THE CITY OF SAN DIEGO

MEMORANDUM

DATE: 03-06-2014

TO: John Simms, Quality Assurance Manager

cc: Patrick O'Donnell, Supervising Criminalist
Frank Healy, Supervising Criminalist

FROM: Shawn Montpetit, DNA Technical Manager

SUBJECT: Material Modification of the Hamilton Nimbus-4 (#1) amplification protocol settings

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During recent casework and in experiments related to the QiaSymphony validation, it became evident that the pipetting performance of the Hamilton Nimbus-4 robotic workstations (both #1 and #2) were not performing up to expectations during low volume pipetting. This was causing lower than expected volume transfers as compared to manual pipetting. Adjustments were made to improve the pipetting performance of the Hamilton Nimbus-4 robotic workstations to equalize the robotic pipetting to manual pipetting. The following is a summary of the experiments conducted to identify the scope of the problem more thoroughly, the changes made to the software on Nimbus #1 to correct the issue, and the performance verifications performed after the changes were made.

Previous memos regarding the modifications made to Nimbus #2, and the investigations into the effect this had on casework have been submitted. This memo specifically relates to Nimbus #1.

The data included within indicates that pipetting performance of the Nimbus-4 robotic workstation #1 is again performing as expected.

Shawn Montpetit
DNA Technical Manager

JMS 3-10-14

March 6, 2014

Material Modification

Identification of the issue

Analysts in the past couple of months have had instances where it appeared that the volumes of liquid transferred by the Hamilton Nimbus-4 pipetting workstation during quantification procedure setup may not have been as expected. In a few instances, samples indicated lower quantification results than suggested after subsequent amplification. These instances were reported to the DNA Technical Manager, however, due to the apparent low occurrence rate and the sporadic nature of the occurrences, no pattern was observed and the problem was not able to be definitively diagnosed as related to the performance of the Hamilton Nimbus-4.

During the QiaSymphony validation replicate samples from several extractions were quantified using the Hamilton Nimbus-4 robotic workstations. A couple of different samples, over several quantification plates, indicated lower than expected DNA yields. In re-quantification of those select samples, the quantification values were found to be higher than previously detected, indicating a volume transfer problem attributable to the Nimbus workstation pipetting. Based on this information, additional studies were conducted to determine the scope and extent of the issue.

Scope and Extent of the Issue

Twelve DNA extracts were combined and then used to make a 1:5 dilution. The purpose of the dilution was to bring the concentration of the samples into a lower concentration, and create enough volume of sample to run several replicates. The dilution was distributed into 75 tubes (1-75). Samples 1-50 had 2uL taken for quant and samples 51-75 had 8uL taken for quant. These samples were run twice on each Nimbus for a total of 4 sets of data.

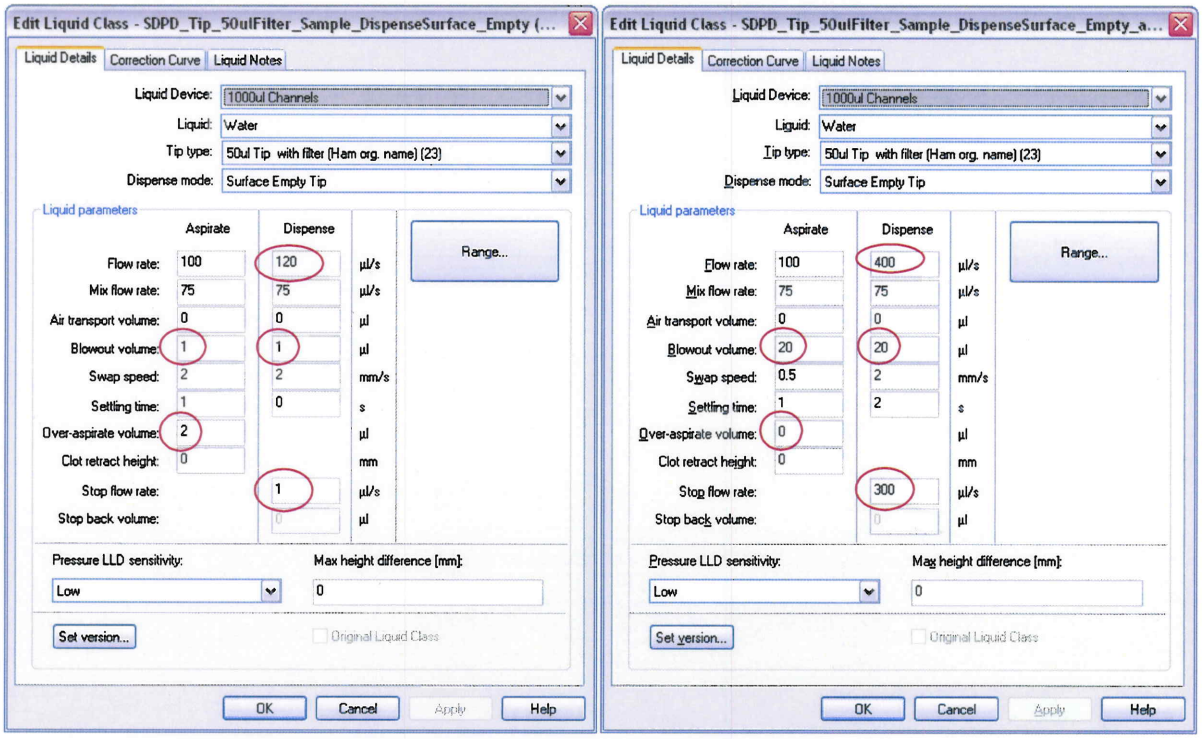
The quantification results indicated that approximately 7% of the time, the 2uL samples had less than 20% of the sample that should have been transferred to the plate. These samples were identified as instances of pipetting error. On average, all 2uL samples had approximately 35% of the sample it should have.

This experiment identified that there were two separate, yet related, issues. The Hamilton Nimbus-4s were consistently transferring far less than the 2uL they were supposed to be transferring, and periodically little to no sample was being transferred. The 8uL samples transferred by both Nimbus instruments were virtually identical to those that had 8uL transferred manually.

Based on this set of data, Hamilton Applications Specialist Laura Fernau was contacted to assist in troubleshooting.

Resolution of the Issue

The Applications Specialist assisted in troubleshooting on-site, and indicated that there were several possible remedies for the issues we were observing. Based on previous experience the applications specialist indicated that the pipetting errors were most likely caused by the low volume not being expelled into the destination well. As a first line approach to correct the dispense issue the liquid class settings were changed to increase the dispensing flow rate and the air gap was increased. This change was made to ensure that any small volumes aspirated would be forced out into the destination well.

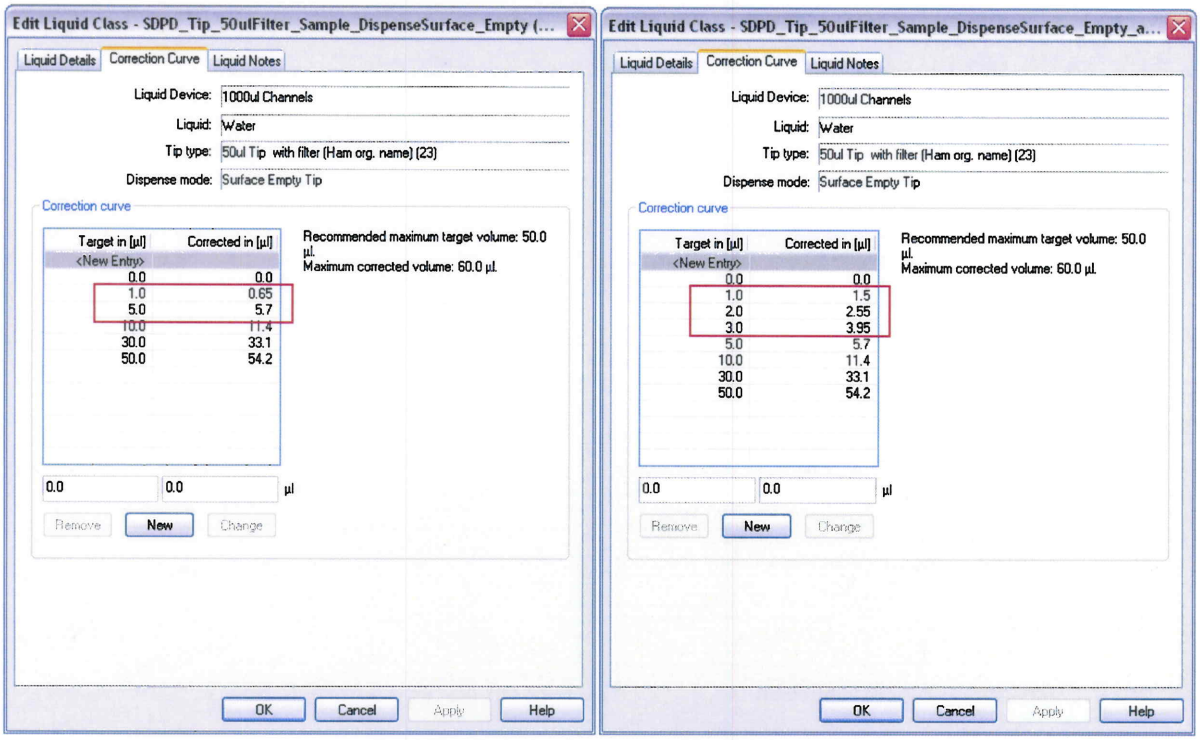


Circled areas above reference the specific changes made to Nimbus #1 (left = before and right =after)

To determine if the adjustments made to the software corrected the pipetting performance several replicates of a dilution of the NIST quantitation standard were quantified via Nimbus setup as well as manual setup.

After each quantification plate was run, the results were assessed to determine equivalence to manual pipetting. Minor adjustments were made to the correction curve until equivalence between manual pipetting and Nimbus pipetting was obtained.

The following is the final adjustments that were made to the correction curve.



Correction Curve changes – left = before and right = after

No pipetting errors were observed. The 2uL on the Nimbus was statistically similar to 2uL manually and the 8uL on the Nimbus was statistically similar to 8uL manually, suggesting that the pipetting disparity between Nimbus pipetting and manual pipetting has been solved.

Discussion and Conclusions

Low volume (<5uL) transfers have inherently more variability associated with them than larger volumes. This phenomenon is true for both robotic and manually pipetting. Volumes less than 1uL are not recommended to be transferred using the Hamilton Nimbus-4 pipetting workstation as per the company specifications for this instrument. 35% of 2uL is 0.70uL, which is outside the dynamic range of the Nimbus-4 as stated by the Hamilton Corporation, therefore the approximately 7% transfer error we had observed may have been due to pipetting such a low volume of liquid.

The changes to the liquid class setting and the pipetting correction curve have solved the sporadic pipetting failures and the changes to the correction curve have addressed the disparity between manual and Nimbus pipetting. Based on the verification plate run indicating the issues had been addressed it is recommended that **Hamilton Nimbus-4 pipetting workstation 1 be reinstated into casework service**.

DNA Technical Manager

02-13-14
DB

To determine if the tweaks that were made to Nimbus #1 makes Nimbus #1 similar to hand pipetting, I did the following experiment:

I took the NIST 2372 Human DNA Quantitation Standard and added 6uL of it to 594uL TE Buffer. I aliquotted 20uL into each of 20 tubes. I had the Nimbus transfer the master mix into all of the wells of the 96-well quant plate. I had the Nimbus transfer 2uL of each of tubes 1 through 10, and 4uL of each of tubes 11 through 20. I then manually transferred 2uL of each of tubes 1 through 10, and I manually transferred 4uL of each of tubes 11 through 20.

THE CITY OF SAN DIEGO

M E M O R A N D U M

DATE: 02-07-2014

TO: John Simms, Quality Assurance Manager

cc: Patrick O'Donnell, Supervising Criminalist
Frank Healy, Supervising Criminalist

FROM: Shawn Montpetit, DNA Technical Manager

SUBJECT: Material Modification of the Hamilton Nimbus-4 amplification protocol settings

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During recent casework and in experiments related to the QiaSymphony validation, it became evident that the pipetting performance of the Hamilton Nimbus-4 robotic workstations were not performing up to expectations during low volume pipetting. This was causing lower than expected volume transfers as compared to manual pipetting. Adjustments were made to improve the pipetting performance of the Hamilton Nimbus-4 robotic workstations to equalize the robotic pipetting to manual pipetting. The following is a summary of the experiments conducted to identify the scope more thoroughly, the changes made to the software to correct the issue, and the performance verification performed after the changes were made.

The data included within indicates that pipetting performance of the Nimbus-4 robotic workstation is again performing as expected.

We are assessing the potential impact to previous casework and will present that information separately.

Shawn Montpetit
DNA Technical Manager

2/14/14

JMS 2-11-14

February 7, 2014

Material Modification

Identification of the issue

A few analysts in the past couple of months have had instances where it appeared that the volumes of liquid transferred by the Hamilton Nimbus-4 pipetting workstation during quantification procedure setup may not have been those that were expected. In a few instances, samples indicated lower quantification results than suggested after subsequent amplification. These instances were reported to the DNA Technical Manager, however, due to the apparent low occurrence rate and the sporadic nature of the occurrences, no pattern was observed and the problem was not able to be definitively diagnosed as related to the performance of the Hamilton Nimbus-4.

During the QiaSymphony validation replicate samples from several extractions were quantified using the Hamilton Nimbus-4 robotic workstation. A couple of different samples over several quantification plates indicated lower than expected DNA yields. In re-quantification of those select samples, the quantification values were found to be higher than previously detected, indicating a volume transfer problem attributable to the Nimbus workstation pipetting. Based on this information, additional studies were conducted to determine the scope and extent of the issue.

Scope and Extent of the Issue

Twelve DNA extracts were combined and then used to make a 1:5 dilution. The purpose of the dilution was to bring the concentration of the samples into a lower concentration, and create enough volume of sample to run several replicates. The dilution was distributed into 75 tubes (1-75). Samples 1-50 had 2uL taken for quant and samples 51-75 had 8uL taken for quant. These samples were run twice on each Nimbus for a total of 4 sets of data.

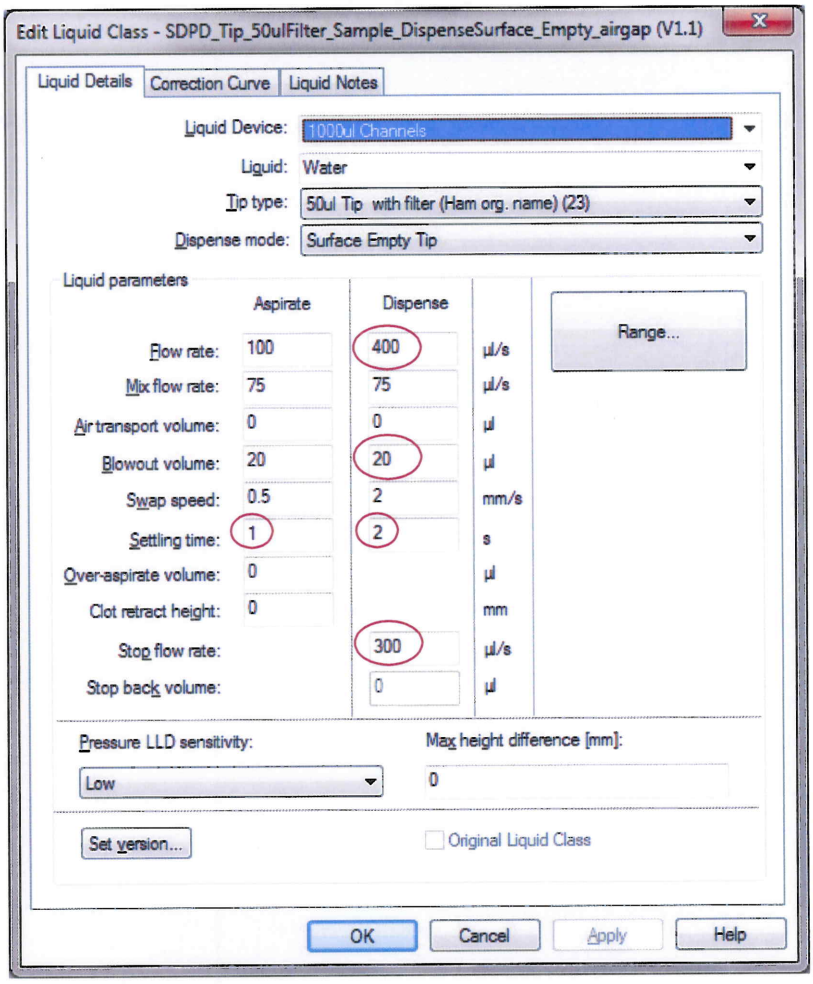
The quantification results indicated that approximately 7% of the time, the 2uL samples had less than 20% of the sample that should have been transferred to the plate. These samples were identified as instances of pipetting error. On average, all 2uL samples had approximately 35% of the sample it should have.

This experiment identified that there were two separate yet related issues. The Hamilton Nimbus-4 was consistently transferring far less than the 2uL it was supposed to be transferring, and periodically little to no sample was being transferred. The 8uL samples transferred by the Nimbus were virtually identical to those that had 8uL transferred manually.

Based on this set of data, Hamilton Applications Specialist Laura Fernau was contacted to assist in troubleshooting.

Resolution of the Issue

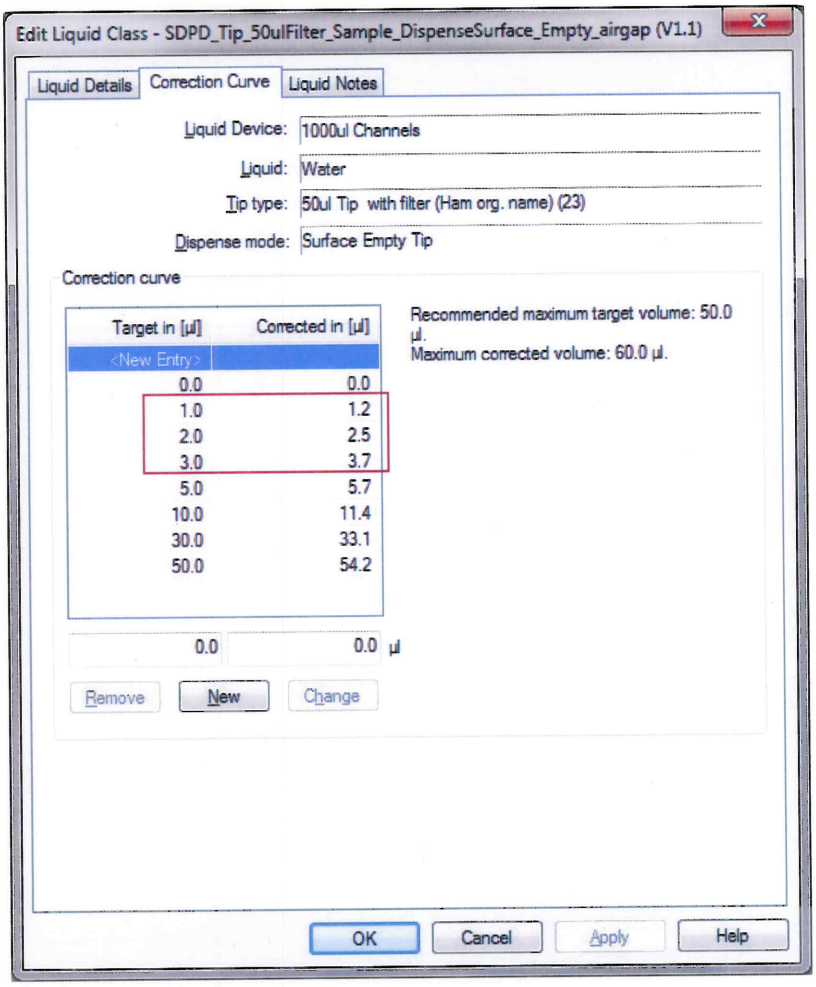
The Applications Specialist assisted in troubleshooting on-site, and indicated that there were several possible remedies for the issues we were observing. Based on previous experience the applications specialist indicated that the pipetting errors were most likely caused by the low volume not being expelled into the destination well. As a first line approach to correct the dispense issue the liquid class settings were changed to increase the dispensing flow rate and the air gap was increased. This change was made to ensure that any small volumes aspirated would be forced out into the destination well. The changes were only made to Hamilton Nimbus-4 pipetting workstation-2, which was subsequently taken off-line to continue testing possible solutions.



A second quantification plate was run to determine the effects of the changes to the liquid class. Six DNA extracts were combined and then used to make a 1:5 dilution. The resulting dilution was distributed into 40 tubes. Samples 1-30 had 2uL taken for quant and samples 31-40 had 8uL taken for quant, both with the Nimbus (1a-40a) and manually (1b-40b).

The results indicated that no samples had any pipetting errors. Therefore it appeared that the transfer problem had been addressed by the liquid class change. Again the 2uL volume transferred by the Nimbus and 2uL manual were still statistically different based on t-test.

The Application Specialist made alterations to the Correction Curve to result in the volume transferred being more equivalent to manual pipetting. The correction increased the pipetted volume for the 1, 2, and 3uL volumes. An additional quantification plate consisting of 20 samples from which 2uL was transferred to the plate by the Nimbus. 10 samples had 2uL taken for quant manually. In addition, a NIST B dilution sample was distributed into 16 tubes. NIST_1 through NIST_8 had 2uL transferred by the Nimbus and NIST_9 through NIST_12 had 8uL transferred by the Nimbus. NIST_1 and NIST_2 also had 2uL transferred manually and NIST_9 and NIST_10 had 8uL transferred manually.



No pipetting errors were observed. The 2uL on the Nimbus was statistically similar to 2uL manually and the 8uL on the Nimbus was statistically similar to 8uL manually, suggesting that the pipetting disparity between Nimbus pipetting and manual pipetting has been solved.

February 7, 2014

Discussion and Conclusions

Low volume (<5uL) transfers have inherently more variability associated with them than larger volumes. This phenomenon is true for both robotic and manually pipetting. Volumes less than 1uL are not recommended to be transferred using the Hamilton Nimbus-4 pipetting workstation as per the company specifications for this instrument. 35% of 2uL is 0.70uL, which is outside the dynamic range of the Nimbus-4 as stated by the Hamilton Corporation, therefore the approximately 7% transfer error we had observed may have been due to pipetting such a low volume of liquid.

The changes to the liquid class setting and the pipetting correction curve have solved the sporadic pipetting failures and the changes to the correction curve have addressed the disparity between manual and Nimbus pipetting. Based on the verification plate run indicating the issues had been addressed it is recommended that Hamilton Nimbus-4 pipetting workstation 2 be reinstated into casework service.

It is also recommended that Hamilton Nimbus-4 pipetting workstation 1 be taken out of service until such time as the same changes can be made to the liquid class settings and correction curve for that instrument and performance verification can be done for that instrument.

DNA Technical Manager

2/11/14

2-11-14

2/14/14

Nimbus Trouble Shooting 2/7/14 Kristin Beyers

Round 1: Identifying the Problem

Twelve DNA extracts ("1000_1" though "1000_12" taken from the QiaSymphony Validation for the CW 1000 ADV protocol) were combined and then used to make a 1:5 dilution. The resulting dilution was aliquotted into 75 tubes (1-75). Samples 1-50 had 2uL taken for quant and samples 51-75 had 8uL taken for quant. These samples were run twice on each Nimbus for a total of 4 sets of data.

Results: Approximately 7% of the time the 2uL samples had less than 20% of the sample it should have (designated as 'errors'). On average, all 2uL samples had approximately 35% of the sample it should have.

Round 2: Laura changed the liquid class in the amplification protocol and adjusted the air gap from 1 to 20uL.

Six DNA extracts ("1:64_1" through "1:64_6" taken from the 'mess-up' dilution for the QiSymphony validation for the Effects of Concentration protocol) were combined and then used to make a 1:5 dilution. The resulting dilution was aliquotted into 40 tubes. Samples 1-30 had 2uL taken for quant and samples 31-40 had 8uL taken for quant, both with the Nimbus (1a-40a) and manually (1b-40b).

Results: No samples indicating an 'error'. 2uL Nimbus and 2uL manual still statistically different based on t-test.

Round 3: Laura Fernau changed the pipetting calibration curve values in the Nimbus Liquid class definition.

Twenty four DNA extracts (All the shake/no shake, SDPD reagents/Qiagen reagents samples taken from the QiSymphony validation for the SDPD reagents v. Qiagent Reagents study) were combined and then aliquotted into 20 tubes. 2uL from all 20 tubes was taken for quant both with the Nimbus and samples 1-10 had 2uL taken for quant manually. In addition, a 1/100 dilution of the NIST B standard was made (2uL NIST B in 198ul TE) and then aliquotted into 16 tubes. NIST_1 through NIST_8 had 2uL sampled on the Nimbus and NIST_9 through NIST_12 had 8uL sampled Nimbus. NIST_1 and NIST_2 also had 2uL sampled manually and NIST_9 and NIST_10 had 8uL sampled manually.

Results: No 'errors' observed. The 2uL on the Nimbus was statistically similar to 2uL manually and the 8uL on the Nimbus was statistically similar to 8uL manually, suggesting the that problem had been solved.

Observation for further study: the 8uL sample was statistically different than the 2uL samples, both manually and on the Nimbus. This suggests that the 8uL might be slightly over estimating the amount of DNA in the sample.

THE CITY OF SAN DIEGO

M E M O R A N D U M

DATE: September 27, 2012

TO: John Simms, Quality Assurance Manager

CC: Patrick O'Donnell, Supervising Criminalist
Frank Healy, Supervising Criminalist

FROM: Shawn Montpetit, DNA Technical Manager

SUBJECT: Validation of the Hamilton Nimbus liquid handling system for automated quantification and amplification setup.

The Hamilton Nimbus is an automated multi-channel pipetting workstation that is being incorporated for PCR setup. The Hamilton Nimbus workstation will be validated for use with quantitative PCR (qPCR) setup for the Quantifiler Human and Quantifiler Duo kits. In addition to the qPCR application, the Hamilton Nimbus will also be validated to perform PCR setup for the Applied Biosystems amplification kits in use.

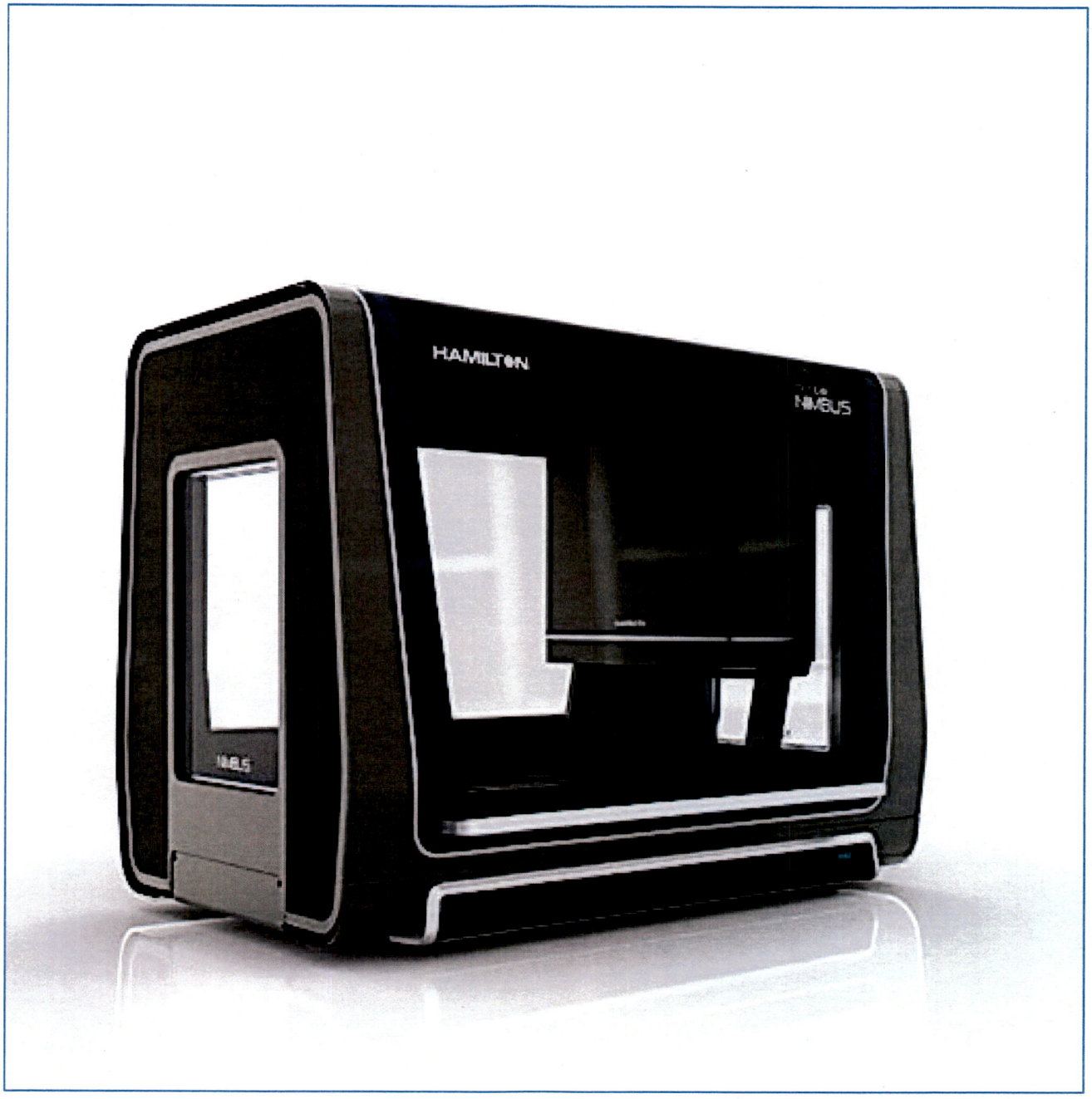
The validation of this robotic workstation will include assessments of the reproducibility and precision as well as contamination assessments for both qPCR and PCR applications using simulated evidence samples.

We expect to complete this validation by December 2012.

If you have any questions regarding this validation proposal, please feel free to contact me.

Shawn Montpetit
DNA Technical Manager

San Diego Police Department
Forensic Biology Section



Validation of the Hamilton Robotics Nimbus 4 Liquid Handling Workstation

SDPD Forensic Science Section – Forensic Biology Unit

Validation of the Hamilton Nimbus 4 Liquid Handling Workstation

Validation Summary

For a number of years the Forensic Biology Unit of the San Diego Police Department Crime Laboratory has been employing a robotic liquid handling workstation for automated pipetting during PCR setup. Currently the laboratory employs the Qiagen® Universal for automated setup for all quantification kit and STR typing kit amplifications. The laboratory has now acquired the Hamilton Nimbus 4 liquid handling workstation to perform this function.

The first part of the validation process involved the method development for both the quantification and STR typing kit assays. The method development process included designing the deck layout, teaching the labware, programming the software to perform the required tasks, and testing the method to determine functionality. The second part of the validation was to test the performance of the liquid handling workstation.

To assess the performance of the Nimbus a number of studies were completed. These studies were based off the validation requirements listed in the *Quality Assurance Standards for DNA Testing Laboratories* published by SWGDAM. Standard 8.3.1 lists the following categories for internal validations: known and non probative evidence samples or mock evidence samples, reproducibility and precision, sensitivity and stochastic studies, mixture studies, contamination assessment. For the purposes of this validation, we determined that the sensitivity and stochastic studies as well as the mixture studies did not apply to the validation of the liquid handing workstation.

The internal validation included studies to determine the reproducibility and precision of the workstation for the quantification and STR typing assays, as well as a contamination assessment using known samples. A final assessment was performed using a set of samples mimicking casework that were carried through the quantification and STR typing methods.

Method development

The most challenging job the Nimbus is being tasked with performing is to aspirate a small volume of liquid in a sample tube to dryness (i.e. aspirate the entire 10 µL of sample for PCR). A significant amount of the method development efforts were spent optimizing this task. It was determined that having the tip descend to the bottom of the tube by tracking down the side of the tube, as opposed to the center, such that it bends slightly is the most effective way to aspirate to dryness given the current hardware.

Another aspect of the method that underwent some significant modifications during the development process is the master mix aspiration step. Testing of the initial master mix aspiration step found that the amount of master mix needed to successfully complete a run was excessive and would result in large amounts (hundreds of microliters) being discarded. Following several small changes, the excess master mix required has been greatly reduced and typically results in only about 50 µL of residual master mix remaining following the run.

Based on the results of preliminary experiments following modifications to the method, the method was deemed complete and the validation proceeded to experiments utilizing samples containing actual DNA.

Reproducibility and Precision

The instrument is able to generate reproducible and precise results that are reflected in the comparison of peak heights of the replicate samples prepared in this study and the relatively small standard deviations obtained about the mean for these samples. There are no significant differences in peak heights for samples that are prepared on the Nimbus and those prepared manually at volumes greater than approximately 4uL. At volumes less than 4uL, there appears to be more variation in the amount of sample transferred. In addition, it appears that the Nimbus workstation is transferring approximately 70% of the volume of manual pipetting at these levels. Based on the increased variability at the low volumes transferred with the Nimbus it is recommended, although not required, that samples that require less than 4uL of input DNA be rounded up to the nearest whole number to account for the reduced volume compared to manual pipetting.

Contamination Assessment

Previously extracted DNA samples and TE blanks were setup on the Nimbus 4 Liquid Handling Workstation for Quantifiler Human DNA quantitation. The samples and blanks were interspersed using two types of patterns. Half the plate was setup in a checkerboard pattern alternating samples and blanks. The second half of the plate was setup alternating rows of samples with rows of blanks. On the first half of the plate the Nimbus transferred 2uL of both samples and blanks. On the second half of the plate the Nimbus transferred 8uL of sample into two columns (7 and 11). The design of this experiment was to determine whether we could detect any contamination of the samples either in the source tubes, or the destination plate.

Previously extracted DNA samples and TE blanks were set up on the Nimbus for DNA amplification using the Identifiler Plus kit. Half of the plate was set up in a checker board pattern (i.e. alternating samples and blanks between each sample) and the other half in a zebra stripe pattern (i.e. alternating samples and blanks between each column). Half of the TE blanks

came from the TE aliquots on the deck and half came from tubes containing TE in sample positions.

Definitive occurrences of cross-contamination between samples on either the source plate or destination plate were not observed with the DNA quantitation and amplification methods on the Nimbus. Based on the lack of any significant levels of detectable DNA in the blanks, in either the quantitation or amplification methods, it does not appear as if liquid handling on the Nimbus workstation is resulting in any significant contamination of the samples tubes or destination plates.

Casework-like Samples

Previously extracted DNA samples from training exercises (buccal swabs, bloodstains, and cigarette butts), quality control runs, proficiency tests (blood and semen stains), and associated reagent blanks were used in this experiment. The samples were set-up on the Nimbus for DNA quantitation with the Quantifiler Human kit. The DNA quantitation results were used to determine the appropriate amount of input DNA for amplification, and the Identifiler Plus amplification was set-up on the Nimbus. Capillary electrophoresis was performed on a 3130 and the resultant data was analyzed in GMID-X. The resultant data was evaluated to verify that the profiles were consistent with those previously obtained for the samples and peak heights were commensurate with the amount of input DNA.

The Nimbus is able to reliably set-up mock case samples for quantitation and amplification. The fact that correct profiles were obtained and blanks were absent of DNA, further supports that contamination is not a concern on the Nimbus. Peaks generated following amplification are consistent with the heights expected based on input amounts of DNA determined from quantitation.

Hamilton Nimbus #2

The initial validation of the Nimbus was performed on the original Nimbus obtained at the SDPD, Nimbus #1. This study demonstrated that the protocol developed on Nimbus #1 was transferrable to the second Nimbus, Nimbus #2 and that performance of Nimbus #2 is equivalent to that of Nimbus #1.

Mock Case Samples analysis was performed on Nimbus #2. Previously extracted DNA samples from training exercises (buccal swabs, bloodstains, and cigarette butts) and associated reagent blanks were used in this experiment. The samples were set-up on Nimbus #2 for DNA quantitation with the Quantifiler Human kit. The DNA quantitation results were used to determine the appropriate amount of input DNA for amplification, and the Identifiler Plus amplification was set-up on Nimbus #2. Capillary electrophoresis was performed on a 3130 and

the resultant data was analyzed in GMID-X. The DNA quantitation results were compared to those originally obtained on Nimbus #1.

The results obtained from the samples run with Nimbus #2 were comparable to those previously obtained on Nimbus #1 during the internal validation. Therefore, Nimbus #2 has been shown to be able to reliably setup of samples for DNA quantitation and amplification.

Conclusions

Based on the results of the validation studies performed on the Hamilton Nimbus 4 Liquid Handling Workstations it is recommended that the instruments be cleared for use in casework.

Coral Luce
Criminalist

Darren Wright
Criminalist

04-30-2013
Shawn Montpetit
DNA Technical Manager

John Simms
Quality Assurance Manager

Purpose

The method developed for use on the Hamilton Nimbus 4 Liquid Handling Workstation (henceforth Nimbus) is the result of collaboration between applications engineers at Hamilton Robotics and criminalists within the SDPD Forensic Biology Unit. Close examination of the method and experimentation have led to multiple modifications to the original method. The testing included simulated runs, dry runs, water runs, and dye experiments of portions of the method and/or the entire method. The end product is a refined method for the Nimbus that performs automated set-up of DNA quantitation via qPCR using the Quantifiler Human/Duo kits and DNA amplification via PCR using the Identifiler Plus, Yfiler, and Minifiler kits.

The basic schematic of the method is as follows (Figure 1):

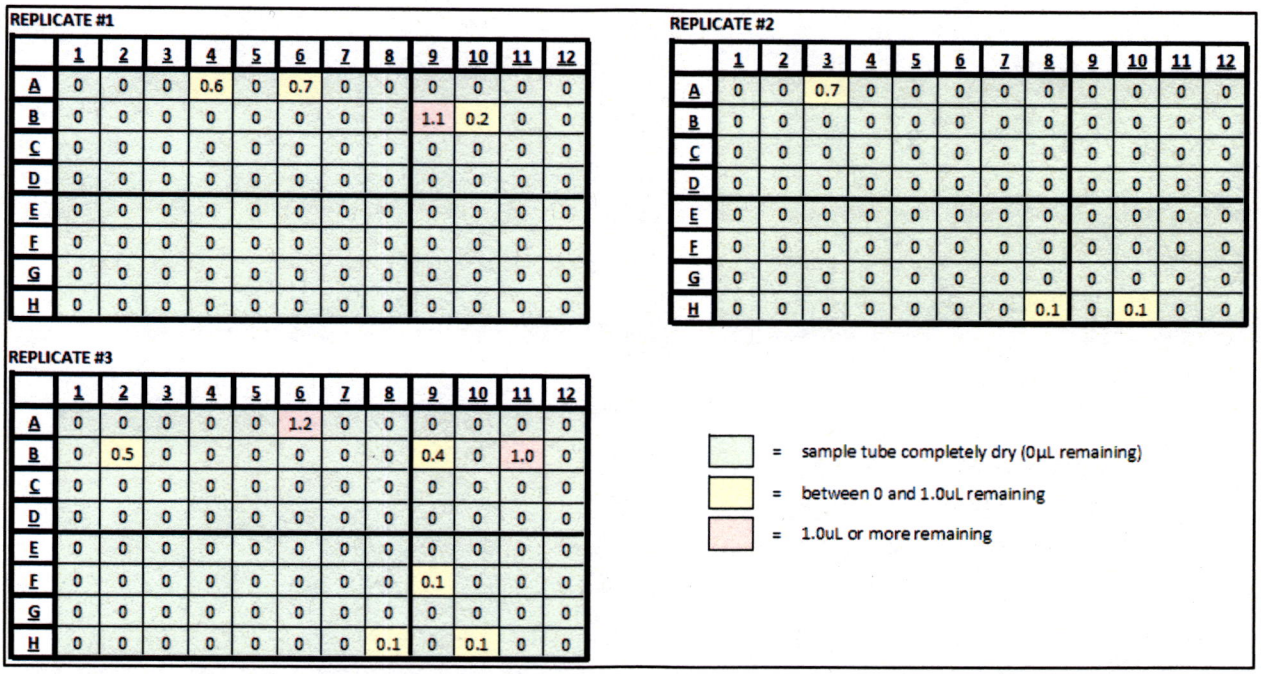
Figure 1: Basic tasks performed by the SDPD qPCR and PCR setup method on the Nimbus.

Many of the modifications to the method have been minor enough so as to not warrant discussion here. However, some of the modifications are critical to the performance of the method and, therefore, will be discussed below.

Discussion

The most challenging job the Nimbus is being tasked with performing is to aspirate a small volume of liquid in a sample tube to dryness (i.e. aspirate the entire 10 µL of sample for PCR). A significant amount of the method development efforts were spent optimizing this task. The initial version of the method performed the sample aspiration step by having the tip descend directly down the center of the tube until it reached the bottom and then aspirating the desired volume of sample. During dry runs and water runs this occasionally resulted in the tip creating a vacuum seal at the bottom of the tube strong enough to lift the tube upward as the tip ascended. On multiple occasions as a result of this vacuum effect, the tube was lifted completely out of the rack and dropped over the top of other open sample tubes. An initial attempt to overcome this vacuum effect involved increasing the bottom height that the tip descended to; however, this resulted in unacceptable levels of residual sample volumes being left behind in the sample tubes. Ultimately, it was found that the vacuum effect could be overcome by having the tip descend to the bottom of the tube by tracking down the side of the

tube, as opposed to the center, such that it bends slightly and does not create a seal at the bottom of the tube. Performing test water runs in which 10 µL of liquid was placed in sample tubes and the entire 10 µL was aspirated by the Nimbus showed that the residual sample volumes using this approach are at an acceptable level (Figure 2). An alternative approach that was tested is the use of a rack with a gasket which holds the sample securely in place thereby eliminating the ability of the vacuum effect to lift the tube. This gasket rack approach allowed the tip to descend down the center of the tube to the bottom, and was also found to successfully overcome the vacuum effect while maintaining acceptable residual volumes. The use of gasket racks may be a solution that is used in the future but it requires the purchase of specially engineered racks.



REPLICATE #1	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0.6	0	0.7	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	1.1	0.2	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0	0	0	0

REPLICATE #2	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0.7	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0.1	0	0.1	0	0

REPLICATE #3	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	1.2	0	0	0	0	0	0
B	0	0.5	0	0	0	0	0	0	0.4	0	1.0	0
C	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0.1	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0.1	0	0.1	0	0

Figure 2: Residual volumes from three replicates of water runs using side of tube sample aspiration.

Another aspect of the method that underwent some significant modifications during the development process is the master mix aspiration step. Testing of the initial master mix aspiration step found that the amount of master mix needed to successfully complete a run was excessive and would result in large amounts (hundreds of microliters) being discarded. Changes to this step included: reducing the depth the tip submerges below the liquid level from 2 mm to 0.5 mm, modifying portions of the method that caused aspiration above and beyond what is necessary, eliminating the error dialogue box when an insufficient volume is detected, modifying the rack to hold the master mix tubes in place during aspiration, and developing a detailed formula to calculate precisely how much master mix needs to be prepared to complete a run. Following these changes, the excess master mix required has been greatly reduced and typically results in only about 50 µL of residual master mix remaining following the run.

Following the modifications to the method, but prior to commencement of running DNA containing validation samples on the Nimbus, both the quantitation and amplification methods were run using TE dyed with food coloring. The purpose of this test was to verify that the instrument and method performed as expected and to visually inspect the volumes transferred (Figures 3 and 4). The method worked as expected and the volumes appeared visually correct.

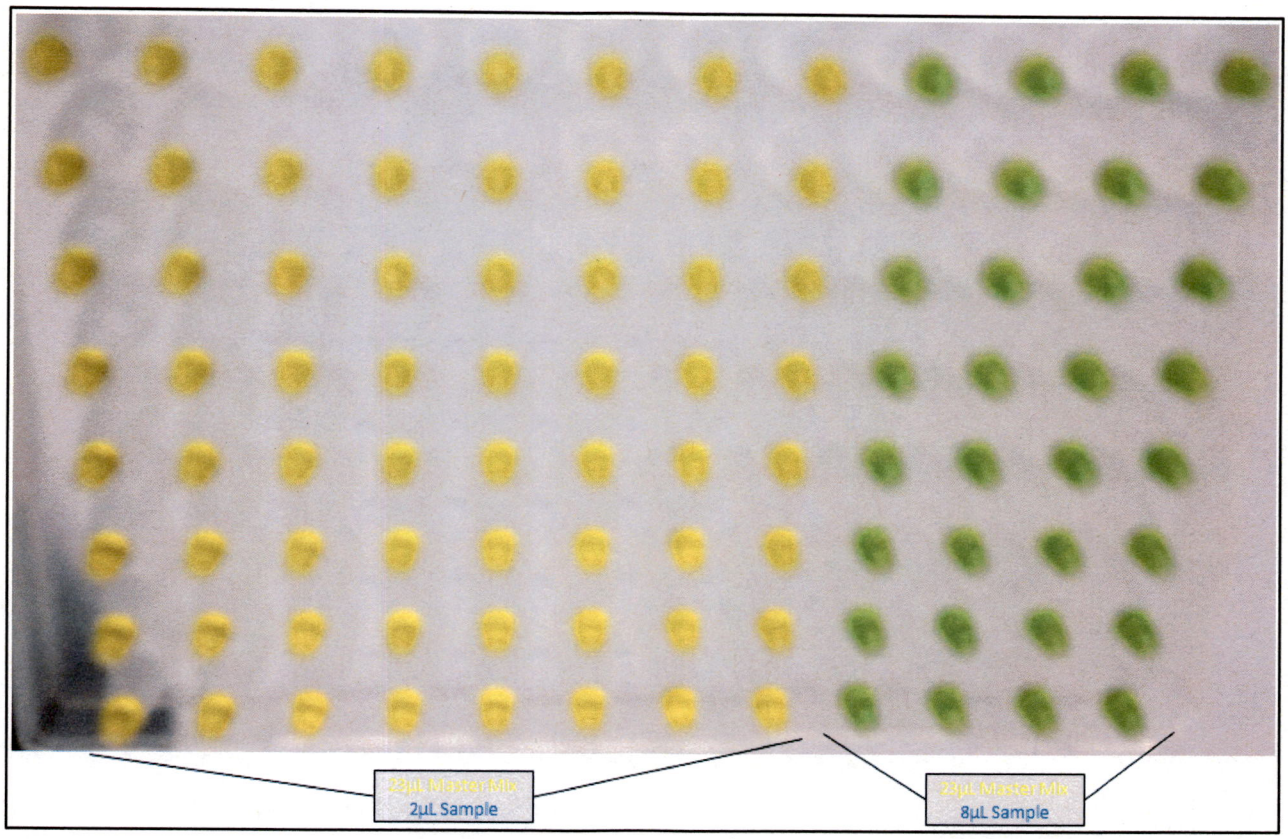


Figure 3: Photograph of PCR plate following quantitation dye experiment (yellow = master mix; blue = sample).

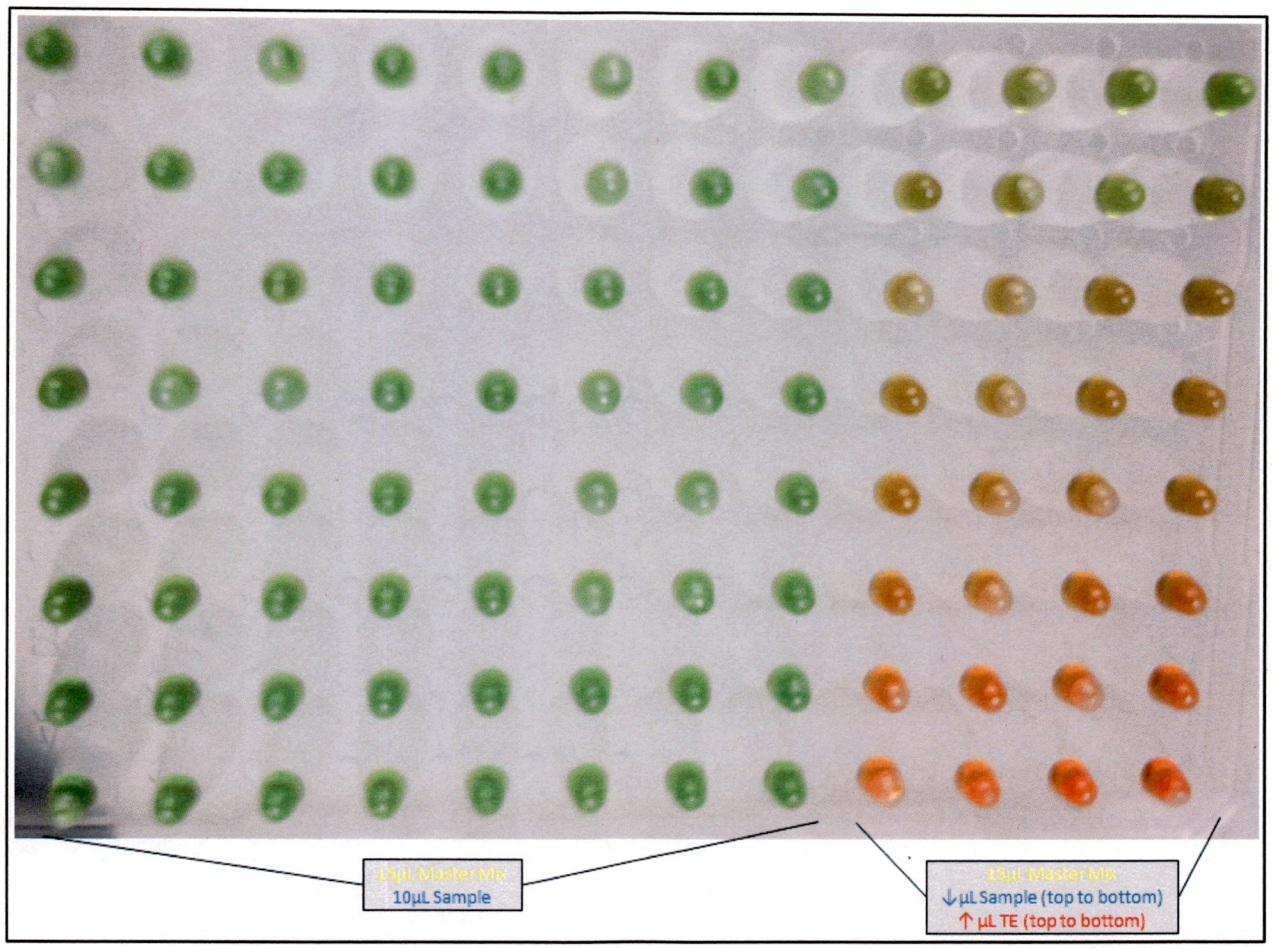


Figure 4: Photograph of PCR plate following amplification dye experiment (yellow = master mix; blue = sample; red = TE).

Conclusions

Based on the results of these experiments following modifications to the method, the method was deemed complete and the validation proceeded to experiments utilizing samples containing actual DNA.

Purpose

The goal of this study is to demonstrate that the Nimbus can reliably set-up plates for DNA quantitation and amplification reproducibly and with precision. This will be demonstrated by comparing the automated set-up process to the manual process with multiple replicates of samples both within the same plate and between different plates.

Materials and Methods

DNA Quantitation

Previously extracted DNA samples were set up for DNA amplification using both the Quantifiler Human and Quantifiler Duo DNA quantification kits. Three distinct, yet related, phenomena were investigated through the analyses of these assays.

The first quantitation plate was designed to examine the performance of the Hamilton Nimbus 4 Liquid Handling Workstation, both as compared with manual pipetting, as well as the reproducibility and precision of the pipetting of the Nimbus 4 within the plate. Half of the first plate was prepared using the Nimbus 4 Liquid Handling Workstation and the second half of the plate was prepared manually using the same set of extracted DNA samples to establish the comparison to manual pipetting. Additionally, within the set of samples pipetted, several samples were prepared in replicate to assess the reproducibility of the instrument within the plate. The plate was prepared using the Quantifiler Duo DNA quantitation kit. 43 samples, 2 calibrators, a TE amplification blank, and 2 reagent blanks were used for the preparation of the first plate. Two different volumes of sample, 2uL and 8uL, corresponding to the two standard volumes used in DNA casework, were investigated in this experiment.

To assess inter-plate reproducibility, two additional plates were prepared using the Nimbus 4. The same set of extracted DNA samples were set up using two separate Nimbus runs. The results of these plates were compared to determine the reproducibility of the Nimbus 4 between quantification runs. The plates were prepared using the Quantifiler Human DNA quantitation kit. 19 samples, 2 calibrators, a TE amplification blank, and 5 reagent blanks were used for the preparation of the first plate.

DNA Amplification

Previously extracted DNA samples were set up for DNA amplification using the Identifiler Plus kit. Half of the plate was setup using the Nimbus and the other half was setup in an identical

fashion manually. Some of the samples were setup over a range of DNA input amounts. Some of the samples were run in replicates with the DNA input amount held constant. One of the samples which was run in replicates with the DNA input amount held constant was also run in replicate at the same input amount on a different PCR amplification setup on the Nimbus for the Contamination Assessment study. The PCR amplification was performed on a 9700 thermal cycler, capillary electrophoresis was performed on a 3130, and the resultant data was analyzed in GMID-X. The resultant data was evaluated to verify that the profiles were consistent with those previously obtained for the samples, peak heights were comparable for replicates and commensurate with DNA input amount, that Nimbus and manually setup samples produced comparable peak heights, and that peak heights are comparable between plates.

Results and Discussion

DNA Quantitation

Comparison with manual sample transfer

The results of the 43 samples, 2 calibrators, a TE amplification blank, and 2 reagent blanks transferred by the Nimbus 4 were compared to the results obtained from manual pipetting. Overall, the results demonstrated a difference in pipetting between the Nimbus and manual pipetting. The results indicate that the Nimbus is, on average, transferring less than the manual pipette (the Eppendorf pipette in the amplification setup hood). The results indicate that volumes transferred manually were generally higher than the volumes pipette by the Nimbus, based on the resulting quantitation values (Table 1).

The results indicated that at the 2uL volume, the Nimbus was transferring approximately 70% the amount of liquid as the Eppendorf pipette. In addition, it was observed that there was less variation in transferring the large volume than when transferring the smaller volume. When transferring the 8uL volume the results were closer to a 1:1 ratio (Nimbus: Manual) than when transferring the 2uL volume. In general, the variation in robotic pipetting increases as the volume transferred decreases, as was observed in this study.

When the results of the Nimbus transferred volumes were examined, the instrument demonstrated that it is consistently transferring similar amounts. The quantitation values of replicate samples within the plate demonstrated consistent results. The standard deviation of replicate transfers was generally less than 0.1ng/uL, and when 10 replicates were examined, the standard deviation was approximately 0.11ng/uL (Tables 2 and 3).

Figure 1: Nimbus vs. manual sample transfer.

Sample	Volume Pipetted	Nimbus		Manual		Fold Difference Manual/Nimbus (Human Only)
		Human	Male	Human	Male	
Calibrator 1	2	7.215	7.815	12.570	12.138	1.74
Calibrator 2	2	2.099	1.881	3.071	3.013	1.46
TE_Amp_Blank	2	undet.	undet.	undet.	undet.	-
Sample 1	2	0.751	0.795	1.068	0.880	1.42
Sample 1.1	2	0.362	0.362	0.569	0.519	1.57
Sample 1.1.1	2	0.513	0.431	0.740	0.622	1.44
Sample 1.2.1	2	1.152	1.289	1.463	1.572	1.27
Sample 1.2.2	2	0.518	0.592	0.755	0.704	1.46
Sample 1.3	2	0.148	0.180	0.355	0.326	2.40
Sample 1.3.1	2	0.307	0.307	0.519	0.441	1.69
Sample 1.4	2	0.467	0.419	0.736	0.686	1.58
Sample 1.4.1	2	0.647	0.739	0.764	0.844	1.18
Sample 1.5	2	0.641	0.659	1.014	0.925	1.58
Sample 1.6a	2	0.410	0.391	0.629	0.591	1.53
Sample 1.6b	2	0.794	0.737	1.142	1.150	1.44
Sample 2	2	0.256	0.246	0.487	0.500	1.90
RB	2	undet.	undet.	undet.	undet.	-
Sample 1(2)	2	0.614	0.620	0.987	0.869	1.61
Sample 1.1(2)	2	0.338	0.346	0.559	0.435	1.65
Sample 1.1.1(2)	2	0.576	0.434	0.754	0.668	1.31
Sample 1.2.1(2)	2	1.163	1.217	1.510	1.613	1.30
Sample 1.2.2(2)	2	0.545	0.607	0.770	0.718	1.41
Sample 1.3(2)	2	0.166	0.147	0.263	0.194	1.59
Sample 1.3.1(2)	2	0.220	0.207	0.313	0.260	1.42
Sample 1.4(2)	2	0.424	0.344	0.600	0.451	1.42
Sample 1.4.1(2)	2	0.531	0.553	0.806	0.812	1.52
Sample 1.5(2)	2	0.710	0.675	1.021	0.754	1.44
Sample 1.6a(2)	2	0.317	0.314	0.467	0.430	1.47
Sample 1.6b(2)	2	0.896	0.809	1.033	0.877	1.15
Sample 2(2)	2	0.267	0.227	0.389	0.345	1.45
RB(2)	2	undet.	undet.	undet.	undet.	-
Sample 1.2.1(3)	2	1.359	1.373	1.820	1.812	1.34
Sample 1.2.1(4)	2	1.109	1.028	1.602	1.591	1.45
Sample 1.2.1(5)	2	1.078	1.181	1.598	1.530	1.48
Sample 1.2.1(6)	2	1.336	1.376	1.669	2.000	1.25
Sample 1.2.1(7)	2	1.359	1.269	1.772	1.686	1.30
Sample 1.2.1(8)	2	1.291	1.321	1.659	1.691	1.29
Sample 1.2.1(9)	2	1.110	1.166	1.654	1.600	1.49
Sample 1.2.1(10)	2	1.145	1.160	1.693	1.514	1.48
PTO	8	2.132	2.179	1.839	2.173	0.86
SAM	8	9.871	10.167	10.159	9.914	1.03
SAM_RNA	8	2.710	2.790	2.622	2.590	0.97
PTO_RNA	8	1.051	1.153	1.189	1.229	1.13
2b(09-572)1/50	8	0.037	0.049	0.037	0.020	1.00
SAM-2(1/50)	8	0.044	0.052	0.061	0.030	1.39
1(09-572)	8	0.103	0.212	0.120	0.262	1.17
2(09-572)	8	3.115	3.132	3.193	3.226	1.03
4NS(12-571)	8	1.642	0.165	1.729	0.102	1.05
					Total Average	1.40
					Average 8uL	1.07
					Average 2uL	1.49

Table 2: Reproducibility within a single plate

Within Plate Reproducibility							
Sample	Volume (uL)	Human	Male	Human(2)	Male(2)	Std. Dev Human	Std Dev Male
Sample 1	2	0.751	0.795	0.614	0.620	0.096	0.124
Sample 1.1	2	0.362	0.362	0.338	0.346	0.017	0.011
Sample 1.1.1	2	0.513	0.431	0.576	0.434	0.045	0.002
Sample 1.2.1	2	1.152	1.289	1.163	1.217	0.008	0.051
Sample 1.2.2	2	0.518	0.592	0.545	0.607	0.019	0.010
Sample 1.3	2	0.148	0.180	0.166	0.147	0.013	0.023
Sample 1.3.1	2	0.307	0.307	0.220	0.207	0.061	0.071
Sample 1.4	2	0.467	0.419	0.424	0.344	0.030	0.053
Sample 1.4.1	2	0.647	0.739	0.531	0.553	0.082	0.132
Sample 1.5	2	0.641	0.659	0.710	0.675	0.048	0.011
Sample 1.6a	2	0.410	0.391	0.317	0.314	0.066	0.054
Sample 1.6b	2	0.794	0.737	0.896	0.809	0.072	0.051
Sample 2	2	0.256	0.246	0.267	0.227	0.008	0.013
RB	2	undet.	undet.	undet.	undet.	-	-

Table 3: Reproducibility of 10 replicates

Precision - 10 replicates			
Sample	Volume (uL)	Human (ng/uL)	Male (ng/uL)
Sample 1.2.1	2	1.152	1.289
Sample 1.2.1(2)	2	1.163	1.217
Sample 1.2.1(3)	2	1.359	1.373
Sample 1.2.1(4)	2	1.109	1.028
Sample 1.2.1(5)	2	1.078	1.181
Sample 1.2.1(6)	2	1.336	1.376
Sample 1.2.1(7)	2	1.359	1.269
Sample 1.2.1(8)	2	1.291	1.321
Sample 1.2.1(9)	2	1.110	1.166
Sample 1.2.1(10)	2	1.145	1.160
	Average	1.210	1.238
	Std Dev	0.113	0.109
	Max	1.359	1.376
	Min	1.078	1.028

Inter-plate Reproducibility

The 19 samples, 2 calibrators, a TE amplification blank, and 5 reagent blanks were prepared on two separate plates for analysis in two distinct 7500 runs. The data were compared to determine the reproducibility of the performance of the Nimbus liquid handling workstation. In order to produce a meaningful comparison across the data set, the quantitation data were evaluated based on the subsequent volume of the samples that would be used for amplification with the Identifiler Plus amplification kit. Each of the samples in the experiment was evaluated based on the theoretical target amount of 0.8ng DNA and the required volume of sample required to obtain the target value was compared across both plates (Table 4).

Table 4: Inter-plate Reproducibility

Plate 1	Plate 1 Quant value	Plate 2	Plate 2 Quant value	Difference ng/uL	% Difference	Vol. Amped based on Plate 1	Vol. Amped based on Plate 2	Difference in volume amped
Calibrator 1	9.616	Calibrator 1	10.186	0.570	5.6%			
Calibrator 2	2.250	Calibrator 2	2.501	0.250	10.0%			
TE_Amp_Blank	undet.	TE_Amp_Blank	undet.	-	-			
Sample 1	0.935	Sample 1	0.953	0.017	1.8%	0.86	0.84	0.02
Sample 1.1	0.318	Sample 1.1	0.407	0.089	22.0%	2.52	1.96	0.55
Sample 1.1.1	0.551	Sample 1.1.1	0.613	0.062	10.1%	1.45	1.30	0.15
Sample 1.2.1	0.898	Sample 1.2.1	0.915	0.017	1.8%	0.89	0.87	0.02
Sample 1.2.2	0.987	Sample 1.2.2	1.257	0.270	21.5%	0.81	0.64	0.17
Sample 1.3	0.278	Sample 1.3	0.348	0.070	20.1%	2.88	2.30	0.58
Sample 1.3.1	0.393	Sample 1.3.1	0.515	0.122	23.7%	2.03	1.55	0.48
Sample 1.4	0.541	Sample 1.4	0.521	0.020	3.9%	1.48	1.54	0.06
Sample 1.4.1	0.423	Sample 1.4.1	0.601	0.178	29.6%	1.89	1.33	0.56
Sample 1.5	0.765	Sample 1.5	0.892	0.127	14.2%	1.05	0.90	0.15
Sample 1.6a	0.473	Sample 1.6a	0.557	0.085	15.2%	1.69	1.44	0.26
Sample 1.6b	0.994	Sample 1.6b	0.991	0.003	0.3%	0.80	0.81	0.00
Sample 2	0.464	Sample 2	0.496	0.033	6.6%	1.73	1.61	0.11
RB	undet.	RB	undet.	-	-	-	-	-
1(09-572)b	0.009	1(09-572)b	0.020	0.011	55.2%	91.07	40.80	50.27
2(09-572)	1.182	2(09-572)	0.942	0.240	25.5%	0.68	0.85	0.17
RBa	undet.	RBa	undet.	-	-	-	-	-
PTO_RNA	0.273	PTO_RNA	0.504	0.230	45.7%	2.93	1.59	1.34
SAM_RNA	0.788	SAM_RNA	0.916	0.128	14.0%	1.02	0.87	0.14
RB_RNA	undet.	RB_RNA	undet.	-	-	-	-	-
SAM	0.507	SAM	0.671	0.164	24.5%	1.58	1.19	0.39
RB1	undet.	RB1	undet.	-	-	-	-	-
PTO	0.248	PTO	0.362	0.114	31.5%	3.23	2.21	1.02
RB2	undet.	RB2	undet.	-	-	-	-	-

Based on this assessment, the results from the separate quantitation assays were comparable. Over the 19 samples in the data set, 16 samples had less than 0.58uL difference in amplification volume between the two plates, two samples differed by approximately 1uL, and a very dilute sample indicated a drastic difference of 50uL. Overall these results indicate a high level of concordance between the two plates. The largest difference between the two data sets occurred in a sample that has picogram quantities of DNA per microliter. This amount of DNA is within the stochastic region of the assay, where large differences in volume do not necessarily correlate to extreme differences in the overall amplification results. In this particular instance, the sample would have required concentration and consumption in order to attempt to produce a complete DNA profile. In the other instances where the volume of sample amplified differed by approximately 1uL, the likely difference in the robustness of the profile would not have been very significant and the samples likely would still have been within the dynamic range of the capillary electrophoresis instruments.

DNA Amplification

All samples produced the expected profiles. Overall peak heights appear to be fairly consistent between samples setup on the Nimbus and manually (Table 5). There does seem to be more variation between Nimbus and manual setup at lower input volumes. However, even with the increased variability in peak heights at lower input volumes, full DNA profiles were still obtained for all samples. For samples with varied DNA input ranges, peak heights generally increased with increasing input amount for both Nimbus and manual setup with few anomalies. Samples amplified in replicate with constant DNA input amount produced constant peak heights, as demonstrated by the relatively low standard deviations. The amount of variation between these replicate samples is comparable between the Nimbus and manual setup methods.

Table 5: Peak height data for reproducibility and precision amplification experiment.

Sample	Total RFU-Nimbus	Total RFU-Manual	Nimbus RFU/Manual RFU
9947A_Control-7uL	41,731	44,497	94%
TE_Amp_Blank	0	0	n/a
SAM-1uL	12,963	33,995	38%
SAM-2uL	44,842	62,152	72%
SAM-3uL	60,698	78,861	77%
SAM-4uL	79,004	105,559	75%
SAM-5uL	100,664	126,632	79%
SAM-6uL	129,786	149,243	87%
SAM-7uL	199,663	151,074	132%
SAM-8uL	154,764	155,297	100%
2(13-571)-2uL	11,940	20,838	57%
2(13-571)-3uL	20,446	27,051	76%
2(13-571)-4uL	26,699	33,410	80%
2(13-571)-5uL	43,565	44,145	99%
2(13-571)-6uL	48,789	47,282	103%
2(13-571)-7uL	55,055	64,496	85%
2(13-571)-8uL	95,313	67,891	140%
2(13-571)-9uL	85,480	79,190	108%
PTO-3uL	12,746	26,848	47%
PTO-4uL	90,599	28,343	320%
PTO-5uL	36,066	26,583	136%
PTO-6uL	30,724	43,978	70%
PTO-7uL	97,112	44,086	220%
PTO-8uL	59,479	52,495	113%
PTO-9uL	94,888	62,621	152%
PTO-10uL	72,994	65,256	112%
1(13-571)-10uL	20,396	32,985	62%
1(13-571)-10uL	20,733	25,592	81%
1(13-571)-10uL	31,680	25,782	123%
1(13-571)-10uL	24,735	21,031	118%
1(13-571)-10uL	21,629	25,922	83%
1(13-571)-10uL	23,170	25,418	91%
1(13-571)-10uL	23,796	26,794	89%
1(13-571)-10uL	22,760	27,948	81%
	AVG Nimbus- 23,612	AVG Manual- 26,434	89%
Std. Dev.	3,583	3,316	
2(12-571)-10uL	52,163	57,126	91%
2(12-571)-10uL	48,113	52,178	92%
2(12-571)-10uL	68,438	51,004	134%
2(12-571)-10uL	52,706	54,432	97%
2(12-571)-10uL	49,948	52,296	96%
2(12-571)-10uL	52,254	44,589	117%
2(12-571)-10uL	55,988	49,018	114%
2(12-571)-10uL	60,504	45,413	133%
	AVG Nimbus- 55,014	AVG Manual- 50,757	108%
Std. Dev.	6,608	4,279	
1(12-571)-1.5uL	14,106	29,926	47%
1(12-571)-2.5uL	14,621	45,336	32%
1(12-571)-3.5uL	42,752	48,851	88%
1(12-571)-4.5uL	59,875	72,321	83%
*For manual the volumes were rounded up using the WellAware			

The sample used to assess inter-plate reproducibility, 2(12-571), had a total RFU average of 55,014 and standard deviation of 6,608 for the first plate. The second plate had an average of 45,037 and 7,013 RFU, respectively.

Conclusions

The instrument is able to generate reproducible and precise results that are reflected in the comparison of concentrations and peak heights of the replicate samples prepared in this study and the relatively small standard deviations obtained about the mean for these samples. There are no significant differences in concentrations and peak heights for samples that are prepared on the Nimbus and those prepared manually, or between different plates prepared on the Nimbus. Based on the increased variability at the low volumes transferred with the Nimbus it is recommended, although not required, that samples that require less than 4uL of input DNA be rounded up to the nearest whole number to account for the reduced volume compared to manual pipetting.

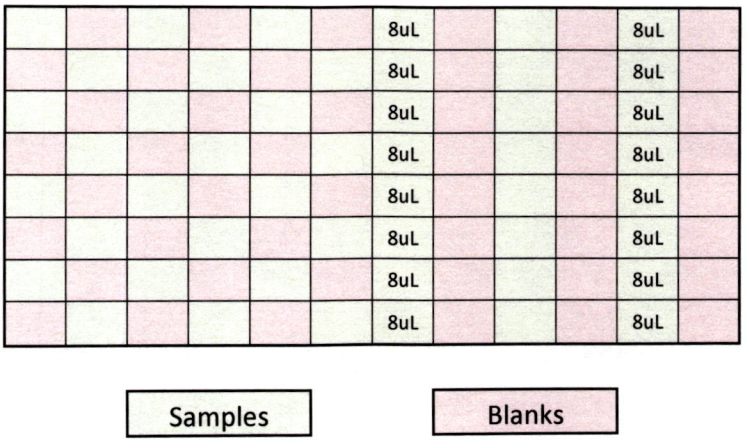
Purpose

The goal of this study is to demonstrate that the Nimbus 4 Liquid Handling Workstation can set up plates for DNA quantitation and amplification without the occurrence of cross-contamination between samples at either the source position or destination plate.

Materials and Methods

DNA Quantitation

Previously extracted DNA samples and TE blanks were setup on the Nimbus 4 Liquid Handling Workstation for Quantifiler Human DNA quantitation. The samples and blanks were interspersed using two types of patterns. Half the plate was setup in a checkerboard pattern alternating samples and blanks. The second half of the plate was setup alternating rows of samples with rows of blanks. On the first half of the plate the Nimbus transferred 2uL of both samples and blanks. On the second half of the plate the Nimbus transferred 8uL of sample into two columns (7 and 11). The design of this experiment was to determine whether we could detect any contamination of the samples either in the source tubes, or the destination plate.



DNA Amplification

Previously extracted DNA samples and TE blanks were set up on the Nimbus for DNA amplification using the Identifiler Plus kit. Half of the plate was set up in a checker board pattern (i.e. alternating samples and blanks between each sample) and the other half in a zebra stripe pattern (i.e. alternating samples and blanks between each column). Half of the TE blanks

came from the TE aliquots on the deck and half came from tubes containing TE in sample positions. The PCR amplification was performed on a 9700 thermal cycler, capillary electrophoresis was performed on a 3130, and the resultant data was analyzed in GMID-X. The resultant data was evaluated to verify that the profiles were consistent with those previously obtained for the samples with no extraneous peaks attributable to contamination and that blank samples had no DNA types detected.

Results and Discussion

DNA Quantitation

Two samples out of the 48 blanks (cells B8 and G10) had 0.001ng/uL detected in them. This level of DNA is well within the concentration where stochastic effects or artifacts are possible. The detection of this amount of DNA is therefore not definitive in determining if contamination had occurred, nor where it may have occurred. A higher amount of DNA in a cell would be more telling in terms of contamination, and if contamination were to occur in casework samples, the amplification for Identifiler Plus of the concentrated samples is likely going to yield far more information with regards to contamination than the single picogram level results from the quantitation assay. Based on the lack of any significant levels of detectable DNA in the blanks, it does not appear as if liquid handling of samples on the Nimbus workstation is resulting in any significant contamination of the samples tubes or destination plates.

DNA Amplification

All blank samples had no DNA types detected. All DNA containing samples produced the expected profiles with no extraneous peaks attributable to contamination.

Conclusions

Definitive occurrences of cross-contamination between samples on either the source plate or destination plate were not observed with the DNA quantitation and amplification methods on the Nimbus. Based on the lack of any significant levels of detectable DNA in the blanks, in either the quantitation or amplification methods, it does not appear as if liquid handling on the Nimbus workstation is resulting in any significant contamination of the samples tubes or destination plates.

Purpose

Following completion of all other validation studies, a final verification was performed using mock case samples to ensure that the quantitation set-up and amplification set-up methods on the Nimbus result in the expected quality of DNA profiles.

Materials and Methods

Previously extracted DNA samples from training exercises (buccal swabs, bloodstains, and cigarette butts), quality control runs, proficiency tests (blood and semen stains), and associated reagent blanks were used in this experiment. The samples were set-up on the Nimbus for DNA quantitation with the Quantifiler Human kit. The DNA quantitation results were used to determine the appropriate amount of input DNA for amplification, and the Identifiler Plus amplification was set-up on the Nimbus. Capillary electrophoresis was performed on a 3130 and the resultant data was analyzed in GMID-X. The resultant data was evaluated to verify that the profiles were consistent with those previously obtained for the samples and peak heights were commensurate with the amount of input DNA. This experiment was performed twice using different mock case samples each time.

Results and Discussion

Initial quantitation runs in which calibrator C_t values were outside the expected range and non-concordant results were obtained, were determined to be the result of user error.

Subsequent to remedying the user error issues, DNA typing results for all samples were consistent with the known profiles based on previous testing of the samples. All blank samples produced "undetermined" quantitation results and had no DNA types detected. Quantitation calibrators and positive amplification controls produced expected results. With the exception of a single sample, the peak heights obtained were commensurate with the amount of input DNA based on quantitation. The exception, was a sample that produced a quantitation result of 5.275 ng/µL and resulted in a partial profile being detected from amplification of 10 µL of a 1/100 dilution.

Conclusions

The Nimbus is able to reliably set-up mock case samples for quantitation and amplification. The fact that correct profiles were obtained and blanks were absent of DNA, further supports that contamination is not a concern on the Nimbus. Peaks generated following amplification are

consistent with the heights expected based on input amounts of DNA determined from quantitation. The single exception of this observed during validation was possibly caused during dilution of the sample which was quantitated, rather than an issue with the Nimbus itself. The fact that initial quantitation efforts suggested user error, emphasizes the critical nature of training and use of proper technique.

SDPD Forensic Science Section – Forensic Biology Unit

Validation of the Hamilton Nimbus 4 Liquid Handling Workstation

Performance Check of Nimbus #2

Purpose

The initial validation of the Nimbus was performed on the original Nimbus obtained at the SDPD, Nimbus #1. The SDPD has acquired a second Nimbus, Nimbus #2. The goal of this study is to demonstrate that results obtained on Nimbus #1 are reproducible on Nimbus #2 and that values from the internal validation can still be obtained.

Materials and Methods

One of the experiments from the Mock Case Samples study was replicated on Nimbus #2. Previously extracted DNA samples from training exercises (buccal swabs, bloodstains, and cigarette butts) and associated reagent blanks were used in this experiment. The samples were set-up on Nimbus #2 for DNA quantitation with the Quantifiler Human kit. The DNA quantitation results were used to determine the appropriate amount of input DNA for amplification, and the Identifiler Plus amplification was set-up on Nimbus #2. Capillary electrophoresis was performed on a 3130 and the resultant data was analyzed in GMID-X. The DNA quantitation results were compared to those originally obtained on Nimbus #1. The electropherogram data was evaluated to verify that the profiles were consistent with those previously obtained for the samples and peak heights were commensurate with the amount of input DNA and comparable to those obtained previously on Nimbus #1.

Results and Discussion

DNA quantitation results were comparable between samples setup on Nimbus #1 and #2. DNA typing results for all samples were consistent with the known profiles based on previous testing of the samples and those generated during the validation run on Nimbus #1. All blank samples produced "undetermined" quantitation results and had no DNA types detected. The quantitation calibrators and positive amplification control produced expected results. Peak heights obtained were commensurate with the amount of input DNA based on quantitation and were comparable to those previously obtained using Nimbus #1. Observed variation between peak heights is due to differing amounts of input DNA based on quantitation and runs on different CE instruments with different sensitivity levels. The sample that produced a quantitation result of 5.275 ng/µL and resulted in a partial profile being detected from amplification of 10 µL of a 1/100 dilution on Nimbus #1, produced a quantitation result of 0.850 ng/µL and resulted in full robust profile being detected from amplification of 1 µL of the neat extract on Nimbus #2. This is the only sample where such a large difference was observed between Nimbus #1 and #2. It appears that the initial quantitation result overestimated the amount of DNA present in the sample.

Conclusions

Results obtained on Nimbus #2 are comparable to those previously obtained on Nimbus #1 during the internal validation. Therefore, Nimbus #2 has been shown to be able to reliably setup of samples for DNA quantitation and amplification.