



Intermountain Forensics

VAL #

QTY-VO2
(Supplemental 1.0)

Forensic DNA Technical Leader Approval

Issue Date

7/7/20

Quant Trio – Quant Studio 5 Summary

1. Summary and Conclusions:

Summary:

Commercial quantification kit Quantifiler Trio (Applied Biosystems) was evaluated for use to quantify DNA samples for forensic testing using the QuantStudio 5 instrument (ThermoFisher). The use of Virtual Standard Curves was also examined.

Data indicates that the quantification kit sufficiently detects human DNA and can differentiate human vs. male DNA. The kit can detect DNA at levels far below what is needed to generate DNA profiling data. Normalizing samples based on the quantification results produce expected and interpretable DNA profiles. The use of Virtual Standard Curves will give an increased degree of reproducibility over generating a new standard curve with every plate. A sample that generates a "no result" or zero quant can be considered free of DNA for forensic DNA downstream processing, will not generate a DNA profile and processing of the sample may be stopped.

Conclusion:

The Applied Biosystems Quantifiler Trio Quantification kit utilizing the Applied Biosystem QuantStudio 5 was examined through Contamination Assessment, Reproducibility, Sensitivity, Mixture Studies, Non-Probativ casework like samples and zero=zero studies and as a result of the conclusions contained within this document it is determined that it is suitable for DNA quantification processing in Forensic DNA Casework.

2. Standard Curve

Summary:

Standard Curve dilution series of 50ng/ul, 5ng/ul, 0.5ng/ul, 0.05ng/ul, and 0.005ng/ul was made according to the instructions in the Quantifiler Trio kit User Guide. A set of standard curves were assessed to determine the variation in order to use Virtual Standard Curves to analyze quantification data. Standard Curve data for plates run during the Validation show variation in DNA results dependent on the standard curve that is used to analyze the data, though it is not expected to significantly affect processing unless stop processing thresholds are used but may be a cause for reamplifying samples when general amplification targets are used.

A single standard is required on plates where a Virtual Standard Curve is used. A comparison in variability between the different standards of the standard curve was performed. 5ng/ul had a lower degree of variation than the others and will be used. +/- (3) of the average Standard Deviations or a range of 3.65-6.35 will be used as an indicator of individual plate amplification. No assessment of how long a single standard dilution tube remains viable was performed, however, the Quantifiler Trio User Guide states that that the dilutions can be stored for up to 2 weeks.

Table 1. Variation in Standard Curve Metrics

Large Target	Slope	R ²	Y-Intercept	Efficiency
Standard Set 1	-3.788	0.996	25.725	83.659
Standard Set 2	-3.49	0.998	25.511	93.43
Standard Set 3	-3.686	0.998	25.587	86.772
Standard Set 4	-3.568	0.999	25.41	90.668
Standard Set 5	-3.369	0.993	25.257	98.08
Average	-3.5802	0.9968	25.50	90.52
STDEV	0.163757	0.002387	0.1771	5.629
CV	0.0457	0.0024	0.0069	0.0622
Small Target	Slope	R ²	Y-Intercept	Efficiency



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Standard Set 1	-3.488	0.999	27.893	93.493
Standard Set 2	-3.491	0.994	27.888	93.398
Standard Set 3	-3.448	0.999	27.824	95.014
Standard Set 4	-3.716	1	27.789	85.823
Standard Set 5	-3.416	0.996	27.598	96.219
Average	-3.5118	0.9976	27.80	92.79
STDEV	0.118263	0.002510	0.1203	4.065
CV	0.0337	0.0025	0.0043	0.0438
Y Target	Slope	R ²	Y-Intercept	Efficiency
Standard Set 1	-3.623	0.999	26.645	88.812
Standard Set 2	-3.421	0.992	26.46	96.041
Standard Set 3	-3.596	1	26.569	89.698
Standard Set 4	-3.655	1	26.516	87.745
Standard Set 5	-3.488	0.998	26.324	93.526
Average	-3.5566	0.9978	26.50	91.16
STDEV	0.098409	0.003347	0.1210	3.491
CV	0.0277	0.0034	0.0046	0.0383

Conclusion:

The use of a virtual standard curve will allow an even further degree of replication so that each quant value will result in the same actions for similar “true” DNA amounts across every plate, provide a more consistent analysis, and use of standard amplification targets. As different manufacture lots may have slight variability, each newly received manufacture lot should have a new Virtual Standard Curve created.

The CV values in the sample sets are extremely low and thus the variability in quant metrics from run to run is also very low. This suggests that use of a virtual standard curve is appropriate as run to run variability of these metrics is unlikely to significantly affect results. The use of a virtual standard curve will allow an even further degree of replication so that each quant value will result in the same actions for similar “true” DNA amounts across every plate and provide a more consistent analysis and use of standard amplification targets. This will also decrease costs and increase efficiency.

From these results, the QuantStudio 5 and Applied Biosystems Quantifiler Trio Quantification Kit:

- 1) Has shown the ability to reproduce standard curves very consistently with an extremely low level of variation
- 2) Allows use of virtual standard curves to be utilized
 - a. As different manufacture lots may have slight variability, each newly received manufacture lot should have a new Virtual Standard Curve created as per vendor recommendations

3. Contamination Assessment

Summary:

The NTC from all quantification runs were examined for DNA.

Table 2. Quant Trio NTC data

Quant Plate	Human	Y
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QPCR021920AM-1	0	0
QPCR200221AM-1	0	0
QPCR200420-AM-TRIO	0	0

Conclusion:

All NTC quantifications detected 0 DNA. The protocol and quantification process is shown to be able to be performed without introducing contamination.

From these results, the QuantStudio 5 and Applied Biosystems Quantifiler Trio Quantification Kit:

- 1) Has shown to yield results that are clear of contamination
 - a. The QuantStudio 5 does not appear to cross contaminate
 - b. The Applied Biosystems Quantifiler Trio Quantification kit does not appear to introduce reagent-based contamination
 - c. The quant setup / lab processes associated with QuantStudio 5 and Applied Biosystems Quantifiler Trio quantification kit do not appear to introduce contamination

4. Sensitivity

Summary:

A dilution series for 6 samples was made with dilutions of 1ng/ul, 0.5ng/ul, 0.125ng/ul, 0.0625ng/ul, 0.03125ng/ul, and 0.015625ng/ul. Each was quantified with and amplified, as the total input DNA amount, with Globalfiler and Investigator 24Plex. Sample egrams showed variation in similar target samples. The dilutions were then requantified to obtain a more accurate input.

An assessment to determine amplification activity at lower quantification values and determine whether any stop-at-quantification thresholds can be established.

Table 3. Quant input target and resulting amplification result

Sample ID	QT (Total ng input)	GF	AVG. PHR	Comparable	INV	AVG. PHR	Comparable
ND1-0.015625	0.002	2 (6%)	N/A	No	0 (0%)	N/A	No
ND1-0.03125	0.0046	10 (29%)	47%	No	4 (11%)	N/A	No
ND21-0.015625	0.0049	0 (0%)	N/A	No	2 (6%)	N/A	No
ND18-0.015625	0.016	10 (25%)	53%	No	8 (20%)	N/A	No
ND12-0.015625	0.0116	14(37%)	47%	Yes	18 (47%)	54%	Yes
ND21-0.03125	0.0125	2 (6%)	N/A	No	8 (24%)	N/A	No
ND11-0.015625	0.0398	10 (26%)	N/A	Yes	37 (95%)	70%	Yes
ND1-0.0625	0.0165	31 (89%)	60%	Yes	32 (91%)	60%	Yes
ND12-0.03125	0.0241	25 (66%)	70%	Yes	31 (82%)	70%	Yes
ND18-0.03125	0.0192	32 (80%)	62%	Yes	20 (50%)	68%	Yes
ND11-0.03125	0.0401	---	---	---	34 (87%)	61%	Yes
ND21-0.0625	0.031	29 (85%)	64%	Yes	26 (76%)	56%	Yes
ND11-0.0625	0.1171	34 (87%)	81%	Yes	39 (100%)	77%	Yes
ND1-0.125	0.0505	35 (100%)	72%	Yes	34 (97%)	62%	Yes
ND12-0.0625	0.0462	38 (100)%	70%	Yes	38 (100%)	71%	Yes
ND9-0.015625	0.0375	40 (100%)	81%	Yes	39 (98%)	69%	Yes



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ND21-0.25	0.0947	34 (100%)	74%	Yes	33 (97%)	73%	Yes
ND1-0.25	0.1074	35 (100%)	81%	Yes	35 (100%)	73%	Yes
ND18-0.0625	0.0355	39 (98%)	68%	Yes	36 (90%)	68%	Yes
ND21-0.125	0.0319	31 (91%)	70%	Yes	27 (79%)	74%	Yes
ND12-0.125	0.1281	38 (100%)	86%	Yes	38 (100%)	72%	Yes
ND9-0.0625	0.1159	40 (100%)	85%	Yes	40 (100%)	84%	Yes
ND18-0.125	0.0918	40 (100%)	82%	Yes	40 (100%)	69%	Yes
ND18-0.25	0.1701	40 (100%)	87%	Yes	40 (100%)	78%	Yes
ND11-0.125	0.2315	33 (85%)	75%	Yes	39 (100%)	78%	Yes
ND12-0.25	0.2702	38 (100%)	86%	Yes	38 (100%)	86%	Yes
ND9-0.03125	0.0806	40 (100%)	85%	Yes	40 (100%)	81%	Yes
ND1-0.5	0.2617	35 (100%)	87%	Yes	35 (100%)	85%	Yes
ND11-0.25	0.3349	36 (92%)	82%	Yes	39 (100%)	89%	Yes
ND12-1	0.9205	38 (100%)	87%	Yes	38 (100%)	89%	Yes
ND11-0.5	0.5987	39 (100%)	89%	Yes	39 (100%)	89%	Yes
ND9-0.125	0.2132	40 (100%)	88%	Yes	40 (100%)	88%	Yes
ND21-0.5	0.2564	34 (100%)	86%	Yes	34 (100%)	75%	Yes
ND1-1	0.8571	35 (100%)	89%	Yes	35(100%)	89%	Yes
ND18-1	0.7309	40 (100%)	90%	Yes	40 (100%)	89%	Yes
ND9-0.25	0.4028	40 (100%)	90%	Yes	40 (100%)	86%	Yes
ND12-0.5	0.4181	38 (100%)	91%	Yes	---	---	---
ND18-0.5	0.3088	40 (100%)	87%	Yes	40 (100%)	85%	Yes
ND9-0.5	0.5621	40 (100%)	88%	Yes	40 (100%)	91%	Yes
ND21-1	0.8734	34 (100%)	88%	Yes	34 (100%)	86%	Yes
ND9-1	1.0797	40 (100%)	93%	Yes	40 (100%)	89%	Yes
ND11-1	1.0348	39 (100%)	87%	Yes	39 (100%)	90%	Yes

Conclusion:

Data indicates that the quantification kit is capable of detecting DNA at a lower level than will produce amplification profile results. Full profiles can be obtained with high quality DNA template as low as 0.080 ng and comparable DNA profiles obtained as low as 0.020 ng. Average Peak Height Ratio decreases with the decrease of input DNA with average PHR above 60% down to 0.04ng/ul. These values are not applicable with degraded and/or inhibited DNA profiles.

By obtaining a comparable DNA profile with only 20pg of input DNA, a Stop-at-Quant threshold will not be determined at this time. Data will be collected from casework samples and an assessment from that will be made at a later time.

From these results, the QuantStudio 5 and Applied Biosystems Quantifiler Trio Quantification Kit:



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- 1) Is able to detect miniscule amounts of DNA that may be usable to downstream processes
- 2) Has a limit of detection that exceeds that of downstream processes, thus is able to obtain results even in circumstances where the sample was not able to generate a DNA profile
 - a. The kit does not have a clear delineation of when a DNA profile is obtained, even at low levels, thus no stop at quant value is recommended (i.e. the lab will amplify samples that yield any result from the quant kit)
- 3) Is able to accurately reflect the amount of DNA within the sample and is capable of estimates that can be normalized effectively to generate usable DNA profiles
 - a. Samples above approximately 100pg appear to show consistently full profiles
 - b. Samples above approximately 230pg appear to show profiles with PHR greater than 80%

5. Mixture Samples

Summary:

Differential samples were prepared to test the ability to detect mixtures of male and female DNA. These samples were quantified with Quant Trio. Epithelial Fractions are expected to have a higher Autosomal:Y DNA ratio than Sperm Fractions.

Table 4. Quant detection of Human and Male DNA

Semen Dilution	Sample	Autosomal (ng/ul)	Y (ng/ul)	Ratio
Neat	D13EP	40.11	22.23	1.80
	D13SP	8.05	9.90	0.81
	D9EP	22.45	3.29	6.82
	D9SP	19.02	22.77	0.83
1:10	D14EP	10.17	3.58	2.83
	D14SP	2.12	2.83	0.74
	D10EP	19.03	0.05	352
	D10SP	0.02	0.0006	38.6
1:100	D15EP	12.50	12.38	1.00
	D15SP	0.30	0.37	0.79
	D11EP	6.85	0.08	82.63
	D11SP	0.12	0.16	0.76
1:1,000	D16EP	7.11	0.018	393
	D16SP	0.019	0.020	0.95
	D12EP	4.51	0.006	709
	D12SP	0.014	0.008	1.68

Single source male samples were evaluated to determine the High, Low, and Average Auto:Y ratio. 21 Quantifications from 3 male donors yielded a low of 0.58, a high of 1.49, and an average of 0.97 Auto:Y ratio for Quant Trio.

Table 5. Auto:Y Ratios of 21 single source male samples

Sample	Auto:Y Ratio
ND1-0.015625	0.744634
ND1-0.03125	0.856839
ND1-0.0625	0.829457
ND1-0.125	0.817931
ND1-0.25	0.880741
ND1-0.5	0.890027
ND1-1	0.684827
ND18-0.015625	1.132391
ND18-0.03125	0.58402
ND18-0.0625	0.932837
ND18-0.125	0.958395
ND18-0.25	0.935727
ND18-0.5	0.974688



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ND18-1	0.878949
ND21-0.015625	1.489225
ND21-0.03125	1.093703
ND21-0.0625	1.084073
ND21-0.125	1.414161
ND21-0.25	1.076826
ND21-0.5	1.050927
ND21-1	0.984326

Conclusion:

Quant Trio displays the ability to detect male DNA separately of total human DNA. The Autosomal:Y ratio can give an indication when a mixture of male and female DNA is present. DNA mixtures below can be visualized at quantification:

Table 6. Visualization of mixtures seen at quant

Female DNA	Male DNA	Visualized
High	High	Yes
High	Low	Yes
Low	High	No
Low	Low	Yes

Mixtures of same gender individuals or number of contributors cannot be visualized from quantification data.

From these results, the QuantStudio 5 and Applied Biosystems Quantifiler Trio Quantification Kit:

- 1) Exhibits the ability to detect Female/Male mixtures from the Auto:Y ratio
- 2) Single Source male profiles can present with some variability in the Auto:Y ratio
 - a. Auto:Y ratios above 1.5 to 1 should be considered a likely mixture
 - b. Low female contributor DNA may not be visualized in the quant data
- 3) Does not allow for detection of Male/Male or Female/Female mixture detection

6. Reproducibility and Precision

Summary:

A plate was run with 6 sets of a standard curve standards. The plate was analyzed using one set of standards to create the standard curve, treating the others as unknowns. This was repeated for all standard curve sets. The data indicates that the concentration levels are less variable in the 5ng/ul range and 0.05ng/ul range and most variable in the highest (50ng/ul) and lowest (0.005ng/ul) range. Also indicated is additional support for the use of a virtual standard curve over using a new standard curve for each plate. A consistent curve will improve the consistency of the amplification data obtained and the quantification results.

Table 7. Variation dependent on the Standard Curve used

		Standard 1 (50ng/ul)	Standard 2 (5ng/ul)	Standard 3 (0.5ng/ul)	Standard 4 (0.05ng/ul)	Standard 5 (0.005ng/ul)
Set 1	Average	45.5203323	5.045156121	0.392498687	0.04937651	0.0050876
	STDEV	6.16178834	0.439254342	0.037408549	0.00767394	0.0012237
	CV	0.135	0.087	0.095	0.155	0.241
Set 2	Average	46.6887274	5.444993615	0.433711395	0.03098282	0.00676272
	STDEV	6.41956166	0.489514836	0.040773187	0.00532429	0.00155034
	CV	13.75	8.99	9.40	17.18	22.92



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Set 3	Average	45.0612574	5.016610861	0.49253612	0.04657204	0.00610141
	STDEV	6.08928065	0.471213125	0.047834826	0.00718212	0.00135495
	CV	0.135	0.094	0.097	0.154	0.222
Set 4*	Average	67.9954796	-	0.454286136	0.0434564	0.00334969
	STDEV	3.57681678	-	0.043750516	0.00513106	0.00047565
	CV	0.053	-	0.096	0.118	0.142
Set 5	Average	62.0943022	5.218159318	0.540925816	0.04988327	0.00927397
	STDEV	9.14633	0.40080	0.01341	0.00422	0.00133
	CV	0.147	0.077	0.025	0.085	0.143

*Standard 2 in set 4 failed and was not included in results

Conclusion:

Quantifier Trio displays the ability to obtain similar results from the same sample. The variation observed allows a small window of processing differences if any stop-processing thresholds are established. A greater variation occurs due to which standard curve is used for analysis given strong support for the use of a virtual standard curve.

All data appears accurate to expected result and consistent, replicate to replicate. Only two CVs above .20 were observed (.222 & .241). The most variable samples were that of the lowest DNA concentration which is an expected outcome.

From these results, the QuantStudio 5 and Applied Biosystems Quantifier Trio Quantification Kit:

- 1) Yields consistent (precise) and reproducible quant results
- 2) Quantifies an extract that produces a reproducible DNA profile that is consistent with expected yield

7. Non-Probative Samples

Quant sample sources used:

Item	Quantification Result
Envelope Seal and Stamps (1920's -1960's)	17% 0 DNA 43% <250pg 22% 250pg-1ng 17% >1ng
Buccal Swabs	255-1851.2ng
Semen dilutions	0.0276-1,285ng male
Discarded Clothing (Found Outside)	0.85ng
Soda Can	0.00ng
Shoes	0.00ng
Jewelry	0.01-0.07ng
Hair root	5.17ng
Hair shafts (swabbed)	0.011-0.026ng
Soccer Shin Guard	0.072-0.455ng
Beer Tap Handle	0.47ng
Cigarette Butt	45.7ng
Blood	8.96-461ng
M-Vac Filter	0.07-6.20ng

From these results, we conclude that the QuantStudio 5 and Applied Biosystems Quant Trio Quantification Kit:

- 1) Provides robust quantification capabilities of extracts on a variety of different substrates



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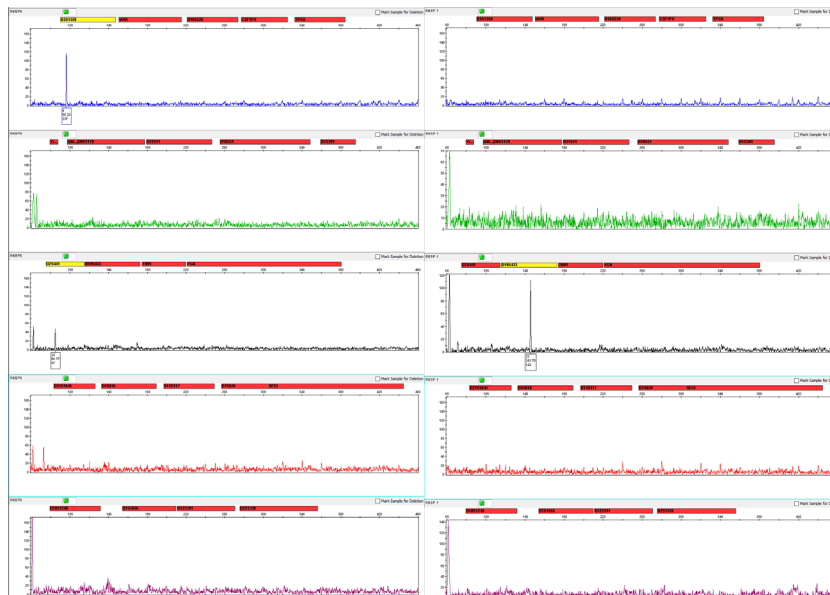
2) Confirms the ability to obtain quantification results of DNA extracts for forensically common items and biological materials

8. Zero equals Zero study

Summary:

Low level quantification samples were compared with their associated amplification e-grams to determine at which input amount consistently results in no DNA peaks calling after amplification with Globalfiler and Investigator 24Plex.

Sample ID	QT (Total ng input)	GF (Called Peaks)	INV (Called Peaks)
E200618DC-END-RB1	0	0	0
D1 EPRB	0	0	0
D1 SPRB	0	0	0
ND1-0.015625	0.002	2	0
ND1-0.03125	0.0046	10	4
ND21-0.015625	0.0049	0	2
RBEP 6	0.006	3	0
RBSP 1	0.006825	1	2
ND18-0.015625	0.016	10	8
ND12-0.015625	0.0116	14	18
ND21-0.03125	0.0125	2	8
ND11-0.015625	0.0398	10	37





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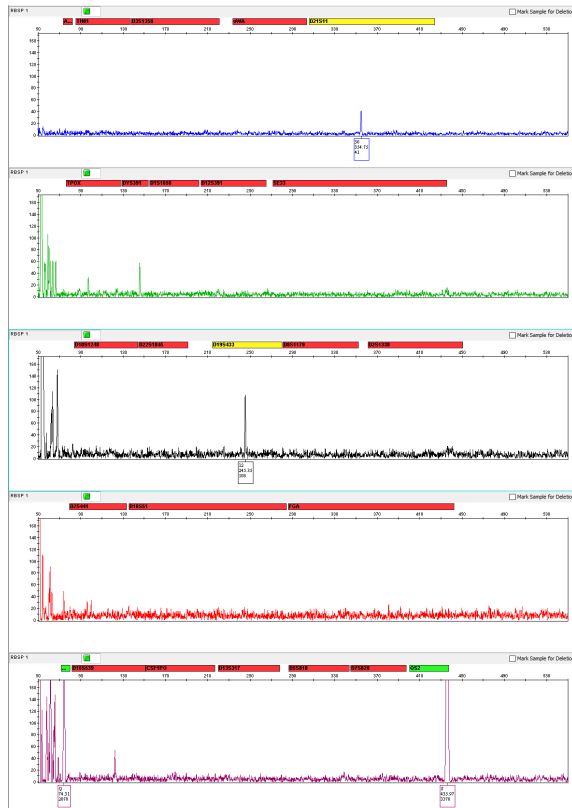
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Conclusion:

No DNA peaks were consistently observed below an input amount of 0.002ng. Subset of zero quant RB's that quanted zero were amplified with no peaks present.

From these results, we conclude that the QuantStudio 5 and Applied Biosystems Quantifiler Trio Quantification Kit:

- 1) Is unlikely to get a usable DNA profile from results below .002ng/ul or less
- 2) Was unable to obtain any peaks or usable DNA profiles from No Result or Zero quants and sporadic peaks from samples below .006ng of total input DNA
 - a. As a result of this and out of an abundance of caution, the laboratory will stop processing (Stop at Quant, SAQ) only when samples yield a No Result or Zero quant

9. Validation Personnel

(upon successful completion of a competency test, integral individuals are deemed to have completed training in the below listed components)

The following individuals were performed the integral to the validation and have met all training requirements as a result of this validation:

Alyssa McElreath
Derek Cutler
Daniel Hellwig

10. Validation Components

Quant Studio 5 (SN: 272523070)
Applied Biosystems Quantifiler Trio Quantification Kit