Internal Validation of the Qiagen® QIAgility® Robotic Liquid Handler

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Abstract

The Qiagen® QIAgility® is an automated liquid handler designed for real-time PCR setup and can be adapted for any step that requires liquid to be transferred. The robot is intended to provide a means to increase casework efficiency, reduce pipetting errors, and improve the quality of DNA standards used in quantitation. An internal validation was performed at the San Mateo County Sheriff's Office Forensic Laboratory (SMCSOFL) according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) validation guidelines. The validation study included studies of reproducibility, contamination, comparison to manual methods, and samples with known profiles. Based on the results, QIAgility® is capable of accurately making DNA standards for quantitation, showed no signs of contamination, performed similar to the validated manual methods already in use, and produced complete DNA profiles for each of the samples with known profiles.

Introduction

The increase in the workload of many forensic laboratories has caused a shift from manual methods towards automation. The Qiagen® QIAgility® is an automated liquid handler designed for real-time PCR setup and can be adapted for any step that requires liquid to be transferred. Based on the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines, the validation study included the following studies: reproducibility, contamination, comparison to manual methods, and samples with known profiles. Automated protocols were designed on the QIAgility® software for the Applied Biosystems® Quantifiler® Human and Quantifiler® Duo DNA Quantification kits, normalization, and amplification and capillary electrophoresis setup using the Applied Biosystems® AmpFℓSTR® Identifiler® kit.

Materials and Methods

Kits and Instrumentation:

Qiagen® QIAgility®
Qiagen® EZ1 Advanced or EZ1 Advanced XL
Applied Biosystems® Quantifiler® Human and Quantifiler® Duo DNA Quantification kits
Applied Biosystems® 7500 real-time PCR instrument
Applied Biosystems® 9700 thermal cycler
Applied Biosystems® AmpFℓSTR® Identifiler® kit
Applied Biosystems® 3130 Genetic Analyzer
Applied Biosystems® GeneMapper® ID software v3.2.1

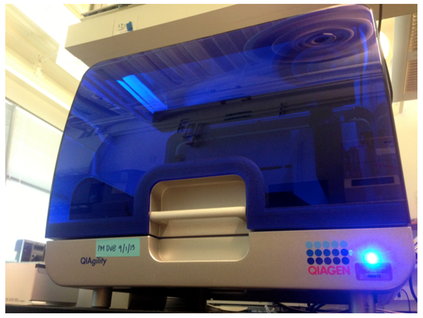

Figure 1: The Qiagen® QIAgility®

Reproducibility Study

Table 1: Expected Concentrations of the Serial Dilutions of the Quantifiler® Duo DNA Standard

Standard	Concentration (ng/µL)
Std 1	50.00
Std 2	16.70
Std 3	5.560
Std 4	1.850
Std 5	0.620
Std 6	0.210
Std 7	0.068
Std 8	0.023

Contamination Study

A checkerboard plate was designed that alternated wells of extracted samples and blank wells of TE^{-4}

Known Profiles

Seventeen samples with known profiles were used to ensure that complete profiles were produced when using the QIAgility®

Comparison Study

Five samples were used for comparison between the instrument and manual techniques for quantitation setup, normalization, amplification setup, and capillary electrophoresis setup being employed by the analysts

Mixture Study

Ten mixtures were created using three female quality control samples

Table 2: Ratios of the two person and three person mixtures used for the study

Sample Name	Ratio
Mx1	1:1
Mx2	3:1
Mx3	5:1
Mx4	1:1:1
Mx5	1:5:1
Mx6	5:1:1
Mx7	5:5:1
Mx8	1:3:1
Mx9	3:1:1
Mx10	3:3:1

Results

Reproducibility Study

The first and second dilutions of the Quantifiler® Duo DNA standard dilution series exhibited the greatest amount of variation from the expected values of 50 ng/µL and 16.67 ng/µL respectively. The first dilution of each of the three series of samples showed the greatest amount of variability between each replicate.

Table 3: Quantifiler® Duo standard quantitation results

Dilution #	Concentration (ng/µL)	Concentration (ng/µL)	Concentration (ng/µL)	Mean	Standard Deviation
1	53.25	56.37	57.8	54.84	2.703
2	14.54	14.02	14.39	14.56	0.5335
3	5.58	5.64	5.65	5.65	0.06164
4	1.85	1.67	2.03	1.877	0.1569
5	0.615	0.6	0.693	0.604	0.07588
6	0.187	0.178	0.296	0.2172	0.05398
7	0.0675	0.0666	0.0588	0.06175	0.006424
8	0.0173	0.0167	0.0239	0.02265	0.007452

Contamination Study

The results of the checkerboard-patterned quantitation plate which was evaluating the potential for contamination showed that 38 out of the 38 blank wells yielded a quantitation value of 0.00 ng/µL for both total DNA and male DNA.

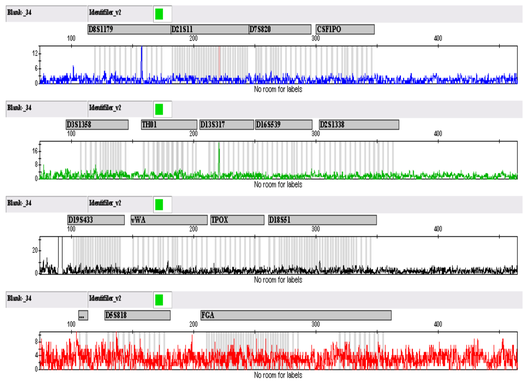

Figure 2: Blank 34 that showed minimal potential contamination at loci D8S1179 and D13S317 below the 50 RFU threshold

Known Profiles

Full profiles of each of the known samples were obtained after capillary electrophoresis and analysis by GeneMapper® ID regardless of the sample type.

Table 4: The expected concentrations of the serial dilution of sample "WL" made by the Qiagen® QIAgility®

Sample Name	Expected Concentration (ng/µL)	Actual Concentration (ng/µL)
WL 1- liquid blood	4.89	4.78
WL 2	1.63	1.76
WL 3	0.543	0.521
WL 4	0.181	0.19
WL 5	0.0604	0.0783
WL 6	0.0201	0.0247
WL 7	0.0067	0.0138
WL 8	0.00224	0.0033

Comparison Study

After the amplification with Identifiler® and analysis of the data from the 3130 Genetic Analyzer with GeneMapper® ID, it was determined that the manual quantitation underestimated the amount of DNA in each sample which led to overamplification.

Table 5: Quantitation results for the samples using the QIAgility® and manual methods

Sample Name	QIAgility® Concentration (ng/µL)	Manual Concentration (ng/µL)
QC134 AB	1.97	1.06
QC135 SG	3.17	2.51
QC136 KM	3.13	1.61
QC137 HV	0.789	0.398
QC138 SW	0.363	0.219

Mixture Study

Due to a malfunction with the Applied Biosystems® 9700 thermal cycler, the mixture study was unable to be completed with accurate results.

Workflow

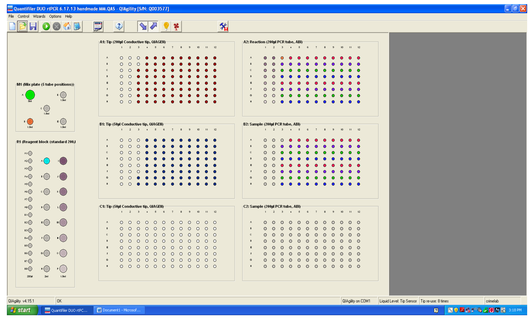

Figure 3: Example protocol in the QIAgility® software for the Quantifiler® Human DNA Quantitation kit

Conclusions

This validation demonstrated that the QIAgility® produces accurate and reliable results comparable to the manual processes in place at the San Mateo County Sheriff's Office Forensic Laboratory.

- The instrument is capable of creating accurate and reproducible DNA standards for quantitation
- No cross-contamination was demonstrated
- Comparable to or better than the manual processes for quantitation setup, normalization, amplification setup, and capillary electrophoresis setup currently being used by the laboratory
- Full profiles of each of the known samples were obtained, which indicated that the QIAgility® could be used with samples having a wide range of concentrations, and a full profile will still be produced.

Future studies involving the QIAgility® would include creating new protocols for any newly validated kits being implemented into the laboratory workflow.

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