The Shift from Manual to Automated, a Cost-Benefit Analysis of the Qiagen® QIAgility® at the West Virginia State Police Forensic Laboratory

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1. Abstract

Automation is known to reduce the risks of potential human error and environmental contamination as well as reduce the analyst's time to process a sample. One notable instrument that demonstrates this is Qiagen®'s QIAgility®. However, at the West Virginia State Police Forensic Laboratory (WVSPFL), it was unknown if the high throughput benefits of using the QIAgility® in a forensic DNA laboratory outweighed the costs of using and maintaining the instrument. As such, a cost-benefit study was performed.

Using WVSPFL's validated laboratory settings, the first notable difference when shifting from manual to automated methods is the cost of consumables. As such, the study compared the costs of the consumables for manual and QIAgility® set-up for quantitation, normalization, and amplification procedures for thirty, fifty, and eighty samples. The second notable difference is the time spent performing the forensic DNA procedures. Thus, the study also compared the time required to perform a quantitation, normalization, and amplification. By determining the difference in time, the study evaluated if using the QIAgility® could save forensic DNA laboratories money and calculated how much hands-on laboratory time was eliminated to free up analysts. This was based on the estimated annual salary of an analyst, the estimated time an analyst takes to perform a particular DNA procedure, and the estimated time an analyst works in the laboratory during the year.

The QIAgility® was found to introduce a higher consumable cost into the forensic DNA workflow if fifty or fewer samples were processed using the QIAgility®; fifty samples introduced an additional cost of $17.52 and thirty samples introduced an additional cost of $38.54. However, for eighty samples, the QIAgility® was found to cost less than a manual set-up by

$31.86. The results demonstrated that the difference in cost was attributed primarily to the use of a 96-well plate, the number of reaction overages, and the use of Qiagen® conductive filtered tips.

Regarding the time study, the QIAgility® was observed to save an estimated total time of 107 minutes for eighty samples, 67 minutes for fifty samples, and 40 minutes for thirty samples. This was based on the difference of total time required to perform a quantitation, normalization, and amplification for manual set-up and QIAgility® set-up. Additionally, the study estimated that for an analyst on an annual salary of $30,000, $40,000, and $60,000, the cost per minute was $0.25, $0.34, and $0.51, respectively. These differences in time and cost of an analyst per minute were integrated into the cost-benefit study, where it was determined that if a DNA forensic laboratory processed 30, 50, or 80 samples using the QIAgility®, the laboratory could save an estimated cost of $10 - $21, $17 - $34, and $26 - $55, respectively. Overall, this study determined that the additional cost of consumables could potentially be offset by increasing the number of samples and by taking into consideration cost reductions associated with the elimination of laboratory time to process a sample. Future studies may incorporate additional variables to investigate other cost reduction factors.

In conclusion, the WVSPFL Biochemistry Section believes that the known benefits and monetary benefits of using the QIAgility® outweighed the costs of using and maintaining the QIAgility®. The Biochemistry Section hopes to streamline the DNA workflow as well as decrease the chance of human error and human contamination during quantitation, normalization, and amplification set-up with the use of the QIAgility®. As a result of this study, the QIAgility® will be incorporated into the DNA workflow at the WVSPFL Biochemistry Section [1].

2. Introduction

Forensic laboratories are constantly seeking approaches in improving their respective DNA workflow to reduce casework backlogs and the risks of potential human error. Since the time for each case can be limited and an analyst can only handle so many samples, many laboratories have stressed the need for a forensic laboratory to balance their DNA workflow to effectively process old and new cases without introducing human error. One method is the incorporation of automation into the DNA workflow [2]. By using automated instruments to perform the laboratory work, analysts can dedicate more time towards analyzing data and writing reports. The utilization of automation has been shown to lower the risk of potential human error in the DNA workflow, because the samples are being liquid handled by an instrument [3]. As A recent instrument that has been introduced into the DNA workflow is Qiagen®'s QIAgility® (Qiagen®, Hilden, Germany). The QIAgility® is a liquid handler manufactured by Qiagen® capable of performing DNA set-up for DNA quantitation, normalization, polymerase chain reaction (PCR) amplification, and capillary electrophoresis for a full 96-well tray in as little as thirty minutes. In addition, the instrument is manufactured with the capability to function with different chemistry kits used for DNA forensic analysis [4]. Using the QIAgility® can help remove potential human error during protocol set-up as well as help a laboratory streamline the DNA workflow.

Quantitation helps analysts by determining the concentration of the sample post-extraction and if dilution or concentration steps are required prior to amplification. By quantitating the sample, the desired sample DNA concentration can be better achieved to accommodate the amplification kit to be used. By correlating the sample's concentration to the

amplification kit's desired DNA concentration range, issues such as amplification artifacts, dropout, and peak height ratio imbalance can be avoided.

PCR amplification is the process of copying a specific region of the DNA template through the use of enzyme *Thermus Aquaticus* (*Taq*), primers, deoxy-nucleotide triphosphates (dNTPs), nuclease-free water, and buffer. For forensic science purposes, the primers are designed to target and amplify specific short tandem repeat (STR) regions. During the process of copying the STR regions, amplification probes that are specific to each amplification kit are incorporated into each copied STR to aide in downstream DNA analysis.

Recently, the WVSPFL Biochemistry Section purchased a QIAgility® and in accordance to the Federal Bureau of Investigation's Quality Assurance Standards (FBI QAS) and the Scientific Working Group of DNA Analysis Methods (SWGDAM), an internal validation must be conducted prior to the instrument's implementation into the forensic DNA workflow [5], [6]. Each internal validation varies between laboratories, because the validation is dependent on the laboratory's discretion regarding which studies are most relevant and appropriate to their specific methodologies [1]. WVSPFL's purpose of obtaining the QIAgility® is for use in quantitation, normalization, PCR amplification.

In accordance with SWGDAM, FBI QAS, and how the WVSPFL's Biochemistry Section plans to utilize the QIAgility®, the internal validation will encompasses the following studies: accuracy and precision, contamination, sensitivity, mixture, and a comparison study for quantitation, normalization, and PCR amplification set-up. An accuracy and precision study is performed because it is vital to test the QIAgility®'s capabilities as a liquid handler for accuracy, precision, reproducibility, and reliability. A contamination study is performed to assess if the QIAgility® can liquid handle samples without the risk of contamination. The QIAgility® liquid

handles samples that are in close proximity of one another and the movement of liquid from different types of wells can possibly introduce contamination. A sensitivity study is performed to assess if the QIAgility® can reliably liquid handle DNA samples of "high" and "low" concentrations. Similarly, the mixture study is performed to demonstrate if the QIAgility® can liquid handle mixture samples without affecting the mixture's integrity. A concordance study is performed to demonstrate if the QIAgility® can produce better or comparable results to a manual set-up of quantitation, normalization, and amplification. The concordance studies incorporated known and non-probative samples to further verify if the QIAgility® can liquid handle known samples and those that mimic casework samples.

Although the benefits of using the QIAgility® are clear, it is uncertain if those benefits outweigh the costs of using the QIAgility®. As such, a cost-benefit study was performed to investigate this uncertainty. The most notable differences through incorporating the QIAgility® into the DNA workflow are the costs of consumables and the time spent performing the procedures. Hence**,** the study will compare the costs and time needed to perform a quantitation, normalization, and PCR amplification manually versus using the QIAgility®. By comparing the difference in time and costs, it can be investigated if the benefits of the QIAgility® compensate for its costs.

3. Materials and Methods

3.1 Qiagen® QIAgility®

Prior to performing the cost-benefit study, an internal validation was first performed to validate the QIAgility® for use in the laboratory. The QIAgility® was manually calibrated for the tip ejector, plate positions, plate heights, and tip offset in accordance to Qiagen®'s QIAgility® User Manual [7]. According to the manual, the QIAgility® should be set-up away from heat and vibrations as they may skew the instrument's calibrations. However, the WVSPFL Biochemistry

Section's extraction room, where the QIAgility® resides, is within ~30 meters from railroad tracks that generate varying amounts of vibration. Through weekly verifications, the QIAgility® calibrations were slightly shifted during each check and it was uncertain if the vibrations from the train tracks were the cause. As a result, the QIAgility®'s calibrations at the WVSPFL were reviewed on a weekly schedule and updated if the calibrations were inaccurate to ensure the instrument functioned as intended.

The QIAgility® deck was initially set-up as imaged in Figure 1. Blocks A1, A2, and B1 were reserved for the use of QIAgility®'s 200 µl and 50 µl conductive filtered tips. Block M1contained the diluent and pre-mixed master mix. Block R1 held pre-mixed master mix, DNA quantitation standards, and reagents. Block B2 was used solely for samples. Block C1 was used solely for reactions. Block C2 was used for the purpose of holding samples and reactions. Table 1 details the plate types that were calibrated for each block for the validation. Depending on the procedure, the QIAgility®'s deck was changed to accommodate the needs of the analyst.

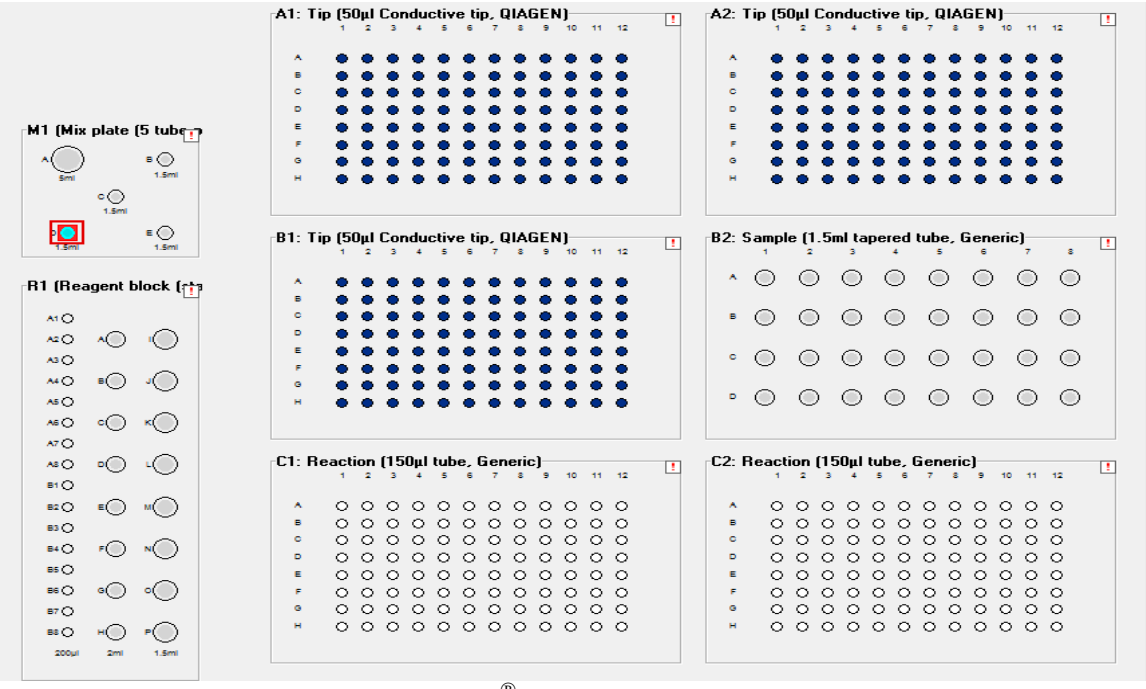

Figure 1. Initial set-up of the QIAgility® deck at the WVSPFL

Table 1. Plate type calibrations performed for each block

Block	**Calibrated Plate Type**
A1	200 µl and 50 µl conductive filtered tips
A2	50 µl conductive filtered tips
B1	50 µl conductive filtered tips
B2	32 generic 1.5 ml screw cap tapered tubes 32 generic 1.5 ml flip cap tapered tubes
C1	96 generic 200 µl tapered skirted
C2	96 generic 200 µl tapered skirted
M1	1x Qiagen® QIAgility® 5 ml, 4x Qiagen® 1.5 ml tapered screw caps 1x Qiagen® QIAgility® 5 ml, 4x Qiagen® 1.5 ml tapered hinged caps 1x Generic 5 ml, 4x Generic 1.5 ml tapered tubes
R1	16x generic 200 µl, 8x generic 2 ml free standing, 8x generic 1.5 ml tapered tubes

The QIAgility® software contains run settings and advanced settings that can be changed depending on the needs of the laboratory. To ensure proper pipetting during a run, the QIAgility®'s pipetting speeds were maintained at certain settings for the validation. Sample speed was maintained at 80 microliters/seconds (µl/s), diluent and master mix speed was maintained at 70 µl/s, reagent speed (normal) was maintained at 150 µl/s, intermediate reaction speed was maintained at 150 µl/s, reagent speed (viscous) was maintained at 70 µl/s, standards speed was maintained at 150 µl/s, and mixing speed was maintained at 150 µl/s. These settings were recommended by Qiagen® specialist Mark Guilliano and the QIAgility® is still capable of performing liquid handling accurately and precisely if all speeds were maintained at 150 µl/s. The QIAgility® can also be programmed to re-use a tip as long as the tip does not introduce contamination. For the validation, the tip re-use setting was set at four times for the maximum number of times a tip may be reused. The tip will only be re-used for the purposes of aliquoting master mix and diluent, not samples. A similar variable is the multi-eject function, where the QIAgility® functions like a repeater pipette and aspirates the same liquid to multiple wells. For the validation, the multi-eject function was not used. Another setting that can be changed is tip air intake. Tip air intake controls the amount of air that is taken up by the pipetting head prior to

taking liquid from the designated well. This helps to ensure no liquid remains inside the walls of the tip during ejection if the tip is to be re-used. For samples, reagent, standards, diluent, master mix, and intermediate reactions, the tip air intake was maintained at 7 µl, in accordance with the QIAgility® manual [7]. The QIAgility® software contains an interlock mechanism that can be turned off or on at three levels of sensitivity: ignore all errors, interrupt run on all serious errors, and interrupt run on all errors. The setting used for the validation was interrupt run on all errors, where if the QIAgility® detects an error such as "Unable to find liquid" or "Lid was lifted during UV'ing," the run will be interrupted and user action will be needed to resolve the error. The QIAgility®'s liquid sensing capabilities settings are: detect with liquid sensing, detect without liquid sensing, and detect with liquid sensing estimate. The QIAgility® was set to detect with liquid sensing for the validation to ensure proper pipetting was performed. The advanced setting contains the tip pickup mode. The QIAgility® can pick up a tip normally (stage one), or pick up a tip using a three stage process. Instead of punching the pipetting head down once, the three stage process guides the pipetting head down to the tip, then punches down two times to ensure the tip is locked securely. For the validation, the one stage tip pick-up mode was used. All other run and advanced settings such as master mix extra volume, standards extra volume, and concentration unit were not changed and remained as is in accordance with Qiagen®'s protocols [7].

3.2 DNA Extraction

All DNA samples used for the validation were previously extracted or extracted with the use of Qiagen®'s EZ1® Advanced Robot or Qiagen®'s EZ1® Advanced XL (Qiagen®, Hilden, Germany). The EZ1® protocols used for the extractions were trace and large volume with elution at 50 µl with TE^{-4} buffer.

3.3 DNA Quantitation

All DNA samples used for the validation were quantified using Applied Biosystems® Quantifiler® Duo kit (Life Technologies™, Foster City, CA) in a 96-well plate. For all of the studies, the QIAgility® was used to set-up all quantitation procedures. In addition, for the concordance study, a quantitation was set-up manually for comparison. The DNA samples were analyzed using Applied Biosystems® 7500 Fast Real-Time® PCR system in conjunction with the Software Detection System (SDS) version 1.2.3 for analysis.

3.4 DNA Normalization

All DNA samples used for the validation were normalized using the QIAgility® in a 96-well plate or 1.7 ml tubes for all studies. In addition, for the concordance study, a normalization was set-up manually for comparison.

3.5 DNA Amplification

All DNA samples used for the validation were amplified using Promega™ PowerPlex® 16 (PP16) amplification kit (Promega®, Madison, WI) in a 96-well plate. For all of the studies, the QIAgility® was used to set-up all amplifications. In addition, for the concordance study, an amplification was set-up manually for comparison. The DNA samples were amplified using Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler (9700) (Life Technologies™, Foster City, CA). The 9700 settings were in accordance with WVSPFL's DNA procedure manual [1].

3.6 Capillary Electrophoresis and DNA Analyzing

All DNA samples in this validation were analyzed using the Applied Biosystems® 3130 Genetic Analyzer (Life Technologies™, Foster City, CA) in conjunction with GeneMapper® ID v.3.2.1 (Life Technologies™, Foster City, CA). All capillary electrophoresis set-ups were

performed manually with injection durations at 5 seconds and 15 seconds. The analytical threshold at the WVSPFL is 100 relative fluorescence units (RFU), but a threshold of 60 RFU and 25 RFU were for investigative purposes. In the contamination study, the threshold was changed to 60 RFU and 25 RFU to investigate if potential contaminants were present in the TE^{-4} blanks under the 100 RFU analytical threshold. In the mixture study, the analytical threshold was changed to 25 RFU to investigate an allele dropout at D18S51.

3.7 Validation study

3.7.1 Accuracy and precision study

In this study, the QIAgility® was programmed to aliquot a full 96-well plate with TE^{-4} buffer for each of the following aliquots: 2 µl, 10 µl, 25 µl, 50 µl, 100 µl. The first set of data for the 50 µl aliquots were deemed to be sporadic and inconsistent. Because it was uncertain if the cause was due to the instrument or due to manual measurements, a second set of data was obtained to verify the QIAgility®'s accuracy and precision. To perform these aliquots, 200 µl and 50 µl Qiagen® conductive tips were used to pipette the liquid and a 5 ml Qiagen® tube and generic 1.7 ml tubes were used as needed. To verify if the QIAgility® was pipetting properly, calibrated pipettes were used to measure the aliquots. Table 2 lists the pipettes used, their respective serial number, when they were calibrated, and when calibrations will be due. Each measurement was recorded and the following statistical data was obtained: trend-line, R-squared (R^2), average, standard deviation and coefficient of variation (%CV).

Table 2. Pipette calibration information

Pipette	**Serial ID**	**Calibrated on**	**Calibrations due**
Gilson Pipetman P-200	DG58104	4/28/15	4/28/16
Gilson Pipetman P-100	DC37183	4/28/15	4/28/16
Gilson Pipetman P-20	DA59801	4/28/15	4/28/16
Gilson Pipetman P-10	DD50520	4/28/15	4/28/16

3.7.2 Contamination study

In this study, the samples used were listed in Table 3. The samples used in the contamination study were previous competency tests or training samples that previous analysts at WVSPFL have extracted, quantified, amplified with PP16, and fully genotyped. The QIAgility® was programmed to aliquot the forty samples and forty TE^{-4} blanks in the pattern shown in Figure 2. This pattern was to demonstrate if cross-contamination existed when the QIAgility® liquid handles samples. The sample set-up was taken from quantitation (Figure 3) to PCR amplification (Figure 4). As pictured, eight samples, highlighted in red, were normalized and loaded the DNA into the amplification tray with the 40 TE^{-4} blanks. Those eight samples were selected, because they bordered a TE^{-4} blank and a sample. For the TE^{-4} blanks, 15 µl of the sample were loaded for amplification, while the 8 samples in red were first normalized to 0.1 ng/µl, then 5 µl of each normalized sample was loaded for amplification. The samples and the forty TE^{-4} blanks were then analyzed via capillary electrophoresis and GeneMapper® ID version 3.2.1 (Figure 5). For capillary electrophoresis, the analytical threshold at the WVSPFL was set at 100 RFU. However, for investigative purposes, the threshold was changed to both 60 RFU and 25 RFU as well.

Table 3. List of sample ID and source of sample

Sample ID	Sample Source	Sample ID	Sample Source
Trn6 J.	Saliva sample	T6 Sperm	Differential sample
Trn16 AS	Saliva sample	Trn5 M:M Sp	Differential sample
Trn16 BH	Saliva sample	T16 JP	Saliva sample
Trn3 C1SP	Differential sample	T16 LP	Saliva sample
CA Swab	Competency sample – blood swab	T19 K1	Saliva sample
CBsp	Competency sample – semen swab	T19 K2	Saliva sample
CBEcell	Differential sample	T19 KB	Saliva sample
CBvicB	Differential sample	T19 MG	Saliva sample
T4 Sperm	Differential sample	T19 JE	Saliva sample
Trn7 Diff #1	Differential sample	Trn3 COSp	Differential sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	Trn6 J.	TE Blank	T4 Sperm	TE Blank	T19 K2	Trn6 J.	TE Blank	T4 Sperm	TE Blank	T19 K2	(blank)	(blank)
B	TE Blank	CA Swab	TE Blank	Trn5 M:M SP	TE Blank	TE Blank	CA Swab	TE Blank	Trn5 M:M SP	TE Blank	(blank)	(blank)
C	Trn16 AS	TE Blank	T6 Sperm	TE Blank	T19 KB	Trn16 AS	TE Blank	T6 Sperm	TE Blank	T19 KB	(blank)	(blank)
D	TE Blank	CBsp	TE Blank	T16 JP	TE Blank	TE Blank	CBsp	TE Blank	T16 JP	TE Blank	(blank)	(blank)
E	Trn16 BH	TE Blank	Trn7 Diff #1	TE Blank	T19 MG	Trn16 BH	TE Blank	Trn7 Diff #1	TE Blank	T19 MG	(blank)	(blank)
F	TE Blank	CBEcell	TE Blank	T16 LP	TE Blank	TE Blank	CBEcell	TE Blank	T16 LP	TE Blank	(blank)	(blank)
G	Trn3 C1SP	TE Blank	Trn3 CO SP	TE Blank	T19 JE	Trn3 C1SP	TE Blank	Trn3 CO SP	TE Blank	T19 JE	(blank)	(blank)
H	TE Blank	CBvicB	TE Blank	T19 K1	TE Blank	TE Blank	CBvicB	TE Blank	T19 K1	TE Blank	(blank)	(blank)

Figure 2. Contamination study – sample set-up in a 96-well tray

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	Trn6 J.	TE Blank	T4 Sperm	TE Blank	T19 K2	Trn6 J.	TE Blank	T4 Sperm	TE Blank	T19 K2
B	STD 2	STD 2	TE Blank	CA Swab	TE Blank	Trn5 M:M SP	TE Blank	TE Blank	CA Swab	TE Blank	Trn5 M:M SP	TE Blank
C	STD 3	STD 3	Trn16 AS	TE Blank	T6 Sperm	TE Blank	T19 KB	Trn16 AS	TE Blank	T6 Sperm	TE Blank	T19 KB
D	STD 4	STD 4	TE Blank	CBsp	TE Blank	T16 JP	TE Blank	TE Blank	CBsp	TE Blank	T16 JP	TE Blank
E	STD 5	STD 5	Trn16 BH	TE Blank	Trn7 Diff #1	TE Blank	T19 MG	Trn16 BH	TE Blank	Trn7 Diff #1	TE Blank	T19 MG
F	STD 6	STD 6	TE Blank	CBEcell	TE Blank	T16 LP	TE Blank	TE Blank	CBEcell	TE Blank	T16 LP	TE Blank
G	STD 7	STD 7	Trn3 C1SP	TE Blank	Trn3 CO SP	TE Blank	T19 JE	Trn3 C1SP	TE Blank	Trn3 CO SP	TE Blank	T19 JE
H	STD 8	STD 8	TE Blank	CBvicB	TE Blank	T19 K1	TE Blank	TE Blank	CBvicB	TE Blank	T19 K1	TE Blank

Figure 3. Contamination study – Quantitation set-up with QIAgility®

	1	2	3	4	5	6	7	8	9	10	11	12
A	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 K2	AmpPOS	(blank)	0	0	0	0
B	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 KB	AmpNEG	(blank)	0	0	0	0
C	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 MG	BLANK	(blank)	0	0	0	0
D	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 JE	(blank)	(blank)	0	0	0	0
E	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn6 J.	(blank)	(blank)	0	0	0	0
F	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn16 AS	(blank)	(blank)	0	0	0	0
G	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn16 BH	(blank)	(blank)	0	0	0	0
H	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn3 C1SP	(blank)	(blank)	0	0	0	0

Figure 4. Contamination study – Amplification set-up with QIAgility®

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn16 BH	(blank)	0	0	0	0
B	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 K2	Trn3 C1SP	(blank)	0	0	0	0
C	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 KB	AmpPOS	(blank)	0	0	0	0
D	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 MG	AmpNEG	(blank)	0	0	0	0
E	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	T19 JE	Ladder	(blank)	0	0	0	0
F	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn6 J.	(blank)	(blank)	0	0	0	0
G	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Trn16 AS	(blank)	(blank)	0	0	0	0
H	TE-Blank	TE-Blank	TE-Blank	TE-Blank	TE-Blank	Ladder	(blank)	(blank)	0	0	0	0

Figure 5. Contamination study – Capillary Electrophoresis Manual Set-Up

3.7.3 Sensitivity study

The sample used in the sensitivity study was a known sample that was previously extracted, quantified, amplified with PP16, and fully genotyped. First, the study defined a high-

level DNA sample as having a concentration of 5 ng/µl or greater. The sample was previously quantitated with Quantifiler® Duo resulted in a concentration of 5.28 ng/µl. Because the sensitivity study's purpose was to verify if the QIAgility® can liquid handle high-level and low-level DNA samples, the QIAgility® was then programmed to perform a serial dilution of the sample. The sample's concentration was serially diluted by 3 folds 6 times and quantitated using Quantifiler® Duo (Figure 6). The quantitation was expected to yield the following concentrations: 5.28 ng/µl, 1.76 ng/µl, 0.58 ng/µl, 0.19 ng/µl, 0.065 ng/µl, 0.0215 ng/µl, and 0.0073 ng/µl. Next, 1 µl of each serially diluted sample was loaded for amplification with PP16 in triplicate to generate statistical data (Figure 7). After amplification, the samples were run using capillary electrophoresis and GeneMapper® ID version 3.2.1 (Figure 8).

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	HBM100 - 1	HBM100 - 1	HBM100 - 1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	STD 2	STD 2	HBM100 - 2	HBM100 - 2	HBM100 - 2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	STD 3	STD 3	HBM100 - 3	HBM100 - 3	HBM100 - 3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	STD 4	STD 4	HBM100 - 4	HBM100 -4	HBM100 -4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	STD 5	STD 5	HBM100 - 5	HBM100 - 5	HBM100 - 5	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	STD 6	STD 6	HBM100 - 6	HBM100 - 6	HBM100 - 6	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	STD 7	STD 7	HBM100 - 7	HBM100 - 7	HBM100 - 7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	STD 8	STD 8	NTC	NTC	NTC	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 6. Sensitivity Study – Quantitation set-up using QIAgility

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	HBM100-3	HBM100-6	AmpNEG.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	HBM100-1	HBM100-3	HBM100-6	AmpNEG.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	HBM100-1	HBM100-4	HBM100-6	BLANK	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	HBM100-1	HBM100-4	HBM100-7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	HBM100-2	HBM100-4	HBM100-7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	HBM100-2	HBM100-5	HBM100-7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	HBM100-2	HBM100-5	AmpPOS.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	HBM100-3	HBM100-5	AmpPOS.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 7. Sensitivity Study – Amplification set-up using QIAgility®

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	HBM100-3	HBM100-6	AmpNEG.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	HBM100-1	HBM100-3	HBM100-6	AmpNEG.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	HBM100-1	HBM100-4	HBM100-6	Ladder	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	HBM100-1	HBM100-4	HBM100-7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	HBM100-2	HBM100-4	HBM100-7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	HBM100-2	HBM100-5	HBM100-7	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	HBM100-2	HBM100-5	AmpPOS.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	HBM100-3	HBM100-5	AmpPOS.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 8. Sensitivity Study – Capillary Electrophoresis manual set-up

3.7.4 Mixture study

The two known samples used for this study were previously quantified, amplified with PP16, and genotyped at the WVSPFL. The two samples were used to generate the mixture ratios as listed in Table 4. These mixture ratios were chosen, because they were believed to most closely mimic sample concentrations often seen in casework samples at the WVSPFL. Using the QIAgility®'s sample pooling function, a sample pool protocol was set-up for a 96-well plate and used to generate the mixture samples (Figure 9). Afterwards, the mixture samples were quantified with Quantifiler® Duo in triplicate and their concentrations were noted (Figure 10). Because the study was to attest if the QIAgility® can liquid handle mixture samples without affecting the samples' integrity, it was deemed less important to confirm if the mixture samples' ratios were properly set-up, but it was more important to determine if the samples were a mixture. Because the samples were confirmed to be mixtures, 5 µl of each mixture sample were first loaded as a single set for amplification with PP16 (Figure 11). Next a second and third set were loaded for amplification using PP16 (Figure 12). This was done due to the potential lack of available sample volumes for the QIAgility® to liquid handle. The amplified products were then analyzed through capillary electrophoresis and GeneMapper® ID version 3.2.1(Figures 13 and 14).

Table 4. Mixture Study – Sample mixture ratios

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Male:Female	**1:1**	**1:3**	**1:4**	**1:8**	**3:1**	**4:1**	**8:1**

	1	2	3	4	5	6	7	8	9	10	11	12
A	M1:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	M3:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	M4:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	M8:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	M1:F3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 9. Mixture Study – sample set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	M1:F1	M4:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	STD 2	STD 2	M1:F1	M8:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	STD 3	STD 3	M1:F1	M8:F1	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	STD 4	STD 4	M3:F1	M8:F1	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	STD 5	STD 5	M3:F1	M1:F3	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	STD 6	STD 6	M3:F1	M1:F3	NTC	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	STD 7	STD 7	M4:F1	M1:F3	NTC	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	STD 8	STD 8	M4:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 10. Mixture Study – Quantitation Set-up with QIAgility®

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	M1:F1	AmpPOS 1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	M3:F1	AmpNEG 1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	M4:F1	BLANK	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	M3:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	M8:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	M1:F3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 11. Mixture Study – Amplification, single set-up with QIAgility®

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	M8:F1	AmpNEG1.2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	M1:F1	M1:F3	BLANK	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	M1:F1	M1:F3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	M3:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	M3:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	M4:F1	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	M4:F1	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	M8:F1	AmpPOS 1.2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 12. Mixture Study – Amplification, second and third set-up with QIAgility®

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	M1:F1	AmpPOS 1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	M3:F1	AmpNEG 1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	M4:F1	Ladder	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	M3:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	M8:F1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	M1:F3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 13. Mixture Study – Capillary Electrophoresis manual single set

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	M8:F1	AmpNEG1.2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	M1:F1	M1:F3	Ladder	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	M1:F1	M1:F3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	M3:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	M3:F1	M1:F4	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	M4:F1	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	M4:F1	M1:F8	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	M8:F1	AmpPOS 1.2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 14. Mixture Study – Capillary Electrophoresis manual second and third set

3.7.5 Concordance study

The samples used in the concordance study were known and non-probative samples. The study contained one questioned sample and all other samples were known samples that have been previously extracted, quantified, amplified with PP16, and fully genotyped. The samples are as follows: one buccal swab, one cigarette filter, two blood swabs, two seminal fluid swabs, and two skin cell samples. The questioned sample was the cigarette filter. These samples were chosen for two reasons: 1) confirm if the QIAgility® can liquid handle casework samples without affecting the samples' integrity and 2) these samples most closely mimic samples often obtained for casework at the WVSPFL.

A quantitation set-up using Quantifiler® Duo of these samples was first performed manually to assess the samples' concentrations (Figure 15). Next, the QIAgility® was used to set-up the same quantitation set-up with Quantifiler® Duo as performed manually. However, due to an error in pipetting, some samples from the manual quantitation set-up were repeated on the plate being used for the automated quantitation in columns 8 – 10 (Figure 16). After quantitation, the samples were normalized using the QIAgility® and manually to a 0.1 ng/µl concentration before amplification with PP16 (Tables 5 and 6). For amplification, the same 96-well plate was used for both set-ups (Figure 17). Columns 1-3 of the plate contained the manual set-up, while columns 7-9 contained the QIAgility® set-up. The manual set-up was first performed and then capped using strip tube caps to prevent potential contamination during the QIAgility® set-up. Afterwards, the amplified products were analyzed using capillary electrophoresis in conjunction with GeneMapper® ID version 3.2.1(Figure 18).

In addition to comparing the DNA set-ups, this study compared manual-made quantitation standard curves made by another analyst to the QIAgility®-made quantitation

standard curves. The standard curves' R^2 and slope values were averaged and compared to the R^2 and slope's optimal values; 0.99 and -3.2, to note which set was closer to the optimal results.

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	Cig.Fil.1	Blood_AKG.3	Ecell_AKG.2	QRC-1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
B	STD 2	STD 2	Cig.Fil.2	Blood_KKP.1	Ecell_AKG.3	QRC-1.2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
C	STD 3	STD 3	Cig.Fil.3	Blood_KKP.2	Semen_DWM-1.1	QRC-1.3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	STD 4	STD 4	Buccal_AR.1	Blood_KKP.3	Semen_DWM-1.2	NTC	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	STD 5	STD 5	Buccal_AR.2	Ecell_BEH.1	Semen_DWM-1.3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	STD 6	STD 6	Buccal_AR.3	Ecell_BEH.2	Semen_DWM-2.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	STD 7	STD 7	Blood_AKG.1	Ecell_BEH.3	Semen_DWM-2.2	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	STD 8	STD 8	Blood_AKG.2	Ecell_AKG.1	Semen_DWM-2.3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 15. Concordance Study – Quantitation set-up for manual #1

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	Cig.Fil.1	Blood_AKG.3	Ecell_AKG.2	QRC-1.1	(blank)	STD 1	STD 1	Blood_KKP.2	(blank)	(blank)
B	STD 2	STD 2	Cig.Fil.2	Blood_KKP.1	Ecell_AKG.3	QRC-1.2	(blank)	STD 2	STD 2	Blood_KKP.3	(blank)	(blank)
C	STD 3	STD 3	Cig.Fil.3	Blood_KKP.2	Semen_DWM-1.1	QRC-1.3	(blank)	STD 3	STD 3	Ecell_BEH.1	(blank)	(blank)
D	STD 4	STD 4	Buccal_AR.1	Blood_KKP.3	Semen_DWM-1.2	NTC	(blank)	STD 4	STD 4	Semen_DWM-1.1	(blank)	(blank)
E	STD 5	STD 5	Buccal_AR.2	Ecell_BEH.1	Semen_DWM-1.3	(blank)	(blank)	STD 5	STD 5	Semen_DWM-1.2	(blank)	(blank)
F	STD 6	STD 6	Buccal_AR.3	Ecell_BEH.2	Semen_DWM-2.1	(blank)	(blank)	STD 6	STD 6	Semen_DWM-1.3	(blank)	(blank)
G	STD 7	STD 7	Blood_AKG.1	Ecell_BEH.3	Semen_DWM-2.2	(blank)	(blank)	STD 7	STD 7	(blank)	(blank)	(blank)
H	STD 8	STD 8	Blood_AKG.2	Ecell_AKG.1	Semen_DWM-2.3	(blank)	(blank)	STD 8	STD 8	(blank)	(blank)	(blank)

Figure 16. Concordance Study – Quantitation set-up for QIAgility® (columns 1-6) and manual #2 (columns 8-10)

Table 5. Concordance Study – Manual normalization

Manual	Action	C1 (ng/ µl)	V1 (µl)	C2 (ng/ µl)	V2 (µl)	Diluent (µl)
Cig. Fil	Amp Neat	0.103				
Buccal_AR	Dilute	8.31	1.203369434	0.1	100	98.79663057
Blood_AKG	Dilute	1.037	4.821600771	0.1	50	45.17839923
Blood_KKP	Dilute	6.153	1.625223468	0.1	100	98.37477653
Ecell_BEH	Amp Neat	0.002333				
Ecell_AKG	Amp Neat	0.0043333				
Semen_DWM-1	Dilute	21.79333	1.147139974	0.1	250	248.85286
Semen_DWM-2	Dilute	25.12	1.194267516	0.1	300	298.8057325
QRC-1	Amp Neat	0				

Table 6. Concordance Study – QIAgility® normalization

QIAgility®	Action	C1 (ng/ µl)	V1 (µl)	C2 (ng/ µl)	V2 (µl)	Diluent (µl)
Cig. Fil	Amp Neat	0.134				
Buccal_AR	Dilute	7.64	1.308900524	0.1	100	98.69109948
Blood_AKG	Dilute	0.947333	5.277975115	0.1	50	44.72202488
Blood_KKP	Dilute	5.22	1.915708812	0.1	100	98.08429119
Ecell_BEH	Amp Neat	0.002333				
Ecell_AKG	Amp Neat	0.003				
Semen_DWM-1	Dilute	22.373	1.117418317	0.1	250	248.8825817
Semen_DWM-2	Dilute	23.15	1.295896328	0.1	300	298.7041037
QRC-1	Amp Neat	0				

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	Blood_AKG.2	Semen_DWM-2.1	(blank)	(blank)	(blank)	BLANK	ABlood_AKG.2	ASemen_DWM-2.1	(blank)	(blank)	(blank)
B	Cig.Fil.1	Blood_AKG.3	Semen_DWM-2.2	(blank)	(blank)	(blank)	ACig.Fil.1	ABlood_AKG.3	ASemen_DWM-2.2	(blank)	(blank)	(blank)
C	Cig.Fil.2	Blood_KKP.1	Semen_DWM-2.3	(blank)	(blank)	(blank)	ACig.Fil.2	ABlood_KKP.1	ASemen_DWM-2.3	(blank)	(blank)	(blank)
D	Cig.Fil.3	Blood_KKP.2	AmpPOS1	(blank)	(blank)	(blank)	ACig.Fil.3	ABlood_KKP.2	AQRC-1.1	(blank)	(blank)	(blank)
E	Buccal_AR.1	Blood_KKP.3	AmpNEG1	(blank)	(blank)	(blank)	Buccal_AR.1	ABlood_KKP.3	AQRC-1.1	(blank)	(blank)	(blank)
F	Buccal_AR.2	Semen_DWM-1.1	QRC-1.1	(blank)	(blank)	(blank)	Buccal_AR.2	ASemen_DWM-1.1	AQRC-1.1	(blank)	(blank)	(blank)
G	Buccal_AR.3	Semen_DWM-1.2	QRC-1.1	(blank)	(blank)	(blank)	Buccal_AR.3	ASemen_DWM-1.2	AAmpPOS1	(blank)	(blank)	(blank)
H	Blood_AKG.1	Semen_DWM-1.3	QRC-1.1	(blank)	(blank)	(blank)	ABlood_AKG.1	ASemen_DWM-1.3	AAmpNEG1	(blank)	(blank)	(blank)

Figure 17. Concordance Study – Amplification set-up for manual (columns 1 - 3) and QIAgility® (columns 7 - 9).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	Blood_AKG.2	Semen_DWM-2.1	Ladder	ABlood_AKG.2	ASemen_DWM-2.1	AmpNEG1	(blank)	(blank)	(blank)	(blank)	(blank)
B	Cig.Fil.1	Blood_AKG.3	Semen_DWM-2.2	ACig.Fil.1	ABlood_AKG.3	ASemen_DWM-2.2	Ladder	(blank)	(blank)	(blank)	(blank)	(blank)
C	Cig.Fil.2	Blood_KKP.1	Semen_DWM-2.3	ACig.Fil.2	ABlood_KKP.1	ASemen_DWM-2.3	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
D	Cig.Fil.3	Blood_KKP.2	AmpPOS1	ACig.Fil.3	ABlood_KKP.2	AQRC-1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
E	Buccal_AR.1	Blood_KKP.3	AmpNEG1	Buccal_AR.1	ABlood_KKP.3	AQRC-1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
F	Buccal_AR.2	Semen_DWM-1.1	QRC-1.1	Buccal_AR.2	ASemen_DWM-1.1	AQRC-1.1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
G	Buccal_AR.3	Semen_DWM-1.2	QRC-1.1	Buccal_AR.3	ASemen_DWM-1.2	AAmpPOS1	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)
H	Blood_AKG.1	Semen_DWM-1.3	QRC-1.1	ABlood_AKG.1	ASemen_DWM-1.3	Ladder	(blank)	(blank)	(blank)	(blank)	(blank)	(blank)

Figure 18. Concordance Study – Capillary electrophoresis manual set-up

3.8 Cost-benefit study

Because each laboratory can choose to program the QIAgility®'s settings differently and the study's purpose was to assess the cost-benefits of using a QIAgility®, the QIAgility®'s protocol set-ups for this study were created mimicking, to a certain degree, the manual set-up of how an analyst performs a quantitation, normalization, or amplification set-up at the WVSPFL. By doing this, more consistent comparisons and results can be obtained between the automated and manual set-ups.

The number of consumables used for quantitation, normalization, and amplification as well as the time it took for each protocol to be completed was evaluated for the QIAgility® by using the virtual mode. The number of consumables used for the manual set-up was assessed through counting and tallying the items used during a manual set-up. The time for each protocol to be completed manually was measured through averaging the different times the DNA analysts at the WVSPFL's Biochemistry Section estimated they need to set-up each procedure with 30, 50, and 80 samples.

Aside from limiting the number of variables, several parameters were set to simplify the comparison. The parameters are as follow: 1) It was assumed that the set-ups for both automated and manual contained no mistakes or errors, 2) The time measured to complete each protocol disregards any time spent on filling out paperwork and time spent on making the QIAgility® protocol, 3) The time measured to complete each protocol disregards potential laboratory interruptions, and 4) The potential issue of breaking up casework batches was disregarded and assumed all samples were taken through quantitation, normalization, and amplification in this study.

The number of samples used for the cost-benefit study contains a quantitation negative control and PCR amplification controls. As such, for quantitation, 79 samples, 49 samples, and 29 samples were chosen with one quantitation negative. For normalization, the sample numbers were 79, 49, and 29. For amplification, the sample numbers were 81, 51, and 31, which contained one AmpPOS and AmpNEG in each sample pool. These numbers were chosen, because batching casework samples can range from 10-15 samples per analyst and a batch may contain 2-4 different analysts. Consequently, to encompass this wide range, 3 different numbers were chosen to assess a range of the potential costs and benefits. Eighty samples were chosen as it was the maximum number of samples a 96-well plate can hold for a DNA quantitation with 2 columns of standards. The number 50 was chosen with respect to the number 80; it was a number that was above half a plate. The number 30 was chosen, because this was the typical number of samples the WVSPFL's Biochemistry Section process at one time.

A manual DNA quantitation set-up uses Quantifiler® Duo quantitation kit, 96 optical well plate, 0.5 ml tubes for quantitation standards, 1.7 ml tube for master mix, pipette tips of varying volumes, a repeater pipette tip, and optical film with applicator. For the number of overage

reactions, the WVSPFL uses an overage of +1 reaction per 10 reactions. The QIAgility® set-up used Quantifiler® Duo quantitation kit, 96 optical well plate, 8-tube strip for quantitation standards, Qiagen® 5 ml or 1.7 ml tube for master mix, pipette tips of varying volumes to make the master mix off the QIAgility® deck, Qiagen® 50 µl conductive filtered tips, and optical film with applicator. For the number of overage reactions, the QIAgility® generated the number of overages needed per set-up.

The manual set-up for DNA normalization used 1.7 ml tubes for samples, Invitrogen (Invitrogen Corporation, Carlsbad, CA) water as diluent, and pipette tips of varying volumes. The QIAgility® set-up uses a 96 optical well plate for samples, Invitrogen water as diluent, Qiagen® 50 µl conductive filtered tips, and 8-strip tube caps.

The manual set-up for DNA amplification uses Promega® PowerPlex® 16 amplification kit, AmpliTaq Gold® polymerase, 0.5 ml micro-amp tubes for samples, pipette tips of varying volumes, UV-protected 1.7 ml tube for master mix, and a repeater pipette tip. For the number of overage reactions, the WVSPFL uses an overage of +1 reaction per 10 reactions. The QIAgility® set-up uses Promega® PowerPlex® 16 amplification kit, AmpliTaq Gold® polymerase, a 96 optical well tray for samples, Qiagen® 50 µl conductive filtered tips, a 1.7 ml tube for master mix, pipette tips of varying volumes to make the master mix off the QIAgility® deck, and 8-strip tube caps. For the number of overage reactions, the QIAgility® generated the number of overages needed per set-up.

By determining the price per unit for each consumable, a comparison of the consumable costs between the QIAgility® set-up and manual set-up could be determined. The costs of some items were disregarded, because they were the same for both processes or considered negligible. This included: the costs for Invitrogen water, waste disposal, optical film, costs associated with

Applied Biosystem® 7500 Real-Time PCR and SDS, costs associated with Applied Biosystems® GeneAmp® PCR system 9700 thermal cycler, and pipette calibrations.

In regards to the time saved from using the QIAgility® versus manually setting up the DNA procedures, the time was quantified into units of estimated annual salary / estimated minutes an analyst works in the laboratories. An estimated annual salary of $30,000, $40,000, and $60,000 was used for the calculations. The estimated minutes an analyst works in the laboratories in a year were derived by accounting the number of holidays observed in West Virginia and excluding Saturdays and Sundays, 247 working days. The number of working days was then divided by eight hours per day and then converted to minutes to account for the number of minutes worked in year. Next, the cost per minute for an analyst to work in a laboratory was determined by dividing the estimated annual salary by the estimated minutes an analyst works in the laboratory in a year. For an analyst with an average salary of $30,000, $40,000, and $60,000, the cost would be $0.25/minute, $0.34/minute, and $0.51/minute in the laboratory, respectively. Lastly, the time difference between setting up each DNA procedure using the QIAgility® and manual set-up was then determined. Determining these two factors can help a DNA forensic laboratory determine the amount of money saved if the QIAgility® is used to set-up a quantitation, normalization, and amplification.

4. Results

4.1 Accuracy and precision study

For the 2 µl aliquots, the QIAgility® produced an average of 1.94 µl when programmed to aliquot into 96 wells using 50 µl conductive filtered tips (Figure 19). The standard deviation for precision was 0.11 and for accuracy was 0.040. The trendline was y = -0.0026x + 2.0699 and

the R^2 value was 0.4807 (Figure 20). The coefficient of variation (%CV) for precision was 5.4% and for accuracy was 2.1%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
B	2 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
C	2 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
D	2 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
E	2 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
F	2 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
G	2.5 µl	2 µl	2 µl	2 µl	2 µl	1.9 µl	2 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl
H	2 µl	2 µl	2 µl	2 µl	1.8 µl	1.9 µl	2 µl	2 µl	1.8 µl	1.8 µl	1.8 µl	1.8 µl

Figure 19. Accuracy and Precision study – 2 µl aliquot data

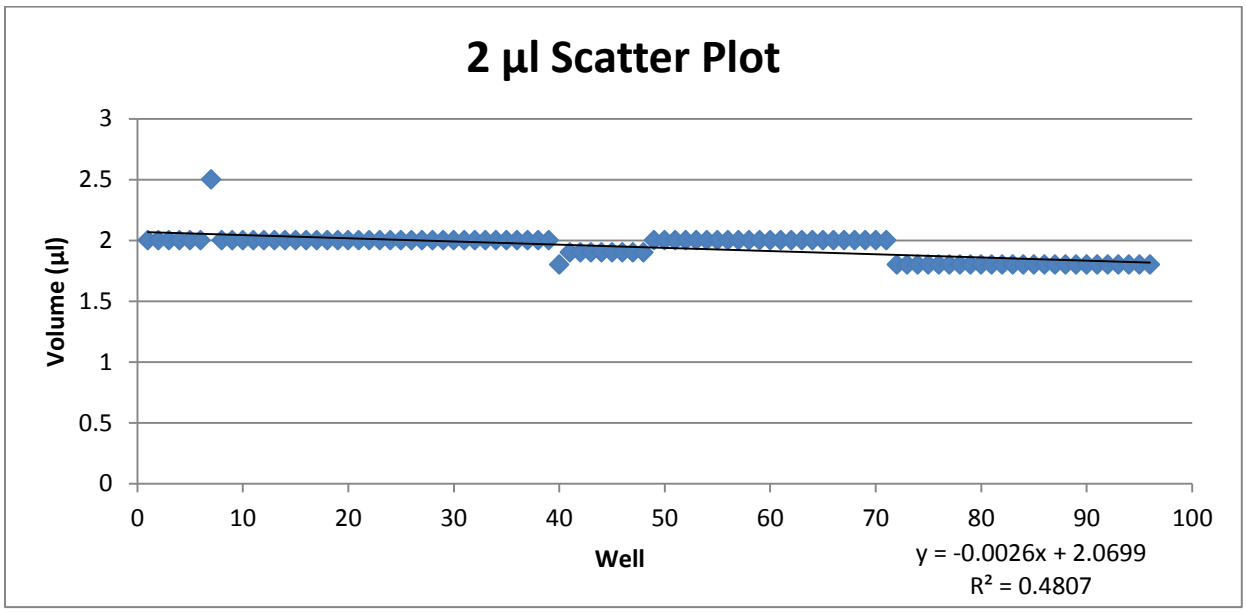

Figure 20. Accuracy and Precision study – 2 µl aliquot scatter plot

For the 10 µl aliquots, the QIAgility® produced an average of 9.93 µl when programmed to aliquot into 96 wells using 50 µl conductive filtered tips (Figure 21). The standard deviation for precision was 0.23 and for accuracy was 0.05. The trendline was y = 0.0006x + 9.9046 and the R^2 value was 0.0054 (Figure 22). The %CV for precision was 2.3% and for accuracy was 0.46%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	9 µl	10 µl	10 µl	10 µl	10.5 µl	10 µl	10 µl	10 µl	10 µl	10 µl	9.5 µl	10.2 µl
B	10 µl	10 µl	10 µl	9.5 µl	10 µl	9.6 µl	9.8 µl	9.5 µl	9.5 µl	10 µl	10 µl	10.2 µl
C	9.5 µl	10 µl	10 µl	10 µl	10 µl	9.6 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10.2 µl
D	10 µl	10 µl	10 µl	10 µl	10 µl	9.6 µl	10 µl	10 µl	10 µl	10 µl	9.5 µl	10.2 µl
E	9.5 µl	11 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	9.5 µl	9.5 µl	10.3 µl
F	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	9.5 µl	10 µl	10 µl	10.2 µl
G	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	9.7 µl	10.2 µl
H	10 µl	10 µl	9.5 µl	10 µl	10 µl	10 µl	10 µl	10 µl	9.5 µl	10 µl	9.7 µl	10.2 µl

Figure 21. Accuracy and Precision study – 10 µl aliquot data

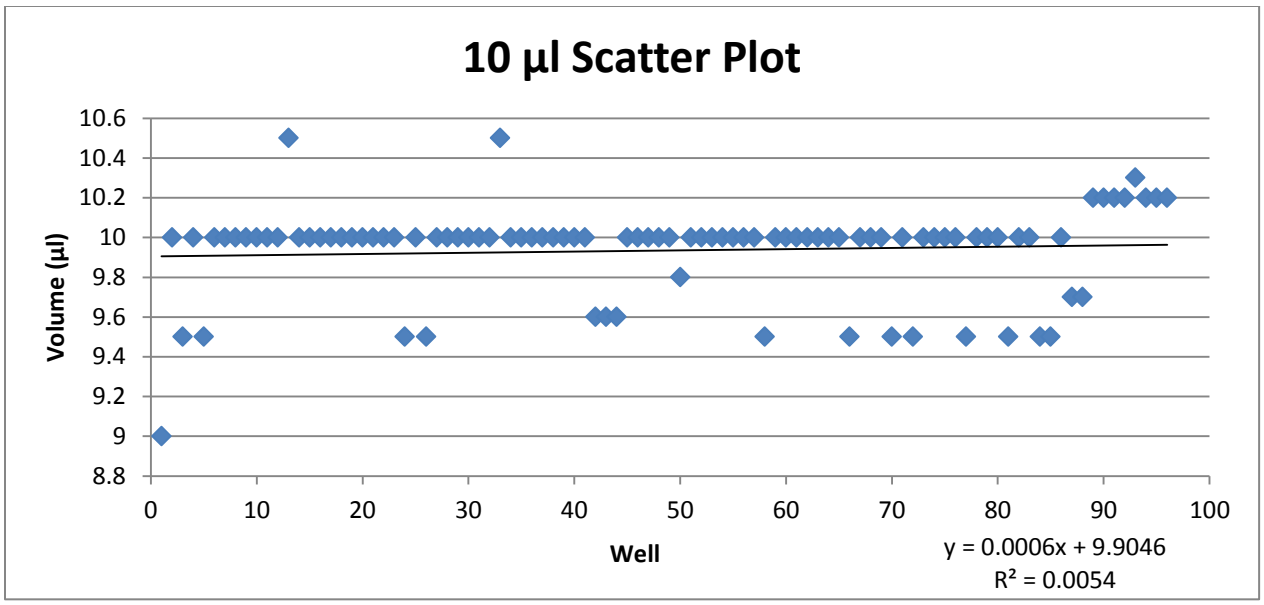

Figure 22. Accuracy and Precision study – 10 µl scatter plot

For the 25 µl aliquots, the QIAgility® produced an average of 25 µl when programmed to aliquot into 96 wells using 50 µl conductive filtered tips (Figure 23). The standard deviation for precision was 0.03 and for accuracy was 0.002. The trendline was y = 0.00001x + 25.002 and R^2 value was 0.0002 (Figure 24). The %CV for precision was 0.12% and for accuracy was 0.00833%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
B	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
C	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
D	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25.3 µl	25 µl	25 µl	25 µl	25 µl	25 µl
E	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
F	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
G	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
H	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl

Figure 23. Accuracy and Precision study – 25 µl Aliquot Data

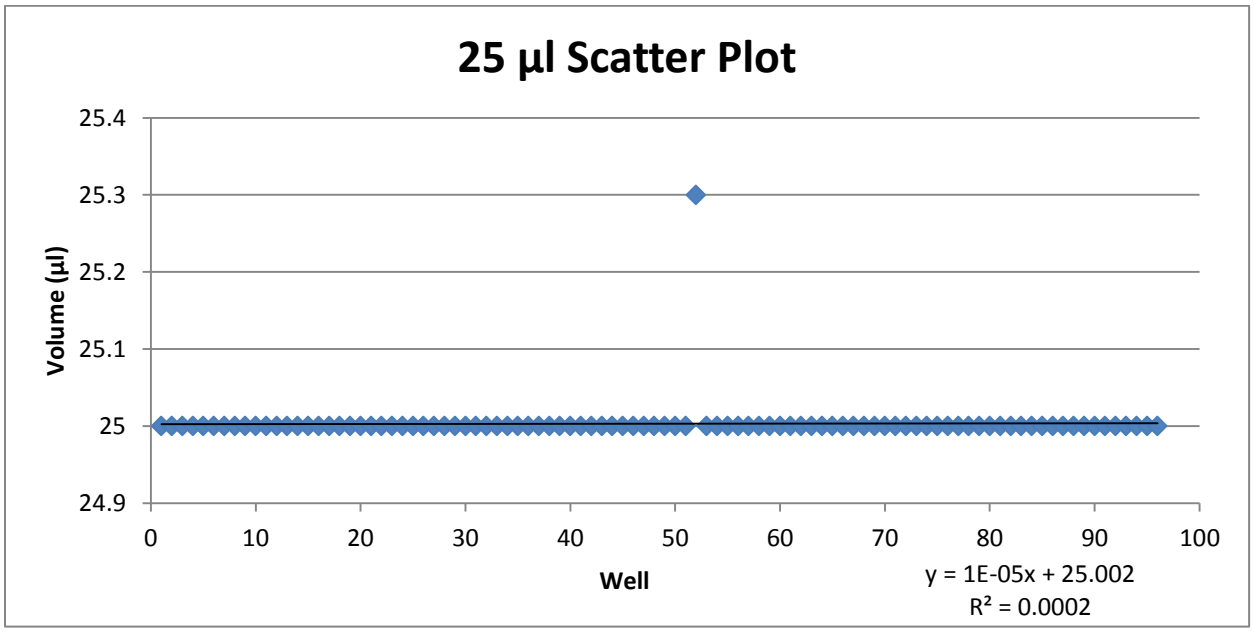

Figure 24. Accuracy and Precision study – 25 µl scatter plot

For the 50 µl aliquots, the QIAgility® produced an average of 50.72 µl when programmed to aliquot into 96 wells using 50 µl conductive filtered tips (Figure 25).The standard deviation for precision was 1.03 and accuracy was 0.51. The trendline was y = -0.0213x + 51.75 and R^2 value was 0.3338 (Figure 26). Because the aliquots were sporadic, the 50 µl aliquots were repeated, which produced an average of 50 µl when programmed to aliquot into 96 wells using 50 µl conductive filtered tips (Figure 27). The standard deviation for precision was 0.06 and accuracy was 0.001. The trendline was y = -0.000009x + 50.003 and R^2 value was 0.00002

(Figure 28). The %CV for the 50 µl for precision was 2.02% and for accuracy was 1.00%. The %CV for the 50 µl repeat for precision was 0.12% and for accuracy was 0.00029%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	49 µl	53 µl	52 µl	52.5 µl	50 µl	50 µl	50 µl	51 µl	50 µl	50 µl	50 µl	50 µl
B	50 µl	53 µl	52 µl	52 µl	50 µl	50 µl	50 µl	51 µl	50 µl	50 µl	50 µl	50 µl
C	50.5 µl	53 µl	52 µl	52 µl	50 µl	50 µl	50 µl	51 µl	50 µl	50 µl	50 µl	50 µl
D	50.5 µl	53 µl	52 µl	52 µl	50 µl	50 µl	50 µl	51 µl	50 µl	51 µl	50 µl	50 µl
E	50.5 µl	53 µl	52 µl	52.5 µl	50 µl	50 µl	50 µl	51 µl	50 µl	50 µl	50 µl	50 µl
F	50.5 µl	53 µl	52 µl	52 µl	50 µl	50 µl	50 µl	51 µl	50 µl	50 µl	50 µl	50 µl
G	50.5 µl	52 µl	52 µl	52 µl	50 µl	50 µl	51 µl	51 µl	50 µl	50 µl	50 µl	50 µl
H	50.5 µl	52.5 µl	51.5 µl	53 µl	51 µl	50 µl	50 µl	51 µl	50 µl	50 µl	50 µl	50 µl

Figure 25. Accuracy and precision study – 50 µl aliquot data

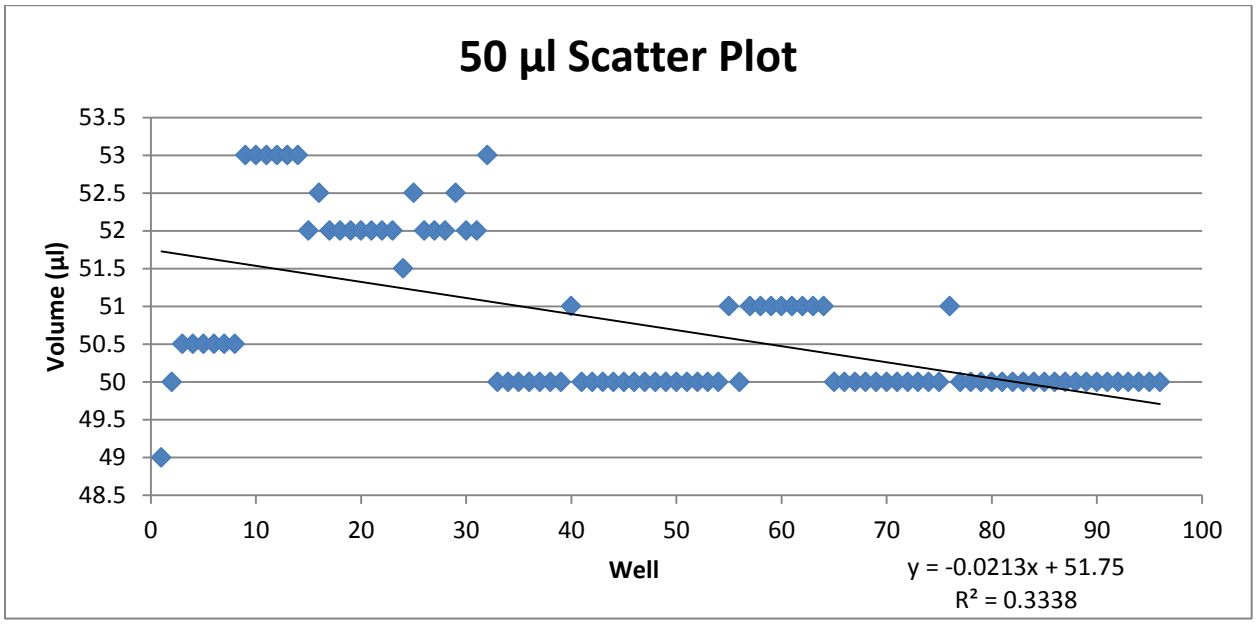

Figure 26. Accuracy and precision study – 50 µl scatter plot

	1	2	3	4	5	6	7	8	9	10	11	12
A	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
B	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
C	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
D	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50.5 µl	50 µl	50 µl	50 µl	50 µl
E	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
F	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	49.7 µl	50 µl	50 µl	50 µl
G	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
H	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl

Figure 27. Accuracy and precision study – 50 µl repeat aliquot data

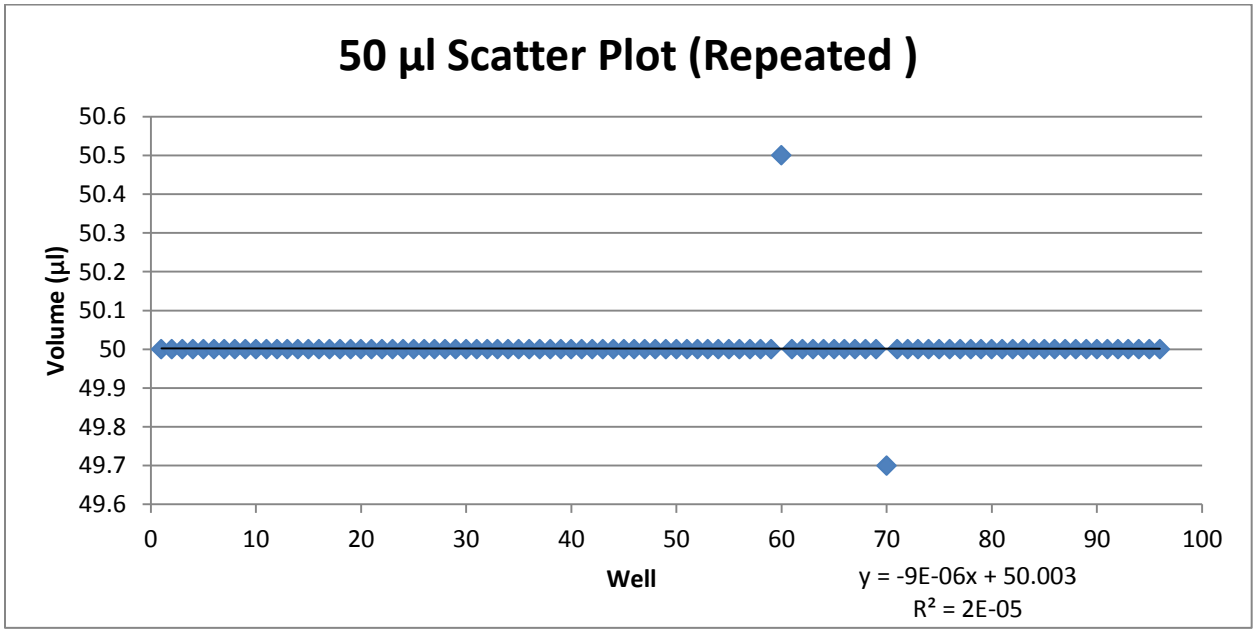

Figure 28. Accuracy and precision study – 50 µl repeat scatter plot

For the 100 µl aliquots, the QIAgility® produced an average of 102.04 µl when programmed to aliquot into 96 wells using 200 µl conductive filtered tips (Figure 29). The standard deviation for precision was 0.23 and accuracy was 1.44. The trendline was y = -0.0019x + 102.13 and R^2 value was 0.055 (Figure 30). The %CV for precision was 0.221% and for accuracy was 1.41%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	103 µl	102 µl	102.5 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl
B	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl
C	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl
D	102.5 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	103 µl
E	102 µl	102 µl	103 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl
F	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	101 µl	102 µl
G	102.5 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl
H	102 µl	102 µl	102.5 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl	102 µl

Figure 29. Accuracy and Precision study – 100 µl aliquot data

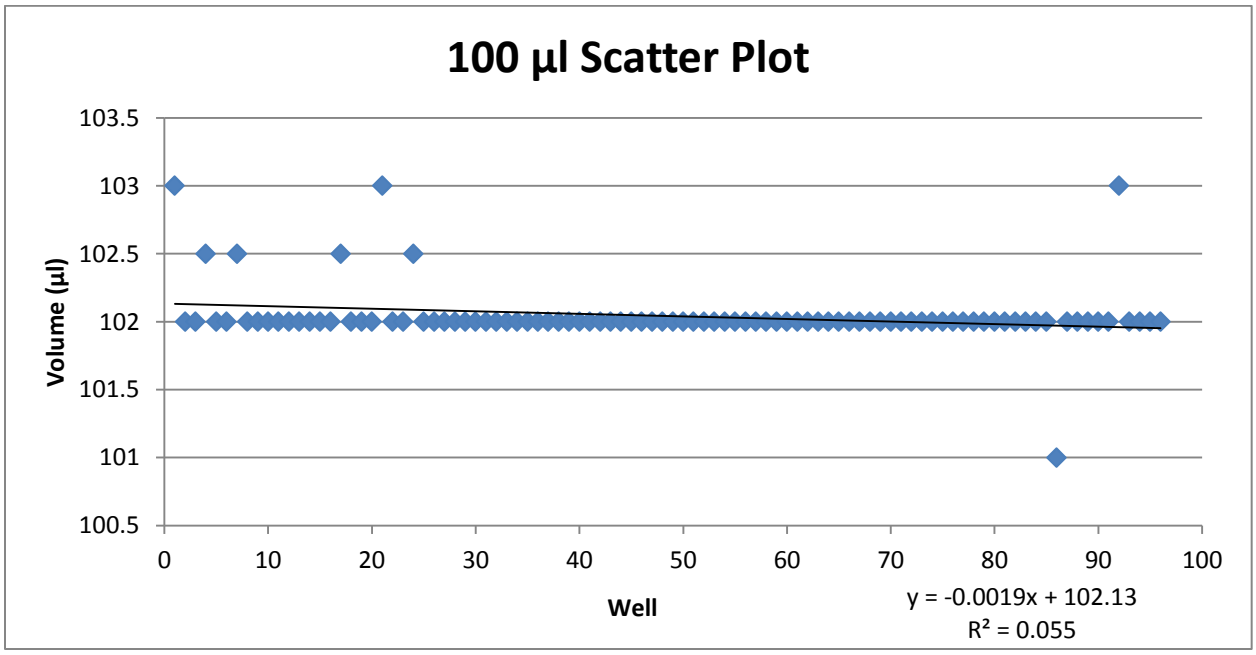

Figure 30. Accuracy and Precision study – 100 µl scatter plot

4.2 Contamination study

All quantitation controls and amplification controls were analyzed and the results were as expected. The eight normalized known samples were genotyped and found to be concordant with the expected known profiles. Initially, the analytical threshold was set at 100 RFU and then at 60 RFU, which yielded all forty TE^{-4} blanks as blanks. However, when the threshold was changed to 25 RFU, 1/40 was found to have an allele call of 11 at D5S818. An additional capillary

electrophoresis run was performed to determine if the 11 allele was reproducible. The first run produced the 11 allele with an RFU of 57 and repeated run produced the same allele with an RFU of 36, which indicated reproducibility. Figure 31 depicts the location of TE-4 blank, highlighted in green, with allele 11 contaminant.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Trn6 J.	TE Blank	T4 Sperm	TE Blank	T19 K2	Trn6 J.	TE Blank	T4 Sperm	TE Blank	T19 K2	(blank)	(blank)
B	TE Blank	CA Swab	TE Blank	Trn5 M:M SP	TE Blank	TE Blank	CA Swab	TE Blank	Trn5 M:M SP	TE Blank	(blank)	(blank)
C	Trn16 AS	TE Blank	T6 Sperm	TE Blank	T19 KB	Trn16 AS	TE Blank	T6 Sperm	TE Blank	T19 KB	(blank)	(blank)
D	TE Blank	CBsp	TE Blank	T16 JP	TE Blank	TE Blank	CBsp	TE Blank	T16 JP	TE Blank	(blank)	(blank)
E	Trn16 BH	TE Blank	Trn7 Diff #1	TE Blank	T19 MG	Trn16 BH	TE Blank	Trn7 Diff #1	TE Blank	T19 MG	(blank)	(blank)
F	TE Blank	CBEcell	TE Blank	T16 LP	TE Blank	TE Blank	CBEcell	TE Blank	T16 LP	TE Blank	(blank)	(blank)
G	Trn3 C1SP	TE Blank	Trn3 CO SP	TE Blank	T19 JE	Trn3 C1SP	TE Blank	Trn3 CO SP	TE Blank	T19 JE	(blank)	(blank)
H	TE Blank	CBvicB	TE Blank	T19 K1	TE Blank	TE Blank	CBvicB	TE Blank	T19 K1	TE Blank	(blank)	(blank)

Figure 31. Contamination Study – location of TE^{-4} blank with allele 11 contaminant (green) on sample plate

4.3 Sensitivity study

All quantitation controls and amplification controls were analyzed and the results were as expected. The serially diluted sample was genotyped and found to be concordant with the expected known profile. Beginning with HBM100-1, the samples were quantitated at the following concentrations: 5.59 ng/µl, 1.64 ng/µl, 0.52 ng/µl, 0.13 ng/µl, 0.032 ng/µl, 0.006 ng/µl, and 0.0026 ng/µl. To represent the sensitivity data, a heat map was generated for the 5-second injection (Figure 32) and 15-second injection (Figure 33). Green indicates both alleles at that loci was present, yellow indicates only one sister allele was present, red indicates complete drop-out, and gray indicates internal lane standard (ILS) failure, which resulted in no data.

5 SECONDS	D3S1358		TH01		D21S11		D18S51		Penta_E		D5S818		D13S317		D7S820		D16S539		CSF1PO		Penta_D		Amel		vWA		D8S1179		TPOX		FGA	
Sample I.Allele	13	16	9	9.3	29	30	15	17	7	16	11	11	9	11	8	10	9	13	12	13	12	13	X	Y	15	17	10	12	8	10	20	21
HBM100-1.1	5173	4257	7826	7843	4724	4065	3085	1642	1062	7669	5814	5275	4261	4018	5349	4212	2636	2622	2239	2109	4534	5660	7425	6673	3927	3421	3007	3040	3108	2950
HBM100-1.2	4833	3901	7772	7738	3808	3395	3351	1538	983	7690	5272	4272	4197	3681	5000	3763	2599	2780	1967	1842	4516	5329	7004	6138	4639	3807	2893	3171	2756	2668
HBM100-1.3	5669	4816	7862	7836	4500	4363	4402	1948	1278	7683	6300	5565	5032	4733	6474	5303	3732	3175	2566	2370	5271	6823	7516	7445	5247	4321	3714	3090	3107	3266
HBM100-2.1	3569	2399	5363	4770	2308	2901	2514	1570	1164	6239	3844	3228	3242	3470	3998	3849	2620	1738	2001	1819	3581	4392	4860	4195	2789	2397	2601	2544	2724	2190
HBM100-2.2	3544	3065	4923	5602	3034	2981	2223	1552	1006	6303	3820	3027	3150	3019	3871	3005	2370	1790	1685	1579	3400	3459	4204	4550	2493	2493	2573	2250	2779	2304
HBM100-2.3	3718	3327	5592	6324	3235	3479	3190	1858	1163	7178	4220	4082	3561	3715	4634	3262	2906	2142	1774	2188	3466	4487	5510	4652	2816	2445	2490	2507	2749	2082
HBM100-3.1	1412	1134	1486	1666	1235	1078	1626	826	793	2486	1256	1117	1267	1452	1644	1293	1089	891	1198	1201	1793	2440	1869	1868	1282	879	1651	1572	1647	1370
HBM100-3.2	2008	1952	2689	3137	2524	1747	2592	1601	949	3876	2462	2003	2103	2168	2672	2673	2078	1788	1495	1620	2766	2809	1788	1906	1821	1453	1940	2217	1819	1902
HBM100-3.3	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)
HBM100-4.1	617	574	859	772	300	260	927	239	431	883	519	415	512	422	1051	593	842	514	498	446	860	1497	469	477	366	489	572	561	440	519
HBM100-4.2	514	448	924	629	531	285	872	324	615	988	457	307	594	600	816	586	657	774	682	494	681	879	217	522	375	231	825	524	532	341
HBM100-4.3	705	560	657	676	567	577	1033	1036	627	1290	592	603	439	636	1134	567	581	425	778	847	682	723	289	446	339	565	722	645	475	421
HBM100-5.1	146	107	159	156	drop	drop	71	89	75	237	179	137	101	164	263	71	115	drop	203	drop	171	118	215	86	166	drop	drop	drop	141	308
HBM100-5.2	drop	drop	94	170	74	drop	131	121	233	232	67	113	101	140	347	199	72	143	97	263	144	414	113	192	79	drop	142	337	162	drop
HBM100-5.3	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)	(LS)
HBM100-6.1	drop	drop	99	drop	drop	drop	drop	68	75	drop	drop	147	67	166	168	drop	drop	drop	63	67	drop	88	drop	87	76	drop	104	drop	drop	92
HBM100-6.2	93	96	89	89	drop	60	135	drop	drop	91	115	80	112	72	125	drop	drop	drop	drop	89	drop	72	166	drop	drop	drop	70	125	drop	drop
HBM100-6.3	drop	70	drop	drop	drop	drop	103	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	138	drop	drop	drop	drop	drop	drop	drop	drop	drop
HBM100-7.1	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop
HBM100-7.2	drop	drop	drop	drop	drop	drop	drop	drop	drop	110	drop	drop	74	86	86	drop	drop	63	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop
HBM100-7.3	drop	60	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop	drop

Figure 32. Sensitivity study – 5 second injection Heat Map

15 SECONDS	D3S1358		TH01		D21S11		D18S51		Penta_E		D5S818		D13S317		D7S820		D16S539		CSF1PO		Penta_D		Amel		vWA		D8S1179		TPOX		FGA	
Sample 1 Allele	13	16	9.3	9	29	30	15	7	16	11	9	11	8	10	9	13	12	13	12	13	X	Y	15	17	10	12	8	10	20	21		
HBM100-1.1	ILS	ILS	ILS	ILS	ILS	ILS	(ILS)	ILS	(ILS)	(ILS)	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS		
HBM100-1.2	ILS	ILS	ILS	ILS	ILS	(ILS)	ILS	ILS	(ILS)	(ILS)	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS		
HBM100-1.3	ILS	ILS	ILS	ILS	ILS	(ILS)	ILS	ILS	(ILS)	(ILS)	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS	ILS		
HBM100-2.1	7951	7822	9280	8839	7690	7679	7798	5190	3850	7487	7492	7605	6901	7436	6219	7644	7656	5736	6564	6121	7307	7087	7258	7312	7485	7424	7535	4207	7550	6587		
HBM100-2.2	7811	7756	8089	8713	7008	7600	5992	4272	2777	7409	7664	7139	6649	7633	6541	7739	6477	4803	4665	4378	7494	7334	7426	7392	7117	7068	6634	3896	6869	6035		
HBM100-2.3	7740	7735	8287	9054	6398	7587	7737	4870	3017	7629	7487	7653	6915	7543	6516	7832	7537	5579	4666	5680	7554	7285	7383	7506	7556	6603	6108	3823	6625	5106		
HBM100-3.1	3670	3037	3542	3969	3271	2877	4303	2265	2175	7283	2861	2558	3476	4041	4344	3416	2960	2411	3244	3190	4186	5495	5097	5048	3680	2521	4387	4157	4119	3524		
HBM100-3.2	5291	5121	6329	7385	6532	4547	6697	4345	2602	7256	5517	4458	5768	6012	6436	7048	5602	4751	4065	4384	6116	6174	4717	4977	5132	4098	4982	3934	4484	4775		
HBM100-3.3	4244	2866	4662	3813	3009	2802	4532	2614	1410	6337	4135	2788	3288	4034	5748	3806	2692	3126	2611	3635	4319	4477	3871	4704	3041	2356	3112	3589	2910	2782		
HBM100-4.1	1786	1635	2249	2040	863	729	2235	627	1194	2681	1322	1090	1504	1248	2911	1606	2167	1290	1355	1223	2199	2689	1320	1368	1100	1437	1586	1563	1042	1269		
HBM100-4.2	1590	1392	2601	1790	1632	876	2702	1032	1962	3210	1236	831	1897	1882	2568	1854	2067	2441	2178	1602	1846	2330	652	1563	1197	748	2526	1576	1628	1033		
HBM100-4.3	2136	1700	1827	1926	1744	1737	3183	3287	2002	4184	1581	1691	1405	2023	3528	1743	1859	1324	2512	2732	1882	1945	877	1323	1115	1693	2262	2047	1452	1292		
HBM100-5.1	384	236	401	412	103	143	193	236	205	667	440	328	280	440	708	202	312	113	571	116	428	284	586	214	474	148	71	103	378	832		
HBM100-5.2	170	93	245	459	211	drop	353	331	645	721	176	289	302	390	964	569	190	400	280	734	377	1049	310	373	227	drop	387	937	442	127		
HBM100-5.3	395	453	528	592	423	340	609	371	398	1088	535	225	572	461	542	570	95	224	421	114	386	176	658	760	549	124	430	319	205	284		
HBM100-6.1	drop	118	265	67	77	drop	176	179	112	drop	94	410	185	451	434	84	drop	153	178	drop	216	drop	248	209	120	285	drop	drop	248			
HBM100-6.2	236	235	203	197	drop	141	314	drop	85	193	234	172	281	181	304	drop	78	125	130	221	drop	151	417	drop	71	drop	189	323	85	drop		
HBM100-6.3	123	194	drop	128	108	156	256	drop	drop	139	drop	61	155	drop	drop	drop	116	drop	drop	102	360	84	85	93	110	drop	drop	drop	drop	drop		
HBM100-7.1	drop	86	136	drop	drop	drop	drop	drop	drop	136	drop	117	drop	drop	118	drop	drop	110	drop	drop	122	drop	drop	drop	drop	111	drop	drop	drop	drop		
HBM100-7.2	89	drop	drop	85	drop	drop	drop	drop	drop	286	drop	drop	213	70	230	157	drop	160	drop	drop	drop	drop	120	drop	drop	drop	drop	114	drop	drop		
HBM100-7.3	85	146	133	drop	drop	89	drop	drop	drop	111	75	drop	drop	drop	drop	drop	drop	drop	drop	91	73	86	drop	drop	drop	drop	drop	drop	drop	67		

Figure 33. Sensitivity study – 15 second injection Heat Map

4.4 Mixture study

All quantitation controls and amplification controls were analyzed and the results were as expected. The mixture samples generated using the QIAgility® were genotyped and determined to be mixtures. During amplification set-up, the procedure was paused due to the QIAgility® being unable to detect liquid for a sample. User-intervention was required and the QIAgility® was instructed to pipette from the bottom of the sample's well. The QIAgility® progressed, but upon review of the capillary electrophoresis results, there was one sample where amplification did not occur. In addition, there was one allele dropout found in the M8-F1 ratio sample at D18S51. However, when the analytical threshold was changed from 60 RFU to 25 RFU, the allele appeared with an RFU of 50. Both samples' profiles in the mixture were found to be concordant with the expected known profiles.

4.5 Concordance study

All quantitation controls and amplification controls were analyzed and the results were as expected. The samples used in this study were first quantified manually and then quantified using the QIAgility® to perform the set-up. The quantified concentrations for both set-ups are tabulated in Table 7a and 7b. Upon comparison, the quantitation values for both set-ups were comparable of each other. Once normalized and amplified, the samples were genotyped and five of the known samples were found to be concordant with the expected known profiles. The known epithelial cell samples were not amplified, because there was a lack of sample volume to perform the 15 µl load amplification manually and automatically. The unknown sample (cigarette filter) was found to yield a single male contributor. Because the sample was collected from a receptacle outside of the laboratory, the identity of the contributor is unknown.

The R^2 and slope values of the manually-made standard curves and QIAgility®-made standard curves were obtained and averaged (Tables 8, 9 and Tables 11, 12). Upon comparison, the curves were found to be comparable of each other. In Table 10 and Table 13, it can be noted that the manually made standard curves had a lower standard deviation when compared to the optimal values.

Table 7a. Concordance Study – Quantified concentrations for manual set-up

Sample Name	Human (ng/ul)	Average Human (ng/ul)	Male (ng/ul)	Average Male (ng/ul)
Cig.Fil.1	0.077	0.103	0.118	0.139
Cig.Fil.2	0.129		0.166	
Cig.Fil.3	0.103		0.133	
Buccal_AR.1	8.12	8.31	0	0
Buccal_AR.2	8.4		0	
Buccal_AR.3	8.41		0	
Blood_AKG.1	1.14	1.037	0	0
Blood_AKG.2	0.993		0	
Blood_AKG.3	0.978		0	
Blood_KKP.1	6.34	6.153333333	0	0
Blood_KKP.2	6.33		0	
Blood_KKP.3	5.79		0	
Ecell_BEH.1	0.006	0.002333333	0	0.000666667
Ecell_BEH.2	0.001		0	
Ecell_BEH.3	0		0.002	
Ecell_AKG.1	0.003	0.004333333	0	0.000666667
Ecell_AKG.2	0.004		0	
Ecell_AKG.3	0.006		0.002	
Semen_DWM-1.1	20.64	21.79333333	19.89	20.91666667
Semen_DWM-1.2	22.26		20.98	
Semen_DWM-1.3	22.48		21.88	
Semen_DWM-2.1	14.77	25.12	22.99	27.13666667
Semen_DWM-2.2	23.65		22.55	
Semen_DWM-2.3	36.94		35.87	
QRC-1.1	0	0	0	0
QRC-1.2	0		0	
QRC-1.3	0		0	
NTC	0	0	0	0

Table 7b. Concordance study – Quantified concentrations for QIAgility® set-up

Sample Name	Human (ng/ul)	Average Human (ng/ul)	Male (ng/ul)	Average Male (ng/ul)
Cig.Fil.1	0.125	0.134	0.146	0.142333333
Cig.Fil.2	0.156		0.165	
Cig.Fil.3	0.121		0.116	
Buccal_AshliReed.1	7.9	7.64	0	0
Buccal_AshliReed.2	7.35		0	
Buccal_AshliReed.3	7.67		0	
Blood_AKG.1	0.89	0.947333333	0	0
Blood_AKG.2	0.932		0	
Blood_AKG.3	1.02		0	
Blood_KKP.1	5.45	5.22	0	0
Blood_KKP.2	5.47		0	
Blood_KKP.3	4.74		0	
Ecell_BEH.1	0.001	0.002333333	0	0.000666667
Ecell_BEH.2	0		0	
Ecell_BEH.3	0.006		0.002	
Ecell_AKG.1	0.001	0.003	0.002	0.001333333
Ecell_AKG.2	0.001		0.002	
Ecell_AKG.3	0.007		0	
Semen_DWM-1.1	20.98	22.37333333	23.49	24.18666667
Semen_DWM-1.2	21.81		23.11	
Semen_DWM-1.3	24.33		25.96	
Semen_DWM-2.1	24.07	23.15	24.46	23.42666667
Semen_DWM-2.2	24.7		24.02	
Semen_DWM-2.3	20.68		21.8	
QRC-1.1	0	0	0	0
QRC-1.2	0		0	
QRC-1.3	0		0	
NTC	0	0	0	0

Table 8 – Manual-made standard curves

Date Made		R^2	*Slope*	*Intercept*
June, 9th	*Human*	0.992011	-3.165507	28.949343
	Male	0.995769	-3.205455	29.640711
		R^2	*Slope*	*Intercept*
June, 16th	*Human*	0.995256	-3.163386	29.09771
	Male	0.994286	-3.121346	29.602591
		R^2	*Slope*	*Intercept*
June, 23rd	*Human*	0.997387	-3.239275	29.112532
Omitted well H1	*Male*	0.990447	-3.385585	29.768084

Table 9 – Manual-made standard curves averages

Average	R^2	*Slope*	*Intercept*
Human	0.994884667	-3.1828885	29.053195
Male	0.993500667	-3.237462	29.670462

Table 10 – Manual-made standard curves' averages compared to optimal values

Std Dev.	R^2 *(0.99)*	*Slope (-3.2)*
Human	0.003453981	0.012099658
Male	0.002475345	0.026489634

Table 11 – QIAgility® made standard curves

Date Made		R^2	*Slope*	*Intercept*
June, 8th	*Human*	0.98667	-3.212615	28.900305
	Male	0.99888	-3.099287	29.497486
		R^2	*Slope*	*Intercept*
June, 12th	*Human*	0.996735	-3.200044	28.741175
	Male	0.993679	-3.068817	29.366328
		R^2	*Slope*	*Intercept*
June, 30th	*Human*	0.996428	-3.101296	28.46002
	Male	0.994378	-3.087657	29.192984

Table 12 – QIAgility® made standard curves averages

Average	R^2	*Slope*	*Intercept*
Human	0.993277667	-3.171318333	28.7005
Male	0.995645667	-3.085253667	29.352266

Table 13 – QIAgility® made standard curves compared to optimal values

Std Dev.	R^2 *(0.99)*	*Slope (-3.2)*
Human	0.00231766	0.020281001
Male	0.003992089	0.08113791

4.6 Cost-benefit study

First, the price per consumable unit was determined based on prices found online as of July, 2015 [8] (Table 14). Next, the number of consumables used for the manual set-up and QIAgility® set-up was then used to determine the costs of consumables for each DNA procedure:

quantitation, normalization, and amplification (Tables 15-23). Based on the figures, the consumable cost was higher if the QIAgility® was used. This was apparent for all scenarios except for quantitation with 80 samples and amplification with 80 samples. For the difference in time, the QIAgility® was found to take less time to set-up each DNA procedure (Tables 24-26). A negative value indicated the additional cost the QIAgility® introduced if used, while a positive value indicated the amount of money saved by using a QIAgility®. In conjunction with the cost of an analyst on an annual salary of $30,000, $40,000 and $60,000, the QIAgility® was found to save the laboratory a range of $10 - $55; depending on the number of samples processed (Table 27).

Table 14 – Cost-benefit study – price per unit as of July, 2015.

Consumables	**Price ($)**	**Unit**	**Price($) / unit**
1.7 ml	$291.00	5000 tubes	$0.06
10 µl	$76.00	960 tips	$0.08
20 µl	$76.00	960 tips	$0.08
100 µl	$76.00	960 tips	$0.08
200 µl	$76.00	960 tips	$0.08
1000 µl	$84.00	960 tips	$0.09
Repeater pipette tip	$64.80	50 tips	$1.30
96 optical well plate	$2,590	500 plates	$5.18
0.5 ml amp tubes	$746.56	10,000 tubes	$0.07
8-strip tube cap	$37.60	1250 caps	$0.03
8-strip tubes	$957.98	1200 tubes	$0.80
Quantifiler® Duo Kit	$1,548	400 rxns	$3.87
PowerPlex® 16 Kit	$7,359	400 rxns	$18.40
AmpliTaq® Gold	$1,864.00	12 tubes	$155 per tube
AmpliTaq® Gold	$155 per tube	50 µl per tube	$3.10 per µl
50 µl conductive filtered tips	$152	960 tips	$0.16
200 µl conductive filtered tips	$152	960 tips	$0.16
5 ml Qiagen tube	$18.60	50 tubes	$0.37

Table 15 – Cost-benefit study – consumable costs for manual quantitation set-up

	80 samples	Price($)/unit	Cost($)	50 samples	Price($)/unit	Cost($)	30 samples	Price($)/unit	Cost($)
Making Standards									
0.5 ml microamp tubes	(8) tubes	$0.07	$0.56	(8) tubes	$0.07	$0.56	(8) tubes	$0.07	$0.56
10 µl - 200 µl tips	9 tips	$0.08	$0.72	9 tips	$0.08	$0.72	9 tips	$0.08	$0.72
Make Master Mix									
10 µl - 200 µl tips	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32
1.7 ml tube	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06
Repeater pipette tip	1 tip	$1.30	$1.30	1 tip	$1.30	$1.30	1 tip	$1.30	$1.30
96-well optical plate	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18
Pipetting									
10 µl - 200 µl	96 tips	$0.08	$7.68	66 tips	$0.08	$5.28	46 tips	$0.08	$3.68
Quantifiler® Duo Kit									
Reactions	105 rxns	$3.87	$406.35	72 rxns	$3.87	$278.64	50 rxns	$3.87	$193.50
Total Costs			$422.17			$292.06			$205.32

Table 16. Cost-benefit study – consumable costs for QIAgility® quantitation set-up

	80 samples	Price($)/unit	Cost($)	50 samples	Price($)/unit	Cost($)	30 samples	Price($)/unit	Cost($)
Making Standards									
8-strip tube	1 strip tube	$0.80	$0.80	1 strip tube	$0.80	$0.80	1 strip tube	$0.80	$0.80
8-strip cap	1 strip cap	$0.03	$0.03	1 strip cap	$0.03	$0.03	1 strip cap	$0.03	$0.03
Making Pre-mixed Master Mix									
5 ml tube/1.7 ml tube	1 tube	$0.37	$0.37	1 tube	$0.06	$0.37	1 tube	$0.06	$0.37
10 µl - 200 µl tips	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32
96-well optical plate	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18
Pipetting									
50 µl conductive filtered tips	111 tips	$0.16	$17.76	81 tips	$0.16	$12.96	61 tips	$0.16	$9.92
Quantifiler® Duo									
Reactions	102 rxns	$3.87	$394.74	71 rxns	$3.87	$274.77	50 rxns	$3.87	$193.50
Total Cost			$419.20			$294.43			$210.12

Table 17. Cost-benefit study – Cost difference between manual and QIAgility® quantitation set-up

Difference in Quant. Cost	**81 Samples**	**51 Samples**	**31 Samples**
Manual vs QIAgility®	$2.97	-$2.37	-$4.80

Table 18. Cost-benefit study – consumable cost for manual normalization set-up

	79 samples	Price($)/unit	Cost($)	49 samples	Price($)/unit	Cost($)	29 samples	Price($)/unit	Cost($)
1.7 ml tubes	79 tubes	$0.06	$4.74	49 tubes	$0.06	$2.94	29 tubes	$0.06	$1.74
10 µl -200 µl tips	158 tips	$0.08	$12.64	98 tips	$0.08	$7.84	58 tips	$0.08	$4.64
Total Cost			$17.38			$10.78			$6.38

Table 19. Cost-benefit study – consumable cost for QIAgility® normalization set-up

	79 samples	Price($)/unit	Cost($)	49 samples	Price($)/unit	Cost($)	29 samples	Price($)/unit	Cost($)
96-well plate	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18
1.7 ml tube	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06
50 µl conductive filtered tips	158 tips	$0.16	$20	98 tips	$0.16	$15.68	58 tips	$0.16	$9.28
Total Cost			$25.24			$20.92			$14.52

Table 20. Cost-benefit study – Cost difference between manual and QIAgility® normalization set-up

Difference in Cost	**81 Samples**	**51 Samples**	**31 Samples**
Manual vs QIAgility®	-$7.86	-$10.14	-$8.14

Table 21. Cost-benefit study – consumable costs for manual amplification set-up

	81 samples	Price($)/unit	Cost($)	51 samples	Price($)/unit	Cost($)	31 samples	Price($)/unit	Cost($)
Making Master Mix									
10 µl - 200 µl tips	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32
1.7 ml tube	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06
Repeater pipette tip	1 tip	$1.30	$1.30	1 tip	$1.30	$1.30	1 tip	$1.30	$1.30
Samples									
0.5 ml microamp tubes	81 tubes	$0.07	$5.67	51 tubes	$0.07	$3.64	31 tubes	$0.07	$2.24
Pipetting									
10 µl - 200 µl tips	81 tips	$0.08	$6.48	51 tips	$0.08	$4.08	31 tips	$0.08	$2.48
PowerPlex® 16 kit									
Reactions	89 rxns	$18.40	$1,637.60	56 rxns	$18.40	$1,030.40	34 rxns	$18.40	$625.60
AmpliTaq® Gold	71.2 µl	$3.10	$220.72	44.8 µl	$3.10	$138.88	27.2 µl	$3.10	$84.32
Total Cost			$1,872.14			$1,178.67			$716.31

Table 22. Cost-benefit study – consumable costs for QIAgility® amplification set-up

	81 samples	Price($)/unit	Cost($)	51 samples	Price($)/unit	Cost($)	31 samples	Price($)/unit	Cost($)
Making Pre-mixed Master Mix									
5 ml tube/1.7 ml tube	1 tube	$0.37	$0.37	1 tube	$0.06	$0.06	1 tube	$0.06	$0.06
10 µl - 200 µl tips	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32	4 tips	$0.08	$0.32
96-well plate	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18	1 plate	$5.18	$5.18
8-tube strip caps	11 caps	$0.03	$0.33	7 caps	$0.03	$0.21	4 caps	$0.03	$0.12
Pipetting									
50 µl conductive filtered tips	82 tips	$0.16	$13.12	52 tips	$0.16	$8.32	32 tips	$0.16	$5.12
PowerPlex® 16 kit									
Reactions	87 rxns	$18.40	$1,600.50	56 rxns	$18.40	$1,030.40	35 rxns	$18.40	$644
AmpliTaq® Gold	69.6 µl	$3.10	$215.57	44.8 µl	$3.10	$138.88	28 µl	$3.10	$86.80
Total Cost			$1,835.39			$1,183.37			$741.6

Table 23. Cost-Benefit Study - Cost difference between manual and QIAgility® amplification set-up

Difference in cost	**81 Samples**	**51 Samples**	**31 Samples**
Manual vs QIAgility®	$36.75	-$5.01	-$25.60

Table 24. Cost-Benefit Study – Estimated time for manual set-ups

Manual Set-up	**80 samples**	**50 samples**	**30 samples**
Quantitation + Make Stds	90 mins	80 mins	60 mins
	90 mins	60 mins	45 mins
	60 mins	45 mins	25 mins
	60 mins	45 mins	20 mins
	60 mins	45 mins	20 mins
	45 mins	30 mins	20 mins
Average Estimated Total Time	67.5 mins	50.83 mins	31.67 mins
Normalization + Amplification	240 mins	105 mins	75 mins
	180 mins	120 mins	60 mins
	150 mins	120 mins	60 mins
	150 mins	60 mins	60 mins
	120 mins	60 mins	45 mins
	120 mins	90 mins	45 mins
Average Estimated Total Time	160 mins	92.5 mins	57.5 mins

Table 25. Estimated times for QIAgility® set-up

QIAgility®	**80 samples**	**50 samples**	**30 samples**
Quantitation + Make Stds	38 mins	27 mins	20 mins
Secondary check time	5 mins	3 mins	1 min
Estimated Total Time	43 mins	30 mins	21 mins
Normalization	38 mins	22 mins	15 mins
Secondary check time	5 mins	3 mins	1 min
Amplification	29 mins	18 mins	11 mins
Secondary check time	5 mins	3 mins	1 min
Estimated Total Time	77 mins	46 mins	28 mins

Table 26.Cost-Benefit Study – Time difference between using and not using QIAgility®

Time difference between QIAgility® and Manual set-up	**80 samples**	**50 samples**	**30 samples**
Quantitation	24.5 mins	20.83 mins	10.67 mins
Normalization and Amplification	83 mins	46.5 mins	29.5 mins
Total time saved	107.5 mins	67.33 mins	40.17 mins

Table 27. Cost-Benefit Study – Money saved by using QIAgility®

Money saved by using QIAgility®	**80 samples**	**50 samples**	**30 samples**
$30,000 Annual salary	$26.88	$16.83	$10.04
$40,000 Annual salary	$36.55	$22.89	$13.66
$60,000 Annual salary	$54.83	$34.34	$20.49

5. Discussion and Conclusions

5.1 Accuracy and precision study

It must be noted that variations in this study may not be due to the QIAgility®, but it may be due to the pipettes used and the analyst measuring the aliquoted volumes. However, it is not believed those variations are due to manual measurement. In the future, additional measurements by another analyst or verifying the pipettes' calibrations can be performed to identify the source of variation.

One of the developmental validations Qiagen® performed was the accuracy and precision study (Table 28). To determine if the QIAgility® was accurate, precise, reliable, and reproducible, WVSPFL's results were compared to Qiagen®'s results [9].Upon comparison, it can be noted that a few of the %CV obtained at WVSPFL were higher than Qiagen®'s %CV. As such, the study investigated the potential downstream effects if the QIAgility® did not liquid handle each aliquot accurately or precisely. The study used the furthest measured volume from each aliquot scenario to note for potential downstream effects (Table 29). Based on the results, it can be noted that even if the QIAgility® pipettes the furthest measured volume, the downstream effects can be considered negligible. As such, it was concluded that the QIAgility®'s liquid handling capabilities are accurate, precise, reliable, and reproducible.

Table 28. Qiagen®'s developmental validation results for accuracy and precision

Volume (µl)	Average	Std Dev	%CV
1	0.922	0.056	6.11
2	1.989	0.067	3.372
5	4.991	0.034	0.678
10	10.064	0.032	0.315
20	20.092	0.024	0.119
50	50.063	0.079	0.159
100	100.174	0.106	0.106
150	150.146	0.081	0.054
200	200.014	0.099	0.049

Table 29. Downstream effects with loss of accuracy and precision of the QIAgility®

		C1	V1	V2	C2	
Results	*QIAgility® action*	*Initial [DNA] (ng/µl)*	*DNA added (µl)*	*Final Volume (µl)*	*Final [DNA] (ng/µl)*	*[DNA] for amp - 2 µl load*
Expected	Pipette 100 µl	25.5	2	102	0.5	1
Observed	Pipette 103 µl	25.5	2	105	0.49514	0.99029
Expected	Pipette 50 µl	13	2	52	0.5	1
Observed	Pipette 53 µl	13	2	55	0.47272	0.94544
Observed	Pipette 49 µl	13	2	51	0.509893	1.019786
Expected	Pipette 25 µl	6.75	2	27	0.5	1
Observed	Pipette 25.3 µl	6.75	2	27.3	0.494505	0.98901
Expected	Pipette 10 µl	3	2	12	0.5	1
Observed	Pipette 10.5 µl	3	2	12.5	0.48	0.96
Observed	Pipette 9 µl	3	2	11	0.55	1.09
Expected	Pipette 2 µl	1	2	4	0.5	1
Observed	Pipette 2.5 µl	1	2	4.5	0.444	0.888
Observed	Pipette 1.8 µl	1	2	3.8	0.526317	1.052634

5.2 Contamination study

The second developmental validation Qiagen® performed was the contamination study [10]. Qiagen®'s study demonstrated that the QIAgility® shows no traces of cross contamination when used to perform a PCR set-up. However, in the study performed at the WVSPFL, a potential contamination was found at D5S818 when the analytical threshold was set at 25 RFU. Upon review, the TE^{-4} blank that contained the potential contaminant was located on well C-01 of the run plate, which came from well B-01 of the amplification plate, which came from well D-01 of the sample plate. The TE^{-4} blank bordered samples Trn16 AS, Trn16 BH, and CBsp.

Because there was insufficient sample volume to perform a second amplification for the TE^{-4} blank, the contamination could not be determined if it was amplification reproducible. Likewise, it could not be determined if the contamination was introduced by the QIAgility® or by external factors.

Although a potential contamination was found, the QIAgility® still passed the contamination study. This is due to the WVSPFL's analytical threshold being set at 100 RFU and given that the potential contaminant's RFUs were 57 and 36, which is below the analytical threshold, they were determined to be of no concern. As such, it was deemed unjustified to repeat the contamination study and the QIAgility® was concluded to not introduce cross-contamination. However, this was monitored throughout the rest of the validation and will be monitored in the future. If similar issues arise, a new contamination study will be repeated to re-assess the QIAgility®.

5.3 Sensitivity study

The results found in this study were consistent with the expected results. In particular, allele dropout was expected for samples with a DNA concentration below 0.1 ng/µl, while full profiles were expected for samples with a DNA concentration greater than 0.1 ng/µl. This was exhibited in Figures 32 and 33, where samples HBM100-5, HBM100-6, and HBM100-7 had concentrations lower than 0.1 ng/µl and dropout was exhibited. All other samples that did not exhibit dropout had a concentration greater than 0.1 ng/µl. As such, the QIAgility® was assessed as being able to handle high-level and low-level concentrations of DNA samples.

5.4 Mixture study

In regards to the sample where amplification did not occur, the amplification controls were amplified as expected. As such, it was concluded that the QIAgility® was unable to pipet

the liquid accurately and precisely due to an insufficient amount of sample volume present for the QIAgility® to detect.

Despite the errors present in this study, the QIAgility® was not attributed as the source of error. The source of error was speculated to be the lack of sufficient sample volume for the QIAgility® to detect. Due to the lack of sample volume, there was a loss of accuracy and precision in the QIAgility®'s sensor, which resulted in the QIAgility®'s inability to liquid handle properly. Although the QIAgility® states how much additional volume should be accounted for, a future study can be performed to truly assess how much additional sample volume is required for the instrument. In conclusion, because the source of error was determined to not be the QIAgility® and the observed results of the mixture samples in this study were found to be consistent to the expected results, the QIAgility® was concluded as being able to handle mixture samples properly.

5.5 Concordance study

In review of the quantitation, normalization, and amplification results, the QIAgility®'s results were found to be comparable to the manual set-up results. Because the results were comparable, the instrument's liquid handling capability was concluded to be equivalent to an analyst's capability.

In review of the quantitation standard curve results, the QIAgility® can be concluded as being able to produce comparable results, but not better results. Even though the standard curves are passing, the QIAgility® made standard curves exhibited higher standard deviation values, which indicates that the QIAgility® made standards are less optimal than the manually-made standards. Even though the manually-made standards were found to be more consistent, it must be noted that the standard curves used for comparison were made on different days and the

manually-made standards made on June 23rd had one well omitted to pass the standard curve. In addition, the manual-made standards were made by one analyst. Other manual-made standard curves made by other analysts could be incorporated into the study to better evaluate if the QIAgility® could make better standards. Due to these differences, another concordance study can be performed in the future to further investigate whether the QIAgility® can produce more optimal standard curves.

5.6 Cost-benefit study

It must first be noted that these evaluations are estimates. The QIAgility® protocols could have been made more efficient, but they were not in order to have the QIAgility® mimick the DNA procedures at the WVSPFL. Any changes to those three variables and addition or subtraction of new variables will require new calculations to be performed to assess if using the QIAgility® is a benefit or a cost. Future cost-benefit studies that may be performed include other variables such as changing the QIAgility® protocols to not mimic the DNA procedures at the WVSPFL or adding in the costs of service checks as a variable.

In review of the consumable costs, the QIAgility® introduced a higher consumable cost into the DNA workflow if 50 or fewer samples were processed using the QIAgility®; 50 samples introduced an additional cost of $17.52 and 30 samples introduced an additional cost of $38.54. However, for 80 samples, the QIAgility® was found to cost less than a manual set-up by $31.86. The results demonstrated that the difference in cost was largely due to the use of a 96-well plate, the number of reaction overages, and the use of Qiagen® conductive filtered tips. To offset the cost of using a 96-well plate, seventy-four 0.5 ml amplification tubes would have to be used. For tips, the costs of Qiagen® conductive filtered tips are unable to be compensated as they are double the cost of regular tips. For the reaction overages, the QIAgility® required fewer overages

than a manual set-up for 80 samples. The instrument needed an overage of six reactions in quantitation and six reactions in amplification, while the manual set-up required an overage of nine reactions in quantitation eight reactions in amplification.

For time, the QIAgility® was found to save an estimated total time of 107 minutes for 80 samples, 67 minutes for 50 samples, and 40 minutes for 30 samples. In conjunction, the study estimated that for an analyst on an annual salary of $30,000, $40,000, and $60,000, the cost per minute was $0.25, $0.34, and $0.51, respectively. The difference in time and cost of an analyst per minute were integrated and evaluated that if a DNA forensic laboratory processed 30, 50, or 80 samples using the QIAgility®, the laboratory could save an estimated cost of $10 - $21, $17 - $34, and $26 - $55, respectively. These results depended greatly on the estimated annual salary of the analyst and number of samples processed. The additional consumable costs could compensated by the amount of money saved based on time. For laboratories that pay analysts at least $40,000 per year and process 50+ samples per run through their DNA workflow, the QIAgility® can be concluded as an asset if incorporated into the laboratory. Current investigations at WVSPFL speculate that the breakeven point for consumable costs is 60 samples. Future studies can be implemented to more accurately determine this point.

Overall, the WVSPFL believe that regardless of the expenses of using the QIAgility®, the non-monetary benefits of the QIAgility® compensates for all the costs. For forensic DNA laboratories, it is imperative to efficiently process samples as fast as possible when needed while minimizing human error. By using the QIAgility®, this goal can be achieved. Similarly, with a quicker turnaround time for samples, more samples can be processed in one day to decrease any potential backlog a lab may have. The WVSPFL plans to incorporate the QIAgility® into their DNA workflow.

6. References

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