Internal Validation of the Qiagen® QIAgility™ Liquid Handling Robot

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Abstract

In a field where accuracy, precision and throughput are of the utmost importance, automated instrumentation is being employed more heavily. The use of automation eliminates human error and enables the scientist to spend his or her time elsewhere. The Qiagen® QIAgility™ is a multifunctional instrument that performs a variety of pipetting tasks that are typically performed manually by the scientist. The instrument has the capabilities to complete three operations vital to the DNA workflow in the Marshall University Forensic Science Center (MUFSC) DNA Laboratory: quantitation and amplification setup, as well as normalization of sample extracts. An internal validation of the robot was completed at MUFSC in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) validation guidelines. The validation included five studies: accuracy, contamination, quantitation comparison, sensitivity, and normalization and amplification comparison. Based on the results, the QIAgility™ is capable of producing adequate and similar results to that of traditional pipetting methods.

Introduction

Implementation of instruments capable of automation in forensic deoxyribonucleic acid (DNA) workflow processes is becoming more prevalent. Automating forensic DNA processes allows a laboratory to be more efficient and increase sample throughput, ultimately leading to a decrease in backlogs. Within each process of the DNA workflow, there are a variety of tasks that involve transfer of precise liquid volumes. The Qiagen® QIAgility™ is a liquid handling instrument capable of performing quantitation, amplification and capillary electrophoresis setup, as well as normalization of sample extracts. Before new instrumentation is implemented into a forensic laboratory, an internal validation is conducted. The Scientific Working Group of DNA Analysis Methods (SWGDAM) describes a validation as "a process by which a procedure is evaluated to determine its efficiency and reliability for forensic casework and/or database analysis." An internal validation was performed at the Marshall University Forensic Science Center (MUFSC) DNA Laboratory to determine reliability, reproducibility and accuracy of the QIAgility™.

Methods & Materials

Instrumentation and Chemistries
Qiagen® EZ1 Advanced XL & DNA Investigator Kit
Qiagen® QIAgility™
Qiagen® Investigator® QuantiPlex HYres Kit
Applied Biosystems™ 7500 Real-Time PCR Instrument
Promega® PowerPlex® 16 System Amplification Kit
Applied Biosystems™ 9700 Thermal Cycler
Applied Biosystems™ 3130*xl* Genetic Analyzer
Applied Biosystems™ Genemapper™ ID software v3.2.1

Accuracy Study
Using 200 µl and 50 µl liquid-level sensing tips, the QIAgility™ was programmed to pipette maximum, half-maximum and a small volume into 52 or 96 wells of a 96-well plate.

Contamination Study
A checkerboard plate was designed with alternating wells of extracted blood samples and negative wells of TE^{-4}.

Quantitation Comparison Study
Study 1: Four columns of Control DNA Z1 standards and three replicates of Standard Reference Material (SRM) components A, B and C were set up manually and by the QIAgility™ on the same 96-well plate. Standards were analyzed in pairs and comparisons were made between R^2 and slope values.
Study 2: Two columns of Control DNA Z1 standards and three replicates of three previously characterized samples were set up by the QIAgility™ and manually on the same 96-well plate. Standards were analyzed in pairs and comparisons were made between R^2 and slope values.

Sensitivity Study
Table 1. A 3X serial dilution of the sample, Q4E, was set up manually. The QIAgility™ added three replicates, followed by a manual addition of three replicates to a 96-well plate for a comparison of average concentrations.

Well	Concentration (ng/µl)	Extract	Diluent (µl)
1	31.200	Neat Extract	0
2	10.400	10µl of Extract	20
3	3.467	10µl of 2	20
4	1.156	10µl of 3	20
5	0.385	10µl of 4	20
6	0.128	10µl of 5	20
7	0.043	10µl of 6	20
8	0.014	10µl of 7	20

Normalization and Amplification Comparison Study
Table 2. Four samples' quantitation values and their desired concentration after normalization. Samples were prepared in triplicate.

Sample Name	Human Initial Conc. (ng/µl)	Male Initial Conc. (ng/µl)	Desired Final Human Conc. (ng/µl)
Q3E	1.660	0.149	0.5
Q1S	6.660	6.040	0.5
Q5E	15.780	2.010	0.5
Q2E	31.200	0.226	0.5

Results & Discussion

Accuracy Study
Overall, the QIAgility™ appeared to lose accuracy throughout the course of each run. This tended to be more prevalent when pipetting greater volumes. Initially, this was very alarming, however, after a closer look at downstream effects on final concentrations, it appeared the variation in pipetting was minimal (Table 3).

Table 3. Downstream effects of loss in accuracy by the QIAgility™.

QIAgility™ Pipettes (µl)	Initial [DNA]	DNA added (µl)	Final Volume (µl)	Final [DNA]	[DNA] to Amp
200	50.5	2	202	0.5	1
193	50.5	2	195	0.518	1.036
100	25.5	2	102	0.5	1
98.8	25.5	2	100.8	0.506	1.012
10	3	2	12	0.5	1
9.2	3	2	11.2	0.536	1.072
50	13	2	52	0.5	1
48.6	13	2	50.6	0.514	1.028
25	6.75	2	27	0.5	1
23	6.75	2	25	0.54	1.08
5	1.75	2	7	0.5	1
4.7	1.75	2	6.7	0.522	1.044

Contamination Study
The QIAgility™ instrument showed no contamination at all loci in each of the 38 blank wells. The four blood samples used in the remaining wells presented complete profiles.

Quantitation Comparison Study
Study 1: After quantifying samples using the Qiagen® Investigator® QuantiPlex HYres kit, Control DNA Z1 standards produced comparable human and male R^2 and slope results between methods. SRM component (A, B and C) concentrations showed significant differences between methods. Questions arose about SRM component sample volumes being inadequate for accurate pipetting by the QIAgility™.

Table 4. Comparison of Control DNA Z1 standards between methods.

System	Statistic	R^2 (Human)	Slope (Human)	R^2 (Male)	Slope (Male)
QIAgility™	Average	0.9984	-3.0845	0.9983	-3.3402
	Std. Dev	0.0004	0.0153	0.0008	0.0181
Manual	Average	0.9959	-3.1911	0.9962	-3.2131
	Std. Dev	0.0029	0.0563	0.0030	0.0484

Table 5. Comparison of SRM component concentrations between methods.

System	Statistic	Human [A] (ng/µl)	Human [B] (ng/µl)	Human [C] (ng/µl)	Male [A] (ng/µl)	Male [B] (ng/µl)	Male [C] (ng/µl)
QIAgility™	Average	*31.26*	*39.25*	*41.91*	*36.85*	*0.00*	*35.56*
	St. Dev	*18.64*	*23.33*	*24.89*	*22.02*	*0.00*	*21.26*
Manual	Average	*23.92*	*30.05*	*32.09*	*27.81*	*0.00*	*26.84*
	St. Dev	*16.71*	*20.96*	*22.37*	*19.10*	*0.00*	*18.44*

Study 2: After quantifying samples using the Qiagen® Investigator® QuantiPlex HYres, the two methods produced similar results. Although quantitation values vary greatly from the original values, percent differences show that the two methods are comparable to one another.

Table 6. Comparison of three previously characterized samples between methods.

QIAgility™				
Sample	**Original Quant. (ng/µl)**	**Average (ng/µl)**	**St. Dev.**	**Percent Difference**
Q1E	18.50	27.14	1.1459	38%
Q2E	17.50	24.47	0.9272	33%
Q5E	16.36	24.88	0.9732	41%
Manual				
Sample	**Original Quant. (ng/µl)**	**Average (ng/µl)**	**St. Dev.**	**Percent Difference**
Q1E	18.5	27.33	0.8890	39%
Q2E	17.5	22.88	0.3963	27%
Q5E	16.36	25.62	0.6773	44%

Sensitivity Study
After quantifying samples of the serial dilution in replicates using the Qiagen® Investigator® QuantiPlex HYres kit, the two methods produced very similar results.

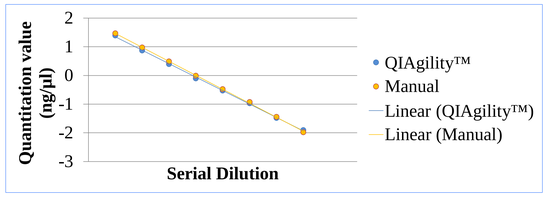

Figure 1. Linear Regression of QIAgility™ vs. Manual Quantitation Values for Q4E Serial Dilution.

Normalization and Amplification Comparison Study
After normalization and amplification using the PowerPlex® 16 Kit, QIAgility™ prepared samples produced slightly higher relative fluorescence units (RFU). However, all profiles of QIAgility™ prepared samples contained sharp, well-defined peaks.

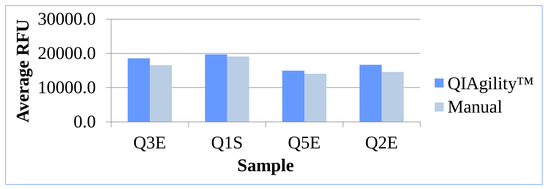

Figure 2. Total average RFU for each set of sample replicates prepared by the two methods.

Conclusion

This validation demonstrated that the QIAgilty™ produced accurate and reliable results, similar to that of traditional pipetting methods at MUFSC DNA Laboratory.

1. Although some loss in accuracy was observed, the downstream effects on final concentrations were not significant.
2. No cross-contamination was present.
3. Both Control DNA Z1 standards and samples produced similar results between methods.
4. The QIAgilty™ can accurately prepare samples with both high and low concentrations.
5. Complete profiles with sharp, well-defined peaks were present after normalization and amplification preparation.

Future work includes an automated system to measure QIAgility™ pipetted volumes in the accuracy study, and a longevity and stability study for a QIAgility™ prepared set of Control DNA Z1 standards. Furthermore, additional studies could include the implementation of newly validated kits.

References

Butler JM. Advanced topics in DNA typing: Methodology. Amsterdam: Academic/Elsevier. 2011. Print.
Kline MC, Duewer DL, Travis JC, Smith MV, Redman JW, Vallone PM, Decker AE, Butler JM. Production and certification of NIST Standard Reference Material 2372 Human DNA Quantitation Standard. Anal Bioanal Chem 2009;394: 1183-1192.
Marshall University Forensic Science Center DNA Laboratory. Database/reference sample interpretation guidelines. Revised November 12, 2013. Analytical Procedures Manual. Huntington, WV.
Marshall University Forensic Science Center DNA Laboratory. PCR amplification using Promega® PowerPlex16® kits. Revised March 14, 2014. Analytical Procedures Manual. Huntington, WV.
Marshall University Forensic Science Center DNA Laboratory. Quantitation of DNA extracts using the Qiagen® Investigator® Quantiplex HYres kit. Revised June 3, 2014. Analytical Procedures Manual. Huntington, WV.
Myers JR. Validation of a DNA quantitation method on the Biomek® 3000. Journal of Forensic Sciences 2010;55.6: 1570-575.
Pasquale FD, Cornelius S, Konig M, Scherer M, Schmid C, Dienemann C, Bochmann L, Prochnow A, Schnibbe T, Engel H. Investigator® Quantiplex Kit: For reliable quantification of human DNA in forensic samples. Forensic Sci. Int. Genet. Supplement Series 2011;3: 413-414.
Qiagen®. Investigator® Quantiplex HYres Handbook. 2014.
Qiagen®. QIAgility® User Manual. 2013.
Scientific Working Group on DNA Analysis Methods. Validation guidelines for DNA analysis methods. Revised December, 2012.
Stangegaard M, Meijer P, Borsting C, Hansen A, Morling N. Biomek® 3000: The workhorse in an automated accredited forensic genetic laboratory. Journal of Laboratory Automation 2012;17.5: 378-86.

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