages		27.13	2.662
Standard Deviation		0.06	0.169
95% Confidence (+/- percent)			12.73
CJMVAL2	K562 (1)	28.03	1.249
CJMVAL1	K562 (1)	28.02	1.332
SAOVAL1	K562 (1)	28.24	1.249
Total Averages		28.10	1.277
Standard Deviation		0.10	0.039
95% Confidence (+/- percent)			6.11
CJMVAL2	K562 (.5)	28.84	0.677
CJMVAL1	K562 (.5)	28.95	0.663
SAOVAL1	K562 (.5)	29.07	0.654
Total Averages		28.96	0.665
Standard Deviation		0.10	0.010
95% Confidence (+/- percent)			2.86

Plate	Sample Name (ng/ul)	CT Average	Quantity Average
CJMVAL2	K562 (.25)	29.64	0.370
CJMVAL1	K562 (.25)	29.88	0.331
SAOVAL1	K562 (.25)	29.80	0.372
Total Averages		29.77	0.358
Standard Deviation		0.10	0.019
95% Confidence (+/- percent)			10.55
CJMVAL2	K562 (.125)	30.58	0.184
CJMVAL1	K562 (.125)	30.71	0.176
SAOVAL1	K562 (.125)	30.88	0.165
Total Averages		30.72	0.175
Standard Deviation		0.12	0.008
95% Confidence (+/- percent)			9.25
CJMVAL2	9947A (0.1)	30.63	0.178
CJMVAL1	9947A (0.1)	30.68	0.182
SAOVAL1	9947A (0.1)	30.79	0.174
Total Averages		30.70	0.178
Standard Deviation		0.07	0.003
95% Confidence (+/- percent)			3.45
CJMVAL2	K562 (0.06)	31.64	0.083
CJMVAL1	K562 (0.06)	31.79	0.079
SAOVAL1	K562 (0.06)	31.93	0.073
Total Averages		31.78	0.078
Standard Deviation		0.12	0.004
95% Confidence (+/- percent)			10.74

The average 95% confidence interval (2 standard deviations/average DNA quantity) demonstrates the approximate range expected for quantitation results between runs when using the Quantifiler Human kits on the ABI 7000 Real Time PCR instrument. The following comparison of the Alabama Department of Forensic Sciences internal validation results and those obtained by ABI during developmental validation illustrate that the ABI 7000 located in the Alabama Department of Forensic Sciences casework section is well suited for obtaining reproducible and reliable human DNA quantitation values.

Reproducibility

Average 95% Confidence Interval	
ADFS	**ABI**
(+/-) 8.18	(+/-) 18.5

Reproducibility: Continued

8.1.3.1(b)   **Has the reproducibility and precision of the procedure been monitored and documented using human DNA controls.**

Experiment:

The K562 (.125 ng/µl), K562 (.5 ng/µl) and K562 (1 ng/µl) samples which were utilized in the initial reproducibility study were subsequently amplified utilizing the Identifiler kit at the approximate concentrations of 0.5ng and 1ng (based on the Quantifiler results). The samples were then run on the ABI310, analyzed and genotyped. The resulting peak heights were then collected, averaged and tabulated, and are given below:

Results:

Locus	Genotype	0.5ng to 0.6ng		1ng to 1.28ng	
		Peak Height (1) Average	Peak Height (2) Average	Peak Height (1) Average	Peak Height (2) Average
D8S1179	12	2225	---	5067	---
D21S11	29, 30, 31	Not Used in Calculations (Tri-allelic)			
D7S820	9, 11	689	662	1746	1318
CSF1PO	9, 10	601	1149	1192	2528
D3S1358	16	3942	---	7108	---
TH01	9.3	2006	---	5005	---
D13S317	8	1133	---	2660	---
D16S539	11, 12	602	1173	1646	2740
D2S1338	17	1034	---	2352	---
D19S433	14, 14.2	512	1079	1063	2143
vWA	16	656	---	1546	---
TPOX	8, 9	406	732	842	1542
D18S51	15, 16	342	333	694	599
Amelogenin	X	819	---	1811	---
D5S818	11, 12	497	845	941	1801
FGA	21, 24	644	132	1487	316

The purpose of this experiment was to determine a preferred amount of DNA template to amplify, based on quantitation values from the ABI 7000, which would subsequently produce peak height values in the preferred range for analysis. This determination supports the reproducibility study requirements while also providing the Alabama Department of Forensic Sciences with quantitation information which can be used downstream at PCR setup to minimize the necessity of re-injections and re-amplifications.

The experimental results indicate that amplifying in the range of 0.5ng to 1ng of template DNA, utilizing the Identifiler Kit and the Quantifiler Human results, will consistently result in peak heights suitable for interpretation. All peak heights that were obtained for the 0.5ng to 1.28ng template samples are above the Alabama Department of Forensic Sciences' peak amplitude threshold of 75 RFU's. Utilizing the template DNA concentration range of 0.5ng to 1ng should limit the amount of pull-up detected during analysis, thus decreasing the number of re-injections and increasing the confidence in allele calls.

Precision

8.1.3.1(b)   **Has the reproducibility and precision of the procedure been monitored and documented using human DNA controls.**

Experiment:

A set of eight standard dilutions of the Quantifiler Human DNA standard was made with the final concentrations ranging from 50ng to 0.023ng. These standards were then run on three separate plates spanning two days. The CT values were compiled, averaged and the standard deviations calculated for each standard.

In addition to the CT values for the Quantifiler Human DNA standard, the CT values for 330 internal positive controls (IPC) were compiled, averaged and the standard deviations calculated as well.

Results:

The CT averages and standard deviations for each dilution of the Quantifiler Human DNA standard are listed below along with the corresponding CT averages and standard deviations which were obtained from ABI's developmental validation and reprinted from the ABI 7000 User's Manual.

Precision of the Human DNA Standard Dilution Series
Quantifiler Human Kit

Quantitation Standard Dilution (ng/µl)	C$_T$ Values ADFS		C$_T$ Values ABI	
	Mean C$_T$	Standard Deviation	Mean C$_T$	Standard Deviation
50	23.28	0.12	23.09	0.1
16.7	24.74	0.18	24.64	0.17
5.56	26.19	0.14	26.19	0.16
1.85	27.59	0.13	27.67	0.17
0.62	29.09	0.25	29.09	0.17
0.21	30.38	0.19	30.31	0.19
0.068	31.65	0.28	31.9	0.28
0.023	33.67	0.85	33.45	0.48

The CT average and standard deviation for the internal positive control (IPC) is listed below along with the CT value range recommended by ABI for the Quantifiler Human Kit.

Precision of the Internal Positive Control – Quantifiler Human Kit

Average CT Value	26.81
Standard Deviation	0.35
Minimum CT Value Detected	26.33
Maximum CT Value Detected	29.98

Recommended CT Value Range (ABI)
20 to 30

Precision

These results demonstrate that the ABI 7000 instrument located in the casework section of The Alabama Department of Forensic Sciences Birmingham Laboratory consistently yields results comparable to those obtained at ABI during the developmental validation process.