An 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 reduces the rate of human error and permits the scientist to spend his or her time elsewhere in the laboratory. The Qiagen® QIAgility™ is a liquid handler that performs a variety of pipetting tasks that are typically performed manually by the scientist. The instrument will be employed 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 normalization of sample extracts. During quantitation setup, the QIAgility™ is responsible for generating a serially diluted set of standards and adding those standards, master mix and samples to each well according to the software program prepared by the scientist. When programmed to perform amplification setup, the QIAgility™ is tasked with adding master mix, samples and TE^{-4} Buffer to a 96-well plate. To normalize samples, the sample extract concentrations are imported into the software. Target concentrations and final volumes are selected by the user. The QIAgility™ software determines the volume needed of sample extract and diluent to reach the final concentration and volume selected by the user. The liquid handler then adds the calculated volume of sample extract and diluent into an empty tube or well.

At the MUFSC DNA Laboratory, an internal validation of the Qiagen® QIAgility™ liquid handling robot was performed. The QIAgility™ was assigned to

perform various setups on a 96-well plate with sample extracts that had been previously quantified and produced full STR profiles. After completion, the same setups were performed manually. For quantitation, standards and samples were amplified using the Qiagen® Investigator® Quantiplex HYres kit on the Applied Biosystems® 7500 Real-Time PCR System and data was analyzed using ABI PRISM® 7500 Sequence Detection Software v1.2.3. Amplification was accomplished using Promega® PowerPlex® 16 on an Applied Biosystems® GeneAmp® PCR System 9700. Capillary electrophoresis was performed on the Applied Biosystems® 3130xL Genetic Analyzer and analysis was completed using Applied Biosystems® GeneMapper® ID v3.2.1.

The QIAgility™ was tested first for precision and accuracy over the course of a 96-well run, followed by a checkerboard contamination check. A quantitation comparison study included statistical comparisons between Control DNA Z1 quantitation standards prepared by the QIAgility™ and manual method. These comparisons were made based on the standard curves generated by the SDS software and direct analysis of the R-squared and slope values. A quantitation sensitivity study established comparisons between extract quantitation values of the two methods, including a serially diluted sample extract that had been previously quantified at approximately 30 ng/µl. A normalization and amplification comparison study determined if the QIAgility™ could produce quality STR profiles, comparable to those produced by traditional methods. Samples that had been recently quantified were normalized by both the QIAgility™ and manually, and then each was set up for amplification. STR profiles were compared by number of allele calls and average peak height per dye channel.

Future studies include automating QIAgilty™ pipetting measurements for better accuracy and elimination of human error. Secondly, a future study could include analysis of QIAgility™ prepared standards using Sequence Detection Software over the course of its recommended lifespan of one week. QIAgility™ prepared standards tended to produce standard curves with slopes on the lower end of acceptability. It would be essential to test the bounds of the lifespan of the standards. Finally, studies and cost analysis should be performed regarding the usage of the extra master mix per QIAgility™ run for quantitation and amplification setup. According to the manufacturer's recommendations, a much larger consumption of master mix is needed for QIAgility™ prepared quantitation and amplification setup than MUFSC DNA Laboratory's traditional methods. This study would be necessary for the laboratory to decide if the cost for overage is worth the benefits of the instrument.

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 [6]. Furthermore, robotics have the ability to reduce the number of human errors, including sample misplacement and improper pipetting techniques [12]. Some instrumentation has been developed to be specific to a single function, such as the Qiagen® EZ1 Advanced XL (Qiagen®, Hilden, Germany) and the Applied Biosystems® GeneAmp® PCR System 9700 (Life Technologies™, Foster City, CA). This allowed for easy assimilation into the DNA workflow, permitting DNA analysts to become familiar with

instruments and instrument protocols. However, single function instrumentation is also limited due to space constraints in the laboratory. Implementation of multifunctional instrumentation, such as the Qiagen® QIAgility™ (Qiagen®, Hilden, Germany), allows for more flexibility and a greater deal of automation in the DNA workflow.

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. The QIAgility™ employs a single-channel pipette to setup as many as 96 reactions in as little as 30 minutes. This instrument is versatile and can support a variety of plate and tube formats, allowing it to work in tandem with a variety of other laboratory instrumentation, such as the Qiagen® EZ1. The QIAgility™ also contains a High-Efficiency Particle Air (HEPA) filter for removal of airborne biohazards and a UV lamp for decontamination after a run [7].

Before new instrumentation is implemented into a forensic laboratory workflow, an internal validation must be 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 efficacy and reliability for forensic casework and/or database analysis” [11]. It is of the utmost importance to verify that procedures a laboratory enacts will be effective and supported with evidence prior to implementation. SWGDAM provides a set of guidelines that laboratories can follow during the validation process to ensure all proper testing is performed. It is each laboratory’s discretion to determine

which validation studies listed in the SWGDAM guidelines are appropriate and relevant to the methodology in use, along with the number of samples that should be tested [11].

An internal validation was performed at the Marshall University Forensic Science Center (MUFSC) DNA Laboratory to determine reliability, reproducibility and accuracy of the QIAgility™. The laboratory obtained one QIAgility™ instrument for the purpose of pre-amplification procedures: quantitation and amplification setup, as well as normalization of sample extracts. The validation was made up of five studies, including an accuracy and precision, contamination, quantitation comparison, sensitivity, and amplification and normalization comparison study. All samples for the validation studies, with the exception of SRM 2372 components A, B and C, were previously quantified and profiled.

The QIAgility™ was responsible for moving a finite amount of liquid from one location to another. The instrument's ability to pipette accurately and precisely is vital to its function. An accuracy study was performed to verify that the QIAgility™ could perform these functions.

A laboratory should confirm that implementation of new methods and instrumentation does not compromise the integrity of samples [10]. The instrument works with samples and reagents in close proximity to one another and movement of liquid from source wells to destination wells could introduce contamination. Furthermore, samples and reagents are located on the worktable and uncapped throughout a run. It is of the utmost importance to ensure there is no cross-contamination of any kind during a run. A contamination study was performed to ensure there was no introduction of cross-contamination during QIAgility™ pipetting tasks.

Quantitation is an important step in the DNA workflow to determine what needs to be done to a sample to reach its amplification target concentration. Accurately quantifying a sample is essential for a clear short tandem repeat (STR) profile to be obtained [7]. Samples were quantified using the Qiagen® Investigator® Quantiplex HYres quantitation kit (Qiagen®, Hilden, Germany), the Applied Biosystems® 7500 Real-Time PCR System (Life Technologies™, Foster City, CA) and the ABI PRISM® 7500 Sequence Detection Software (SDS) v1.2.3 (Life Technologies™, Foster City, CA). Control DNA Z1 from the HYres quantitation kit was used to create a 1:4 serial dilution [9]. Under the manual method, the analyst would use traditional pipetting methods to generate the Control DNA Z1 serial dilution. The concentrations of the dilution are known and used to generate a standard curve under real-time polymerase chain reaction (PCR). The standard curve is used to match a sample's real-time amplification behavior with its corresponding concentration. The QIAgility™ has the capabilities to prepare serially diluted standards. Generation of a consistent and accurate standard curve is important, and improperly prepared standards could have downstream effects on the DNA workflow. If incorrect quantitation results of samples are obtained, the actual concentrations may be over- or under-estimated and lead to poor profiling [1]. A quantitation comparison study was performed to identify if QIAgility™ prepared standards and samples yielded similar results to that of traditional methods. Secondly, it is important that both low and high concentrated DNA samples are quantified accurately. A quantitation sensitivity study was performed to verify that both high and low template DNA samples quantified accurately and comparably to that of traditional pipetting.

Lastly, a normalization and amplification comparison study was performed to ensure that quality short tandem repeat (STR) profiles were produced, comparable to those produced by manual methods. Normalization of samples was performed in Tris EDTA (TE^{-4}) buffer. Normalization is defined as "the process of achieving a DNA concentration that fits the optimal window for analysis" [1]. Normalization is an important step in the DNA workflow, allowing analysts to dilute samples based quantitation results to reach a target DNA concentration, in attempts to obtain the best STR profile possible. Amplifying too high of a DNA concentration can result in unreadable STR profiles or profiles populated with artifacts that could interfere with analysis [1]. Amplification was performed using the Promega® PowerPlex® 16 System amplification kit (Promega®, Madison, WI) with AmpliTaq Gold® DNA Polymerase (Life Technologies™, Foster City, CA) on a Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler. Amplification is a crucial step in the DNA workflow, as it amplifies specific sequences, known as loci, which generate a unique STR profile [1]. It is important that the QIAgility™ has the ability to pipette accurate amounts of samples and reagents for proper amplification to occur.

Methods and Materials

The Qiagen® QIAgility™ liquid handling robot and its software were installed and calibrated in accordance with the manufacturer's instructions prior to use, including position and height, tip offset and volume calibrations [10]. For each plate and sample tube type that was employed on the worktable, a manual plate position calibration and automatic plate height calibration was performed. This was completed to ensure proper

pipetting by the instrument and to reduce the occurrences of insufficient volume liquid errors.

DNA Extraction

All DNA samples chosen for the QIAgility™ validation studies had been previously extracted, quantified, amplified, and yielded full STR profiles when analyzed by the laboratory. DNA samples varied in origin, some being whole blood samples while others were obtained from a previously conducted proficiency test. Whole blood samples were extracted on the Qiagen® EZ1 Advanced XL. Previous proficiency test samples were differentially separated using the Qiagen® QIAcube® (Qiagen®, Hilden, Germany) and extracted on the Qiagen® EZ1 Advanced XL. All extracts were eluted in TE^{-4} buffer. National Institute of Standards and Technology (NIST) traceable Standard Reference Material (SRM) 2372 components A, B and C were used as samples in the quantitation comparison study. As described by NIST, component A is derived from a single human male donor, component B from multiple human female donors and component C from multiple human male and female donors. Each is solubilized in 10 mmol/L Tris HCl and 0.1 mmol/L disodium EDTA that has been added to deionized water and adjusted to pH 8.0 [2].

DNA Quantitation and Analysis

The Qiagen® Investigator® Quantiplex HYres Quantification kit and Applied Biosystems® 7500 Real-Time PCR System were used in the quantitation comparison and sensitivity studies, as well as in the normalization and amplification comparison

study. ABI PRISM® 7500 SDS v1.2.3 was used to analyze data from the 7500 PCR System.

A quantitation setup protocol for the QIAgility™ was designed for Quantiplex HYres. Master mix was prepared manually into a 2 ml screw top tube, in accordance with the manufacturer's recommendations [9]. After preparation, master mix was placed in well H of the reagent block on the worktable prior to quantitation setup. The QIAgility™ was programmed to generate serially diluted standards into seven wells of an eight-well strip tube, in accordance with manufacturer's recommendations [9]. 30 µl Control DNA Z1 of the Quantiplex HYres quantitation kit was manually added to the first well of the eight-well strip tube and placed in reagent block wells A1 through A8 on the QIAgility™ worktable. The instrument generated a 1:4 serial dilution using Control DNA Z1 and diluent, QuantiTect Nucleic Acid Dilution Buffer from the Quantiplex HYres quantitation kit. Dilution buffer was manually added to a 1.5 ml round-bottom screw-cap tube and placed on the QIAgility™ worktable at mix plate well D. A 96-well half-skirted reaction plate was manually positioned onto block C1 of the worktable and the QIAgility™ was programmed to add 18 µl master mix and 2 µl of standard, sample, or TE^{-4} buffer, depending on the reaction composition (Figure 1). After completion, the 96-well reaction plate was manually sealed with optical film, amplified using the 7500 Real-Time PCR System and analyzed with SDS software.

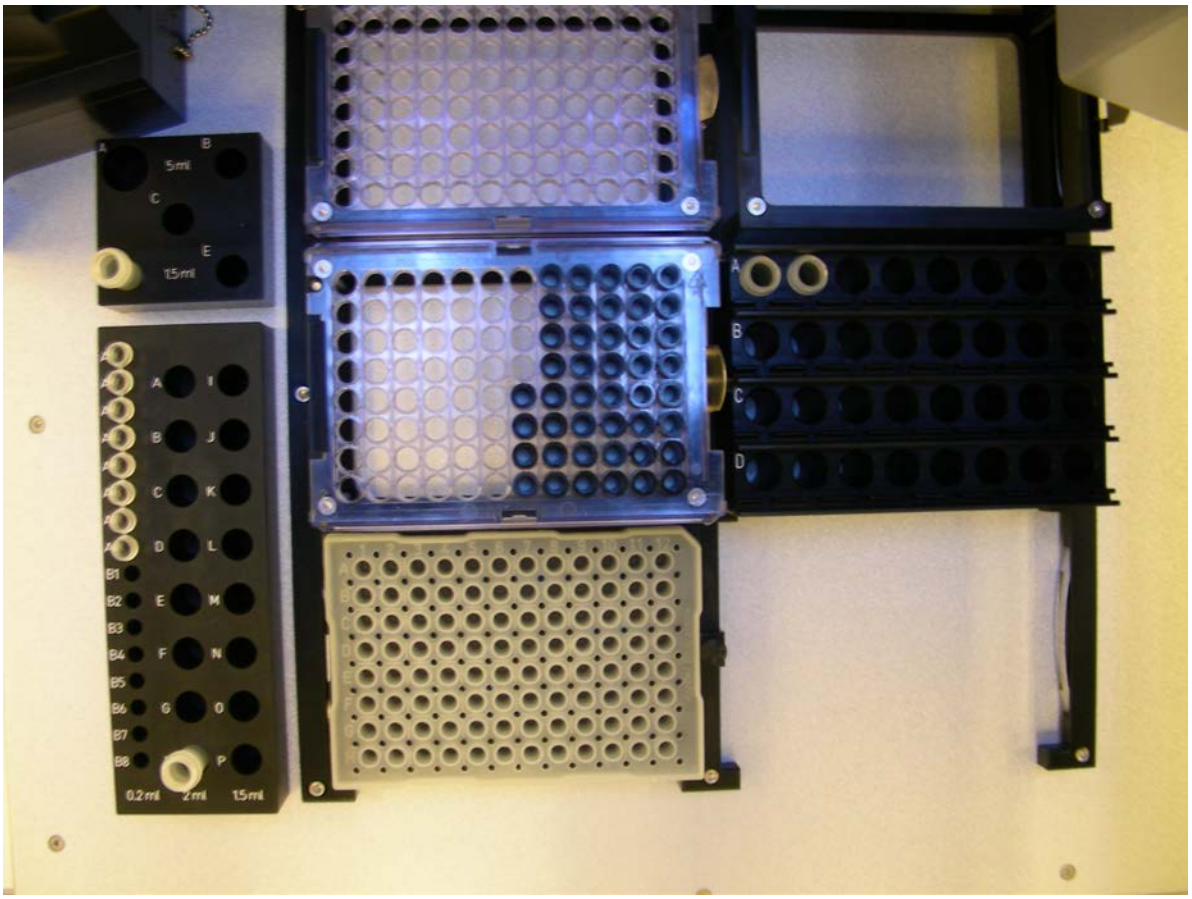

Figure 1. QIAgility™ Worktable Setup for Quantiplex HYres Quantitation Kit.

DNA Amplification

All validation samples that required STR profiling were amplified with the Promega® PowerPlex® 16 System DNA typing kit on the Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler. Samples were amplified in a 96-well half-skirted plate for thirty-two cycles, according to the MUFSC DNA Laboratory PowerPlex® 16 Amplification protocol [4]. All DNA sample templates were targeted for amplification at a concentration of 1.0 ng. Target concentrations were determined based on quantitation results. For quantitation results greater than 1.0 ng, samples were

normalized in TE^{-4} buffer to 0.5 ng/µl and 2 µl of normalized sample and added to reach an amplification target of 1.0 ng.

Capillary Electrophoresis and STR Profiling

After amplification, samples were loaded manually into a 96-well half skirted reaction plate and run on an Applied Biosystems® 3130xL Genetic Analyzer. Samples were analyzed using Applied Biosystems® GeneMapper® ID software v3.2.1. Analysis was completed with an analytical threshold at 100 relative fluorescent units (RFU) for samples and 50 RFU for negative controls, in correspondence to MUFSC guidelines. A stochastic threshold of 200 RFU was also used for analysis [3]. The analytical threshold was reduced to 30 RFU when analyzing blank wells to ensure no low level contamination was present.

Validation Studies

An **accuracy study** was performed to determine accuracy and precision of the QIAgility™ robot over the course of pipetting into 52 or 96 wells. The QIAgility™ was programmed to pipette 200 µl of TE^{-4} into 52 wells on a 96-well plate using 200 µl liquid-level sensing pipette tips. Due to a noticeable loss in accuracy and precision over the course of the initial 200 µl accuracy test, a second 52-well pipetting run of 200 µl per well was performed in reverse order.

Since 200 µl of TE^{-4} was needed per well over 52 wells, a large volume, 10.4 ml, of TE^{-4} needed to be available on the QIAgility™ worktable. The mix plate had the ability to hold five tubes, one 5 ml and four 1.5 ml tubes. Therefore, all five mix plate wells

were utilized to hold TE^{-4}. During the second run, the QIAgility™ was programmed to take TE^{-4} from mix plate tubes in reverse order as the previous run. The QIAgility™ was programmed to pipette 100 µl and 10 µl of TE^{-4} into 52 wells on a 96-well plate using 200 µl liquid-level sensing pipette tips. After completion of QIAgility™ runs, volumes in plate wells were measured via a single-channel manual pipette and recorded to measure the accuracy and precision of the instrument. All single-channel pipettes used in this studied were calibrated prior to use.

Using 50 µl liquid-level sensing pipette tips, the QIAgility™ was programmed to pipette 50 µl of TE^{-4} into 52 wells on a 96-well half-skirted PCR plate. The QIAgility™ was also programmed to pipette 25 µl and 5 µl of TE^{-4} into 96 wells on a 96-well half-skirted PCR plate. After each run, volumes were measured manually.

For each QIAgility™ run, manual measurements were compiled. Averages were determined for comparison to the expected value. Standard deviations were calculated to evaluate precision. Data was represented graphically to observe trends in QIAgility™ pipetting volumes over the course of a run.

A **contamination study** was performed using a checkerboard pattern on a 96-well half-skirted PCR reaction plate. The checkerboard pattern consisted of alternating blank wells and sample extracts. TE^{-4} was in the blank wells and four different extracted single-source blood samples were in the remaining wells (Table 1, Figure 2). No TE^{-4} well was surrounded by two of the same blood extracts; this was purposely constructed to identify the source well of contamination, if it were to occur. Whole blood samples were extracted on the EZ1® Advanced XL using the trace protocol and eluted into 40 µl

TE^{-4}. The QIAgility™ was programmed to setup up a 96-well plate, pipetting TE^{-4} wells first, followed by blood sample extracts into remaining wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}
B	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB
C	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}
D	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM
E	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}
F	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT
G	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}
H	TE^{-4}	CB	TE^{-4}	MK	TE^{-4}	JT	TE^{-4}	BM	TE^{-4}	CB	TE^{-4}	MK

Figure 2. Checkerboard Setup for the Contamination Study.

Table 1. Extracted Blood Sample Names and Concentration

Sample	Concentration (ng/µl)
MK	1.59
CB	2.07
BM	2.53
JT	1.24

The QIAgility™ was programmed to prepare an amplification setup of all TE^{-4} wells and the first four whole blood extracts in a 96-well half-skirted PCR reaction plate with the Promega® PowerPlex® 16 System amplification kit. PowerPlex® 16 master mix was prepared manually in a 2.0 ml flat-bottom tube according to the PowerPlex® 16 protocol [4], and placed on the worktable reagent block at well H. A one to ten dilution was prepared manually of 10 ng/µl 2800M Control DNA, the PowerPlex® 16 System kit positive control, in TE^{-4} buffer. The diluted 2800M was prepared in a 1.5 ml screw-top tube and placed on the mix plate in well C. TE^{-4} was added to two 1.5 ml tubes and placed on the worktable. TE^{-4} in well D of the mix plate was treated as a diluent and was added by the QIAgility™ as a negative control. TE^{-4} in well B was treated as a reagent and added to wells containing samples, positive and negative controls to bring the final reaction volume up to 25 µl. The 96-well amplification reaction plate was placed in block C1 and 96-well checkerboard plate was located in block C2 on the worktable (Figure 3).

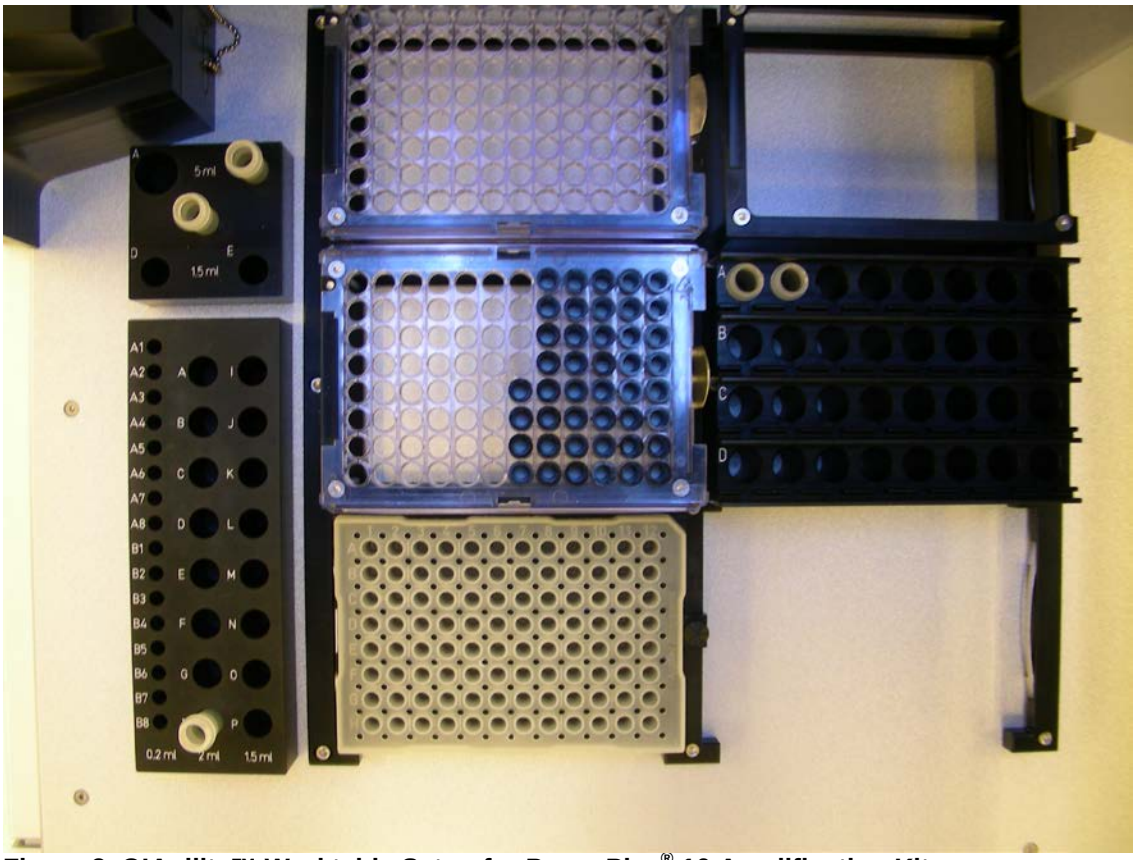

Figure 3. QIAgility™ Worktable Setup for PowerPlex® 16 Amplification Kit.

A 96-well plate was manually setup for capillary electrophoresis and data analysis was completed using GeneMapper® ID with an analytical threshold of 30 RFU. Amplification, capillary electrophoresis and data analysis was performed to verify no amplifiable DNA from any of the blood samples was present in TE^{-4} negative wells. The same was performed on each of the whole blood extracts to verify that correct and full profiles were obtained.

A **quantitation comparison study** was completed using the Qiagen® Investigator® Quantiplex HYres kit. Two studies were performed to compare the QIAgility™ and traditional manual pipetting. In study one, the QIAgility™ was programmed to setup its own serially diluted Control DNA Z1 standards and add four

replicates of these standards to a 96-well plate. On the same plate, the QIAgility™ added three replicates of the three components of NIST traceable SRM 2372 (A, B and C), from stock solutions. According to NIST, each SRM component was quantified at 50 ng/µl using spectrophotometry [2]. Manually, a new set of serially diluted Control DNA Z1 standards were generated and added in replicates of four to the second half of the 96-well plate. Three replicates of the three components of SRM 2372 were added subsequently. After completion, the 96-well plate was topped with an optical adhesive seal and run on the 7500 Real-Time PCR System. Using SDS v.1.2.3, each combination of two sets of standards setup by the QIAgility™ were analyzed. R^2 and slope values of both human and male components of standards were recorded, along with SRM 2372 component human and male concentrations. The same was performed for all combinations of manually prepared standards and comparisons were made (Figure 4).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1 QIAgility™	Standard 1 QIAgility™	Standard 1 QIAgility™	Standard 1 QIAgility™	SRM 2372-A QIAgility™	SRM 2372-C QIAgility™	Standard 1 Manual	Standard 1 Manual	Standard 1 Manual	Standard 1 Manual	SRM 2372-A Manual	SRM 2372-C Manual
B	Standard 2 QIAgility™	Standard 2 QIAgility™	Standard 2 QIAgility™	Standard 2 QIAgility™	SRM 2372-B QIAgility™		Standard 2 Manual	Standard 2 Manual	Standard 2 Manual	Standard 2 Manual	SRM 2372-B Manual	
C	Standard 3 QIAgility™	Standard 3 QIAgility™	Standard 3 QIAgility™	Standard 3 QIAgility™	SRM 2372-C QIAgility™		Standard 3 Manual	Standard 3 Manual	Standard 3 Manual	Standard 3 Manual	SRM 2372-C Manual	
D	Standard 4 QIAgility™	Standard 4 QIAgility™	Standard 4 QIAgility™	Standard 4 QIAgility™	SRM 2372-A QIAgility™		Standard 4 Manual	Standard 4 Manual	Standard 4 Manual	Standard 4 Manual	SRM 2372-A Manual	
E	Standard 5 QIAgility™	Standard 5 QIAgility™	Standard 5 QIAgility™	Standard 5 QIAgility™	SRM 2372-B QIAgility™		Standard 5 Manual	Standard 5 Manual	Standard 5 Manual	Standard 5 Manual	SRM 2372-B Manual	
F	Standard 6 QIAgility™	Standard 6 QIAgility™	Standard 6 QIAgility™	Standard 6 QIAgility™	SRM 2372-C QIAgility™		Standard 6 Manual	Standard 6 Manual	Standard 6 Manual	Standard 6 Manual	SRM 2372-C Manual	
G	Standard 7 QIAgility™	Standard 7 QIAgility™	Standard 7 QIAgility™	Standard 7 QIAgility™	SRM 2372-A QIAgility™		Standard 7 Manual	Standard 7 Manual	Standard 7 Manual	Standard 7 Manual	SRM 2372-A Manual	
H	Quant Neg QIAgility™	Quant Neg QIAgility™	Quant Neg QIAgility™	Quant Neg QIAgility™	SRM 2372-B QIAgility™		Quant Neg Manual	Quant Neg Manual	Quant Neg Manual	Quant Neg Manual	SRM 2372-B Manual	

Figure 4. Quantitation Comparison Study 1 Plate Setup.

Study two was established due to large discrepancies seen in concentration values of SRM 2372 components between the QIAgility™ and manual preparation methods in study 1. To conserve SRM component samples, only 8 µl from each component was taken from stock solutions. Results revealed a significant variation between the SRM component A concentrations prepared by the QIAgility™ and manually. The QIAgility™ was programmed to generate its own set of Control DNA Z1 standards and add two replicates to the first two columns of a 96-well plate. Three previously quantified sample extracts from a past proficiency test were added in three

replicates to the plate by the QIAgility™ for quantitation. The same was performed manually, with generation of a separate set of standards and manual addition in triplicate of the three proficiency test samples. Analysis was completed using the SDS v.1.2.3, and each group of samples was analyzed with its corresponding two sets of standards. R^2 and slope values, as well as sample concentrations were recorded for comparison.

A **sensitivity study** using Investigator® Quantiplex HYres was performed to establish if samples of both high and low concentrations could be comparably prepared by the QIAgility™ to the manual method. A sample extract from a former proficiency test that had previously quantified at a higher concentration was chosen and a 3X serial dilution was performed manually (Table 2).

Table 2. 3X Serial Dilution of QC-QT-LK-Q4E

Well	*Concentration (ng/µl)*	*Extract*	*Diluent (µl)*
1	31.2	Neat Extract	0
2	10.4	10µl of Extract	20
3	3.466666667	10µl of 2	20
4	1.155555556	10µl of 3	20
5	0.385185185	10µl of 4	20
6	0.128395062	10µl of 5	20
7	0.042798354	10µl of 6	20
8	0.014266118	10µl of 7	20

The QIAgility™ was programmed to generate a set of Control DNA Z1 standards and add two replicates to a 96-well plate. The program also included addition of three replicates of the serial diluted extract to the plate. 18 µl of master mix was added to each reaction well, followed by 2 µl of standard, negative control (TE^{-4}) or sample. The same was completed manually on the same half-skirted PCR reaction plate, using a

manually generated set of standards. A separate master mix was created for manual additions. Using SDS v.1.2.3., R^2 and slope values were compared between the two methods. Coefficient of variation was calculated for comparison of variability in relation to the mean for each set of samples between methods. QIAgility™ prepared human quantitation values of each well replicates in the serially diluted sample were averaged and log 10 for each was computed to generate a linear regression for comparison. The same was completed for manually prepared samples and linear regressions were compared using R^2 and slope values.

A **normalization and amplification comparison study** for normalization and amplification was completed using TE^{-4} and the Promega® PowerPlex® 16 System amplification kit, respectively. Four previously extracted, quantified and amplified samples were chosen for the study: QC-QT-CF-Q3E, QC-QT-MM-Q1S, QC-QT-HH-Q5E and QC-QT-LK-Q2E. Those samples were taken from a previous proficiency test and included mixtures with major and minor contributing STR profiles. Since the MUFSC DNA Laboratory already had a stock of prepared mixture samples, major and minor contributing profiles were known and major and minor profiles could be deduced from results. For normalization, samples were chosen with varying concentration between 1.5 ng/µl and 40 ng/µl. Chosen samples were re-quantified to verify concentrations (Table 3).

Table 3. Four Extracts' Human and Male Quantitation Values using Quantiplex HYres		
Sample	Human (ng/µl)	Male (ng/µl)
QC-QT-CF-Q3E	1.660	0.149
QC-QT-MM-Q1S	6.660	6.040
QC-QT-HH-Q5E	15.780	2.010
QC-QT-LK-Q2E	31.200	0.226

A protocol was created for the normalization of sample extracts. Sample extracts and destination 1.5 ml tubes were loaded onto block B2 on the worktable. Sample extracts populated row A and the two sets of 1.5 ml destination tubes populated rows B and C. The QIAgility™ was programmed to normalize the four extracts to a target concentration of 0.5 ng, in replicates. The same concentration was targeted manually. The QIAgility™ was programmed to setup an amplification 96-well PCR reaction plate that included two replicates of each normalized sample, a total of eight samples. Master mix was prepared manually and placed in reagent block, well H, on the QIAgility™ worktable. TE^{-4} was manually loaded into 1.5 ml tubes for the diluent in well D of the mix plate, and reagent in well B of the mix plate. The instrument added 5.8 µl of master mix, followed by 2 µl of normalized sample to each reaction well. 2 µl of diluent was added as a negative control and reagents were used to bring up any final reaction volumes to 25 µl. On the same plate, the remaining manually normalized sample replicates were added by the analyst, a total of eight samples, along with 5.8 µl of master mix per well. 2 µl of each sample was added to the plate for a final target concentration of 1 ng. Amplification was completed on the Applied Biosystem® GeneAmp® PCR System 9700 thermal cycler.

A 96-well half-skirted PCR reaction plate was manually setup for capillary electrophoresis and the plate was run on the 3130xL Genetic Analyzer. Master mix was

generated with addition of 9 µl of Hi-Di formamide and 1 µl of ILS 600 (size standard) per sample. 10 µl of master mix and 1 µl of sample were added to wells of the 96-well PCR reaction plate. Analysis was completed with GeneMapper® ID v3.2.1. Average allele heights, in RFUs, per dye channel were recorded for each sample prepared by the QIAgility™ and manually. Standard deviation, percent coefficient of variation and the number of alleles present were recorded. Only the major contributing profile was considered for statistical comparisons. Since samples had been previously prepared by the laboratory, mixture components were known and profiles of all sources were on file. Major contributing profiles were verified during analysis.

Results

Accuracy Study

Over 52 wells, the QIAgility™ produced an average of 201.6 µl per well with a standard deviation of 1.7 µl using 200 µl tips (Figure 5).

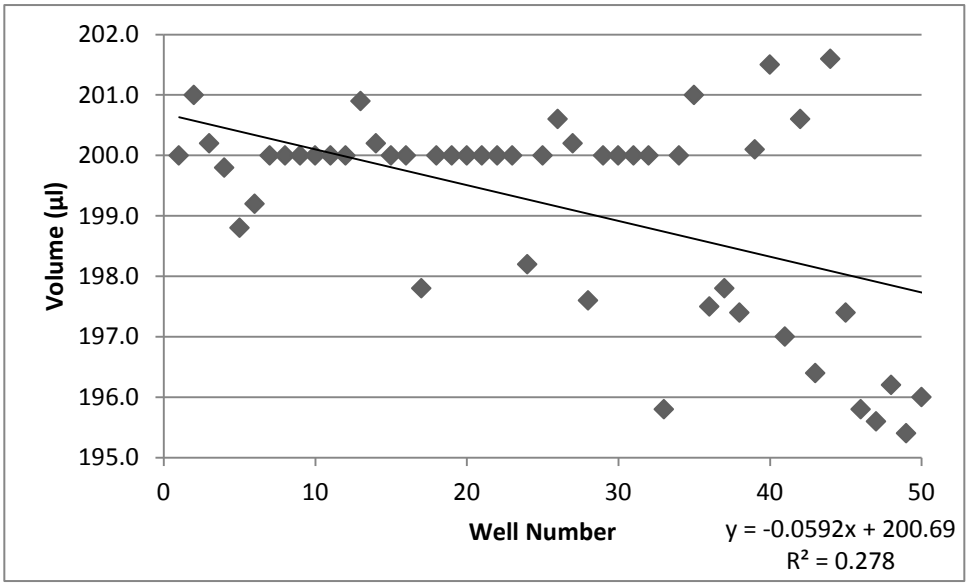

Figure 5. Test 1: Accuracy of QIAgility™ Pipetting 200 µl into each of 52 Wells, using 200 µl tips.

TE^{-4} was pulled from the mix plate tubes in the reverse tube order as the first accuracy test and results were comparable. Over 52 wells, the QIAgility™ produced an average of 197.3 µl per well with a standard deviation of 2.5 µl using 200 µl tips (Figure 6).

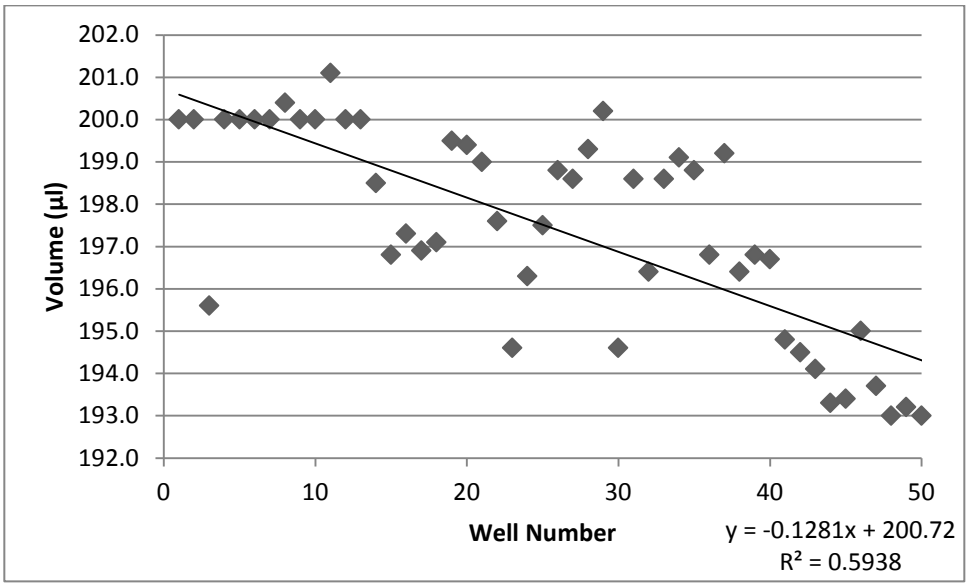

Figure 6. Test 2: Accuracy of QIAgility™ Pipetting 200 µl into each of 52 Wells, using 200 µl tips.

Over 52 wells, the QIAgility™ produced an average of 100.2 µl per well with a standard deviation of 0.69 µl using 200 µl tips (Figure 7).

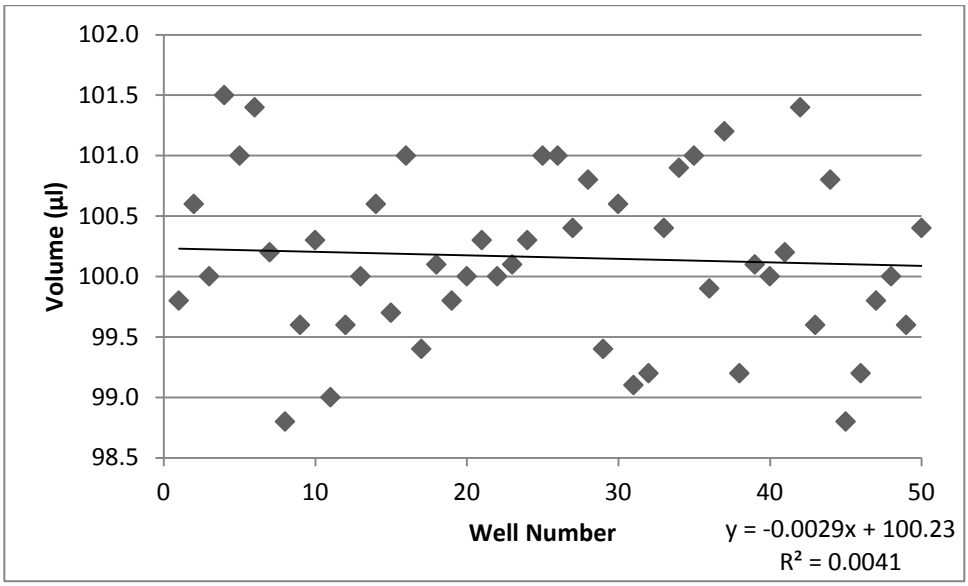

Figure 7. Accuracy of QIAgility™ Pipetting 100 µl into each of 52 Wells, using 200 µl tips.

Over 52 wells, the QIAgility™ produced an average of 9.9 µl per well with a standard deviation of 0.22 µl using 200 µl tips (Figure 8).

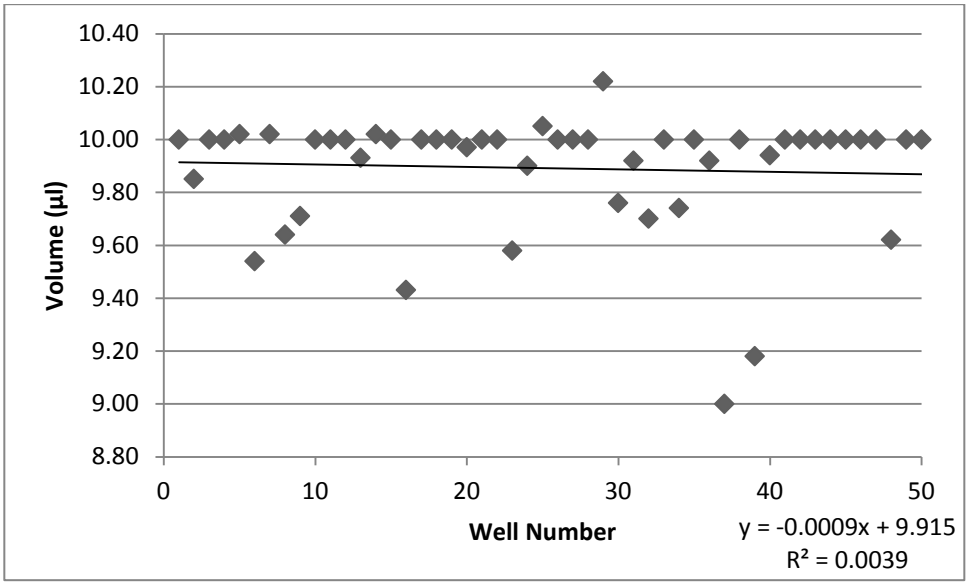

Figure 8. Accuracy of QIAgility™ Pipetting 10 µl into each of 52 Wells, using 200 µl tips.

Over 52 wells, the QIAgility™ produced an average of 49.4 µl per well with a standard deviation of 0.33 µl using 50 µl tips (Figure 9).

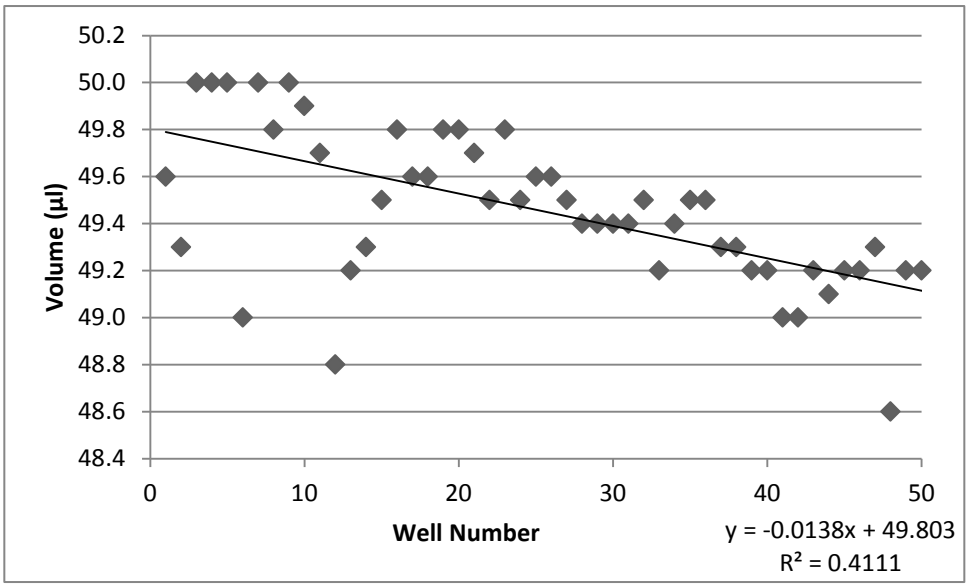

Figure 9. Accuracy of QIAgility™ Pipetting 50 µl into each of 52 Wells, using 50 µl tips.

Over 96 wells, the QIAgility™ produced an average of 23.8 µl per well with a standard deviation of 0.27 µl using 50 µl tips (Figure 10).

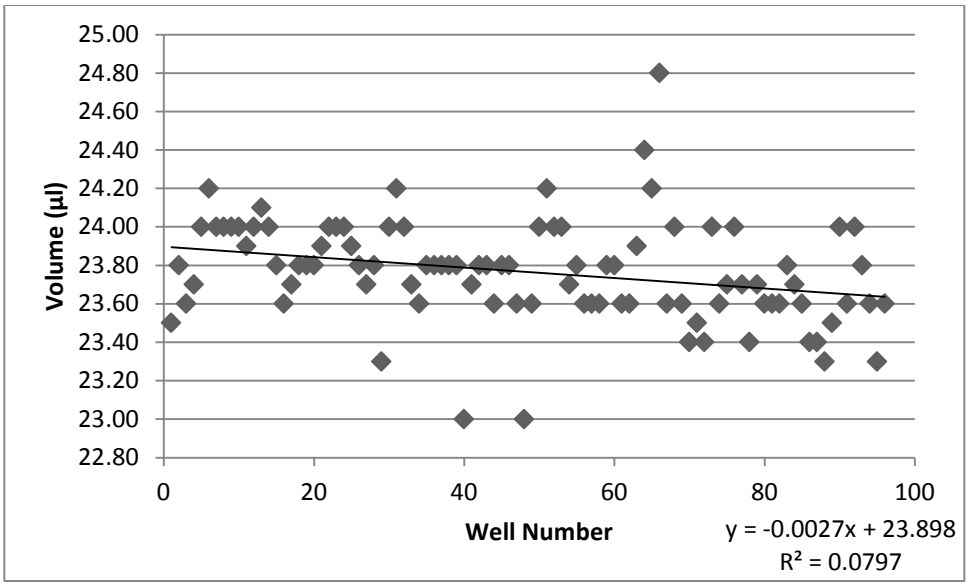

Figure 10. Accuracy of QIAgility™ Pipetting 25 µl into each of 96 Wells, using 50 µl tips.

Over 96 wells, the QIAgility™ produced an average of 5.2 µl per well with a standard deviation of 0.20 µl using 50 µl tips (Figure 11).

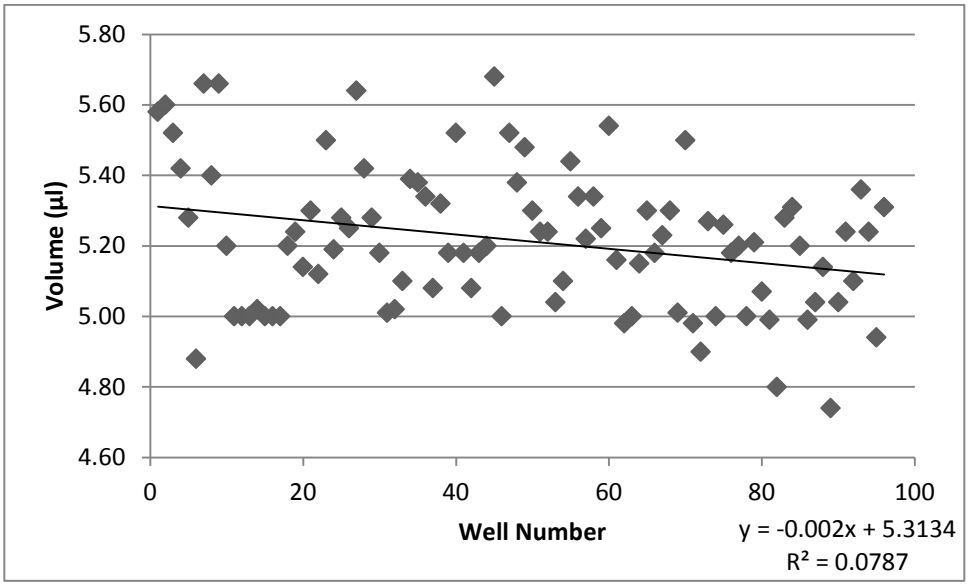

Figure 11. Accuracy of QIAgility™ Pipetting 5 µl into each of 96 Wells, using 50 µl tips.

The furthest measured volume from each run target volume was analyzed and downstream effects to final concentrations were determined (Table 4).

Table 4. Downstream Effect of DNA Workflow with Loss in Accuracy and Precision of the QIAgility™ Instrument					
QIAgility™ Action	**Initial [DNA] (ng/µl)**	**DNA added (µl)**	**Final Volume (µl)**	**Final [DNA] (ng/µl)**	**[DNA] to Amp (ng/µl)**
Pipette 200 µl	50.5	2	202	0.5	1
Pipette 193 µl	50.5	2	195	0.518	1.036
Pipette 100 µl	25.5	2	102	0.5	1
Pipette 98.8 µl	25.5	2	100.8	0.506	1.012
Pipette 10 µl	3	2	12	0.5	1
Pipette 9.2 µl	3	2	11.2	0.536	1.072
Pipette 50 µl	13	2	52	0.5	1
Pipette 48.6 µl	13	2	50.6	0.514	1.028
Pipette 25 µl	6.75	2	27	0.5	1
Pipette 23 µl	6.75	2	25	0.54	1.08
Pipette 5 µl	1.75	2	7	0.5	1
Pipette 4.7 µl	1.75	2	6.7	0.522	1.044

Contamination Study

The four whole blood sample extracts produced the expected full profiles. All TE^{-4} well profiles were clean, none of which produced any alleles from the four blood samples. Some TE^{-4} profiles contained off ladder alleles below the analytical threshold.

Quantitation Comparison Study – Investigator® Quantiplex HYres (Study 1)

All combinations of standards generated by the QIAgility™ and manually passed the MUFSC laboratory requirements, which include an R^2 value of greater than or equal to 0.99 and a slope of -3.3 ± 0.3 [5]. Averages and standard deviations of QIAgility™ and manually prepared standards were similar for both human and male standard curves (Table 5).

Table 5. Comparison of QIAgility™ and Manually Prepared Standard: R^2 and Slope Values for each Combination of Four Rows of Standards					
System	**Standards Used**	**R^2 (Human)**	**Slope (Human)**	**R^2 (Male)**	**Slope (Male)**
QIAgility™	1,2	0.9980	-3.0733	0.9990	-3.3550
QIAgility™	1,3	0.9984	-3.0923	0.9973	-3.3369
QIAgility™	1,4	0.9989	-3.0645	0.9988	-3.3522
QIAgility™	2,3	0.9980	-3.1044	0.9981	-3.3205
QIAgility™	2,4	0.9983	-3.0766	0.9990	-3.3590
QIAgility™	3,4	0.9988	-3.0956	0.9974	-3.3174
QIAgility™	*Average*	*0.9984*	*-3.0845*	*0.9983*	*-3.3402*
QIAgility™	*Std. Dev*	*0.0004*	*0.0153*	*0.0008*	*0.0181*
Manual	7,8	0.9945	-3.1407	0.9940	-3.2118
Manual	7,9	0.9997	-3.2824	0.9985	-3.1326
Manual	7,10	0.9979	-3.2089	0.9991	-3.2600
Manual	8,9	0.9929	-3.1233	0.9938	-3.2162
Manual	8,10	0.9928	-3.1997	0.9928	-3.1938
Manual	9,10	0.9975	-3.1915	0.9991	-3.2644
Manual	*Average*	*0.9959*	*-3.1911*	*0.9962*	*-3.2131*
Manual	*Std. Dev*	*0.0029*	*0.0563*	*0.0030*	*0.0484*

Comparison between SRM 2372 component concentrations varied between QIAgility™ and manually prepared methods (Table 6). Sepcifically, QIAgility™ prepared components produced a significantly lower quantitation value than both manually prepared samples and values that were expected, according to NIST (50 ng/µl). A supplemental study, study 2, was performed to determine if insufficient liquid played a role in varying SRM 2372 component concentrations.

Table 6. Comparison of QIAgility™ and Manually Prepared SRM 2372 Components A, B and C Concentrations with each Combination of Standards							
System	**Standards Used**	**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™	1,2	24.14	42.09	43.98	28.54	0.00	31.02
QIAgility™	1,3	23.99	41.75	43.55	29.54	0.00	32.12
QIAgility™	1,4	24.70	43.13	45.07	30.17	0.00	32.79
QIAgility™	2,3	23.61	40.94	42.76	29.22	0.00	31.78
QIAgility™	2,4	24.30	42.34	44.24	29.85	0.00	32.45
QIAgility™	3,4	24.15	41.94	43.81	30.92	0.00	33.43
QIAgility™	*Average*	*24.15*	*42.03*	*43.90*	*29.71*	*0.00*	*32.27*
QIAgility™	*St. Dev*	*0.36*	*0.72*	*0.76*	*0.82*	*0.00*	*0.83*
Manual	7,8	47.21	59.42	63.48	52.69	0.00	50.85
Manual	7,9	42.75	53.83	57.53	45.30	0.00	43.75
Manual	7,10	39.63	49.64	52.96	44.42	0.00	42.89
Manual	8,9	44.92	56.59	60.49	52.07	0.00	50.25
Manual	8,10	41.58	52.11	55.61	51.10	0.00	49.30
Manual	9,10	37.71	47.28	50.46	43.92	0.00	42.41
Manual	*Average*	*42.30*	*53.15*	*56.76*	*48.25*	*0.00*	*46.58*
Manual	*St. Dev*	*3.46*	*4.46*	*4.80*	*4.11*	*0.00*	*3.95*

Quantition Comparison Study – Investigator® Quantiplex HYres (Study 2)

Both the QIAgility™ and manually prepared standards passed in accordance with MUFSC standards (Table 7) [5].

Table 7. R^2 and Slope Values of Human and Male Standard Curves for QIAgility™ and Manual Prepared Standards				
	Human		*Male*	
System	**R^2**	**Slope**	**R^2**	**Slope**
QIAgility™	0.9990	-3.0956	0.9993	-3.2591
Manual	0.9995	-3.1185	0.9997	-3.3619

The three extracts setup by the QIAgility™ and manual preparation produced considerably higher quantiation values than that of the orginial manual quantitation. However, the two modes of preparation produced similar quantation values to one another. Averages of samples prepared by the two methods were similar and the

QIAgility™ prepared sample quantiation results produced a smaller standard deviation. Percent differences between QIAgility™ prepared sample and original quatitation results were comparable to manually prepared sample and original quantitation results (Table 8).

Table 8. Comparison of Original Quantitation Values to Three Replicates of Three Samples Prepared by the QIAgility™ and Manually

QIAgility™							
Sample	**Original Quant (ng/µl)**	**Replicate 1 (ng/µl)**	**Replicate 2 (ng/µl)**	**Replicate 3 (ng/µl)**	**Average (ng/µl)**	**St. Dev**	**Percent Difference (%)**
QC-QT-JS-Q1E	18.5	28.46	26.4	26.56	27.14	1.146	38%
QC-QT-JS-Q2E	17.5	23.4	25.08	24.92	24.47	0.927	33%
QC-QT-JS-Q5E	16.36	25.42	23.76	25.47	24.88	0.973	41%
Manual							
QC-QT-JS-Q1E	18.5	26.34	27.59	28.06	27.33	0.889	39%
QC-QT-JS-Q2E	17.5	22.47	23.26	22.92	22.88	0.396	27%
QC-QT-JS-Q5E	16.36	26.4	25.28	25.18	25.62	0.677	44%

Quantition Sensitivty Study - Investigator® Quantiplex HYres

Both the QIAgility™ and manually prepared standards passed in accordance with MUFSC standards (Table 9) [5].

Table 9. R^2 and Slope Values of Human and Male Standard Curves for QIAgility™ and Manual Prepared Standards

System	**R^2 (Human)**	**Slope (Human)**	**R^2 (Male)**	**Slope (Male)**
QIAgility™	0.9984	-3.1261	0.9989	-3.3825
Manual	0.9985	-3.0396	0.9996	-3.3069

Quantitation values were similar between serially diluted samples prepared by the QIAgility™ and manually. Generally, QIAgility™ prepared samples did produce

lower quantitation values than manually prepared samples, as well a slightly lower standard deviation values. Similarly to the quantitation comparison study, quantitation values of QIAgility™ and manually prepared standards varied from the original quantitation (Table 10).

Table 10. Comparison of Expected Concentrations with QIAgility™ and Manually Prepared Sample Concentrations Obtained from Serially Diluted Q4E

Previous Quantitation	QIAgility™			Manual		
Expected Concentration (ng/µl)	Conc. (ng/µl)	Standard Deviation	%CV	Concentration (ng/µl)	Standard Deviation	%CV
31.200	24.310	0.609	2.51	28.920	1.500	5.19
10.400	7.267	0.673	9.27	9.260	0.726	7.84
3.467	2.450	0.036	1.47	3.023	0.096	3.18
1.155	0.781	0.065	8.37	0.961	0.039	4.01
0.385	0.291	0.014	4.87	0.329	0.007	1.99
0.128	0.106	0.012	11.1	0.118	0.020	16.9
0.042	0.033	0.002	7.07	0.035	0.002	4.81
0.014	0.012	0.003	20.4	0.010	0.002	19.6

A standard curve was generated for each method's quantiation results, using log 10, for comparison to concentrations that were expected. The results showed a significant correlation for both methods and expected values (Figures 12 & 13).

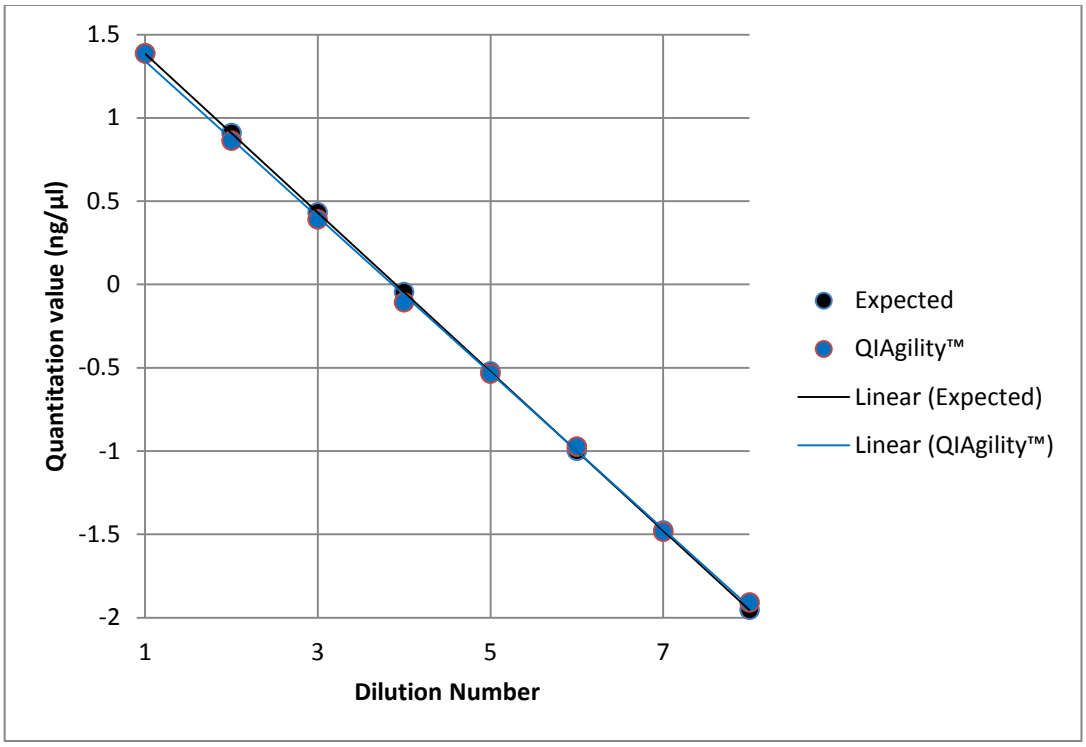

Figure 12. Linear Regression of Expected vs. QIAgility™ Quantitation Values for Q4E Serial Dilution.

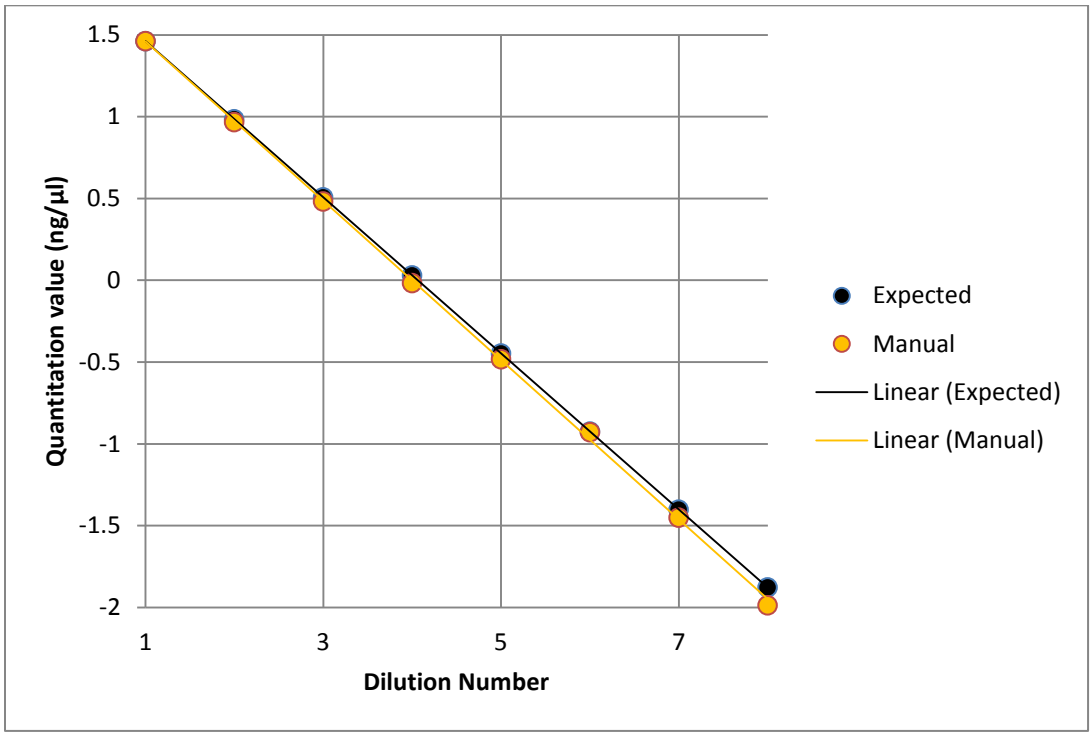

Figure 13. Linear Regression of Expected vs. Manual Quantitation Values for Q4E Serial Dilution.

Correlations were also observed when comparing the two methods to one another (Figure 14).

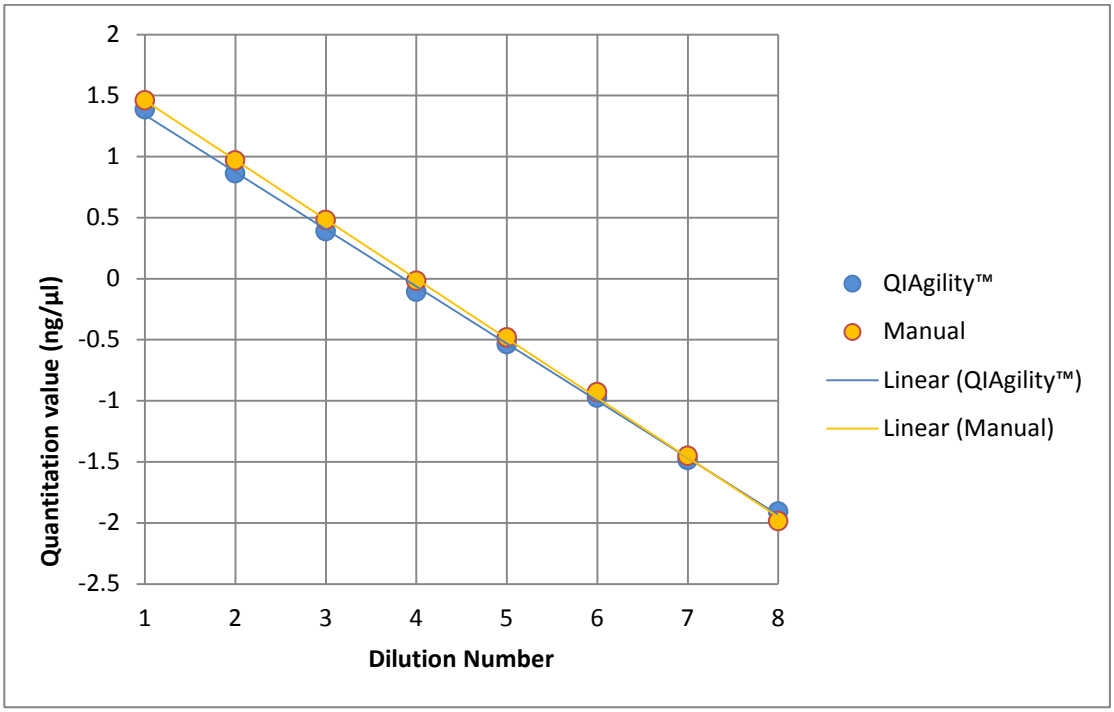

Figure 14. Linear Regression of QIAgility™ vs. Manual Quantitation Values for Q4E Serial Dilution.

Comparisons were also made between the R^2 value and slope of each method's linear regression and compared to the expected R^2 and slope values (Tables 11). In reagards to slope, the QIAgility™ produced a closer value to what was expected. Between the two methods, the R^2 values were comparable.

Table 11. Comparison of R^2 and Slope Between Expected Values, QIAgility™ Prepared and Manually Prepared Values

Expected Slope	QIAgility™ Slope	QIAgility™ Difference	Manual Slope	Manual Difference
-0.4771	-0.468	-0.0091	-0.4871	0.01
Expected R^2	**QIAgility™ R^2**	**QIAgility™ Difference**	**Manual R^2**	**Manual Difference**
1.0000	0.9993	0.0007	0.9996	0.0004

Normalization and Amplification Comparison Study – Promega® PowerPlex® 16 System Amplification Kit

All expected alleles were present in each sample prepared by the QIAgility™ and manually. All peaks were sharp and well-defined. Average relative fluorescent units were summed for each dye channel of each sample. Comparisons between the two methods showed that the QIAgility™ prepared samples had a slightly higher number of average relative fluorescence units per dye channel. Comparison of the percent coefficient of variation indicated that QIAgility™ prepared samples had less variability in relation to the mean (Table 12).

Table 12. Comparison of QIAgility™ and Manually Normalized and Amplification Prepared Sample Extracts

		QIAgility™				Manual			
Sample	**Dye Channel**	**Average RFU**	**St. Dev.**	**% CV**	**Alleles Present**	**Average RFU**	**St. Dev.**	**% CV**	**Alleles Present**
Q3E	Blue	14624.0	724.02	5.0%	9	13284.5	3438.95	25.9%	9
	Green	23250.8	1857.68	8.0%	10	20452.0	5436.30	26.6%	10
	Yellow	17873.3	1302.40	7.3%	8	15960.0	5098.15	31.9%	8
Q1S	Blue	15370.3	1583.95	10.3%	10	15250.5	988.33	6.5%	10
	Green	24759.0	2818.93	11.4%	10	24082.0	1214.82	5.0%	10
	Yellow	19046.8	1430.30	7.5%	7	18002.0	1392.10	7.7%	7
Q5E	Blue	12106.5	1732.51	14.3%	10	11717.3	2611.30	22.3%	10
	Green	18126.0	3764.37	20.8%	11	17236.8	4580.23	26.6%	11
	Yellow	14633.5	2260.69	15.4%	8	13293.8	3756.55	28.3%	8
Q2E	Blue	13159.3	2398.04	18.2%	10	11660.3	4730.74	40.6%	10
	Green	21132.3	3347.77	15.8%	11	19145.8	7802.95	40.8%	11
	Yellow	15808.0	877.95	5.6%	8	12917.8	5224.83	40.4%	8

All dye channels from each sample were averaged together, and the two methods were found comparable. The QIAgility™ prepared samples had a slightly higher RFU count per dye channel, but all peaks were clean and none were produced offscale data (Figure 15).

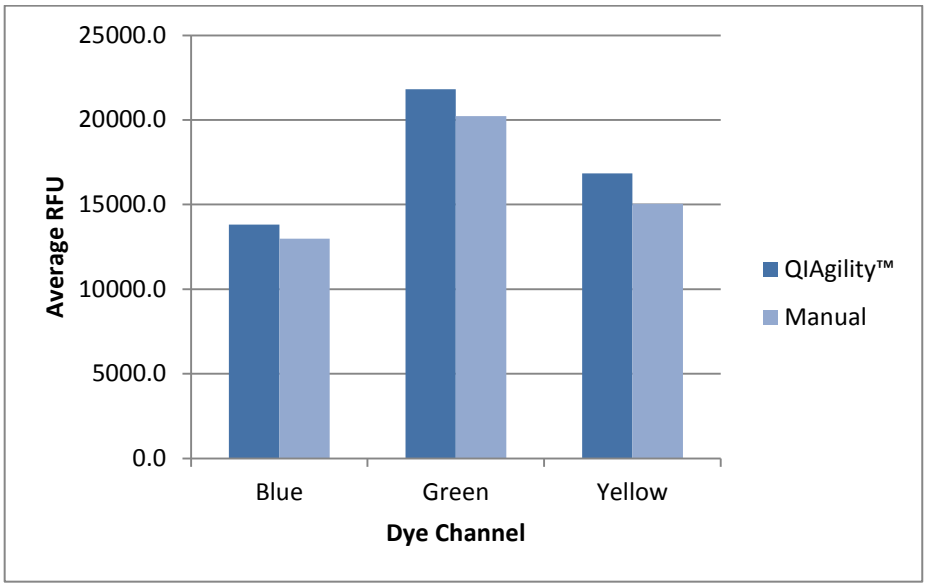

Figure 15. QIAgility™ vs. Manual: Average RFU for each Dye Channel.

Similar results were observed when separating average RFU per sample. The QIAgility™ prepared samples tended to produce slightly higher RFU's per sample (Figure 16).

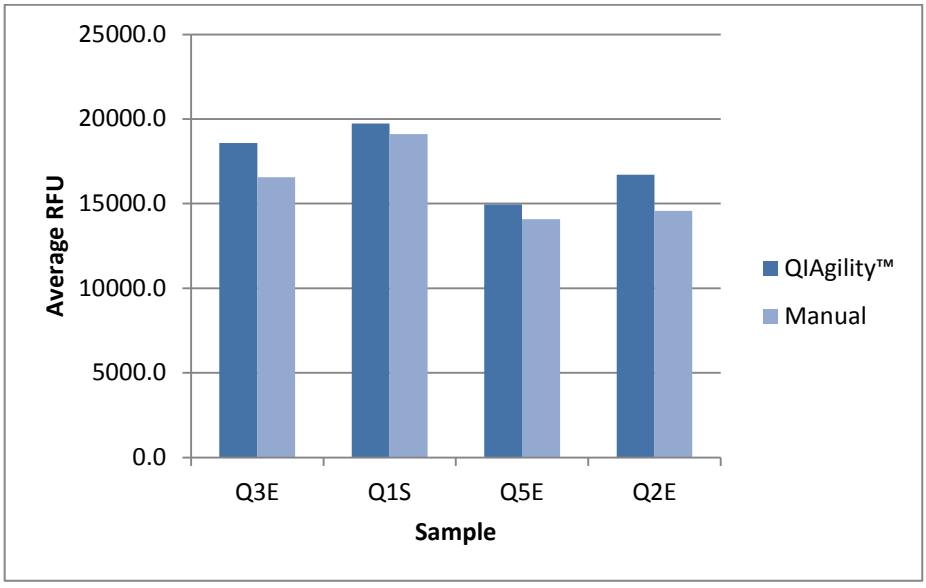

Figure 16. QIAgility™ vs. Manual: Average RFU for each Sample.

Discussion and Conclusions

Accuracy Study

Averages and standard deviations were calculated for each run using 200 µl and 50 µl pipette tips and were within acceptable range. Upon initial observation, the accuracy study indicated a loss in accuracy over the course of a run by the QIAgility™, with both 200 µl and 50 µl liquid-sensing pipette tips. This was confirmed based on the negative slope of trend lines for Figures 3 through 9. Initially, graphical observations were concerning, but when downstream effects of concentration differences were analyzed, the effects were not significant. The downstream changes in concentrations shown in Table 3 were the values observed furthest away from the expected volume and only represent the "worst case scenario" from the study results. Furthermore, the quantitation and normalization processes are only estimates themselves. Some variation may have been due to the measurement method, manual pipetting. It is believed the loss in accuracy observed was not due to manual measurement. A future study may include the use of automation for measuring QIAgility™ pipetted volumes. This would eliminate human error and could lead to more precise measurements.

Contamination Study

The contamination study indicated that the instrument produced no cross-contamination between samples and TE^{-4} wells in a 96-well PCR reaction plate. This was confirmed through amplification with the PowerPlex® 16 System amplification kit, capillary electrophoresis with the 3130xL genetic analyzer and STR analysis with GeneMapper® ID v3.2.1 of blank wells at 30 RFU. A full profile was obtained from the

positive control, 2800M Control DNA, supporting that amplification occurred. Primer peaks were present in the analysis of all blank wells, indicating that PowerPlex® 16 master mix was added to each sample. Blood extracts produced full profiles and were consistent with the profiles on file. No peaks were present in any blank wells above an analytical threshold of 50 RFUs. A few off ladder peaks were observed under analytical threshold; however they were believed to be artifacts due to peak morphology and off-ladder allele call designations. Furthermore, since all blood sample profiles contained only alleles that were called, the off-ladder alleles below analytical threshold were of no concern.

Quantitation Comparison Study – Investigator® Quantiplex HYres Quantitation Kit

Comparisons between QIAgility™ and manually prepared standards revealed very similar results for both human and male in Study 1. R^2 and slope values of all standards prepared fell within MUFSC guidelines and no standards were omitted from the standard curve in order to pass these guidelines. The only concern was observed with the human slope of the QIAgility™ prepared standards. Generally, the human slope of QIAgility™ prepared standards were closer to the lower range of acceptability, -3.0, according to MUFSC guidelines [5]. This may only propose a problem when standards are prepared and then used again at a later time. According to the Investigator® Quantiplex HYres Handbook, serially diluted Control DNA Z1 standards are stable for up to a week after manual preparation [9]. However, since standard curve slopes of QIAgility™ prepared standards were already close to -3.0, concerns arose about how standards would present in the standard curve days after. To determine the stability of

QIAgility™ prepared HYres standards over the course of a week, a future study should be completed. This can be accomplished by re-quantifying HYres standards over the course of a week or possibly longer, and analyzing the generated standard curve's slope, R^2 and Y-intercept.

QIAgility™ prepared SRM 2372 component concentrations varied greatly from manually prepared SRM 2372 components and expected values. Especially low, the average value for QIAgility™ prepared component A quantified at almost half of the average of manually prepared component A. For preservation of SRM 2372 component stock solutions, the researcher removed 8 µl of each component and relocated volumes into separate 1.5 ml screw-cap tubes. The protocol called for 2 µl of each component in replicates of three, a total of 6 µl per component. It is possible that the QIAgility™ was not provided with enough volume of each component to precisely pipette accurate amounts. The second study proved this to be true, as the QIAgility™ and manual method produced similar average quantitation results for the three sample extracts using sufficient volumes. Comparable results can be seen in direct comparison or in percent difference from the original quantitation result (Table 8). Standard deviations for the three replicates of each sample prepared by two systems were within range. Both QIAgility™ and manually prepared standards produced a significantly higher quantitation value than that of the original quantitation. This may have been due to improper vortexing and centrifugation of the samples in the original quantitation setup.

Quantitation Sensitivty Study

QIAgility™ and manually prepared samples of the serial dilution quantitation results were generally comparable. However, manually prepared samples produced higher quantitation values than QIAgility™ prepared samples, as well as values closer to those expected. Smaller standard deviations were observed from QIAgility™ prepared samples. The log 10 of quantitation values for both QIAgility™ and manually prepared samples generated very similar linear regression trendlines. Each system's trendline also compared closely with the expected trendline genereated from the log 10 of expected concentrations of the serial dilution. Futher evidence of similarities between trendlines were observed when comparing the slope and R^2 values. Both QIAgility™ and manually prepared sample concentration trendlines compared similarly to one another and to expected values.

Normalization and Amplification Comparison Study – Promega® PowerPlex® 16 System Amplification Kit

All expected alleles were present in both QIAgility™ and manually normalized and prepared samples. QIAgility™ prepared samples tended to produce a higher average RFU per dye channel and per sample. Generally, the QIAgility™ prepared samples produced smaller standard deviations, with the exception of sample Q1S. QIAgility™ prepared samples also produced a smaller percent coefficient of variation for all samples, with the exception of sample Q1S. A smaller percent coefficient of variation

represents the extent of variability in comparison to the mean. Some variations in RFU values were expected; however, more importantly, all peaks were present, sharp and well defined in all samples prepared by the QIAgility™ and manually.

Concerns about additional cost per QIAgility™ quantitation and amplification setup arose. The QIAgility™ User Manual recommends that an additional 30 µl plus 1 µl additionally per reaction be added to the total volume of master mix to ensure accurate pipetting by the QIAgility™ [10]. This is sufficiently more master mix than is prepared according to MUFSC DNA Laboratory's traditional method [4,5]. A future study that could be beneficial for the laboratory would include a cost analysis of additional master mix used for quantitation and amplification preparation. Additionally, a study can be performed to determine how much additional master mix is needed to produce accurate pipetting results. Researchers could find that only a minimal excess of master mix is needed by the instrument, and this would help reduce the total cost per QIAgility™ run.

The Qiagen® QIAgility™ has been successfully internally validated. The instrument pipettes accurately and precisely, similar to manual methods, and does not produce cross-contamination. The QIAgility™ will be implemented in the MUFSC DNA Laboratory upon approval, for quantitation setup with the Investigator® Quantiplex HYres Quantitation Kit, amplification setup with the Promega® PowerPlex® 16 System Amplification Kit and normalization of sample extracts.

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