SDPD Crime Laboratory – Forensic Biology Unit **December 27th, 2019**

Performance Verification of the QIAgility Liquid Handling System

QIAgility vs. Corbett CAS-1200 vs. Manual Setup Study

Purpose

The QIAgility was installed on December 3rd, 2019. The performance of the QIAgility was evaluated by comparing sample peak heights generated from QIAgility setup to sample peak heights generated from Corbett CAS-1200 and manual setups.

Materials and Methods

One positive control and ten single-source samples amplified using the GlobalFiler kit on October 9th, 2019 were used for comparison and set up on the same plate on the same day. The QIAgility and Corbett setups consisted of 20µL master mix (formamide and GS 600 LIZ sizing standard), 2µL ladder, and 2µL sample. The manual setup was inadvertently prepared using the same volumes as automated rather than 10µL master mix and 1µL ladder and sample as used in casework. The QIAgility protocol was slightly modified to skip the first two rows so that all samples were prepared on the same plate, allowing for direct comparison. The plate was run on the 3500B genetic analyzer, and the samples were analyzed with GeneMapper ID-X software.

Results and Discussion

Overall, the peak heights were lower than expected across all setups methods; however, since this is observed across all setup methods, it is likely due to the age of the samples and is not indicative of an issue with pipetting. The average sample peak heights were calculated and graphed for comparison (figure 1). The fold changes ranged from 0.893 to 1.396 (QIAgility/Corbett) and 0.836 to 1.662 (QIAgility/manual). Variations in peak heights on the 3500 was compared using paired t-tests. T-test between data sets generated values of p=0.068 (QIAgility vs. Corbett) and p=0.998 (QIAgility vs. manual) which establishes that the variation of peak heights between setup methods is not statistically significant and are likely due to expected variation rather from systemic differences in pipetting performance between the different methods. In addition, it should be noted that the results vary from sample to sample as to which method produced the highest and lowest peak heights, which again indicates random variation as opposed to variation favoring one setup method.

In the previous performance check of the Corbett CAS-1200 on October 23rd, 2019, the performance of the Corbett CAS-1200 was established to yield peak heights comparable to casework manual setup (10µL master mix and 1µL ladder and sample). Equivalence of peak heights generated from QIAgility and Corbett setups confirms the equivalence of QIAgility pipetting and manual pipetting using the standard manual setup volumes of 10µL formamide/ISS and 1µL of sample (or ladder).

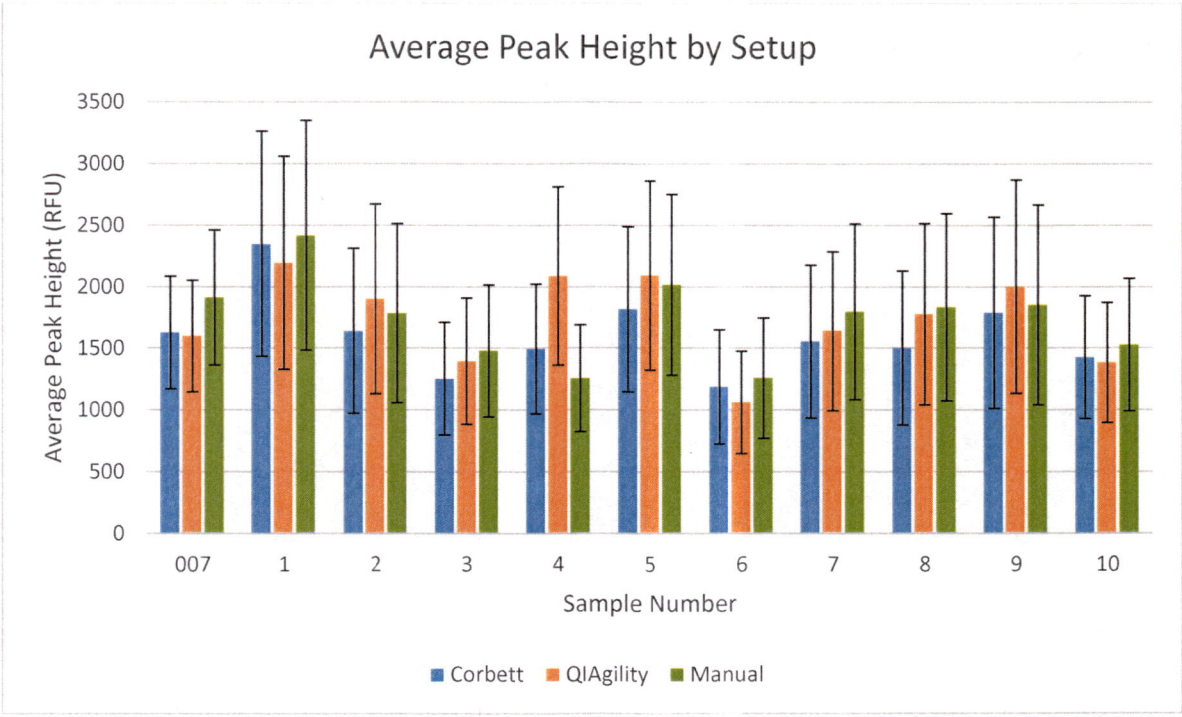


Figure 1

Conclusion

The ranges of overall peak height averages are consistent between QIAgility, Corbett, and manual pipetting using 20µL and 2µL ladder and sample volumes. Statistical analysis of average peak heights of samples prepared using the QIAgility and Corbett instruments indicates comparable pipetting to the manual method; therefore, the QIAgility is approved for casework.

Annaliese Dang 12/27/19

Annaliese Dang
Laboratory Technician

12.27.2019

Shawn Montpetit
DNA Technical Manager

QIAgility Verification
AHD p. 1 of 2

SAN DIEGO POLICE DEPARTMENT
FORENSIC SCIENCE SECTION
3500 Sample Setup Sheet

Case #: QIAgility Verification Date: 12/26/2019 Analyst: AHD

	1	2	3	4	5	6	7	8	9	10	11	12
A	C_Formamide Blank	C_A1-4	Q_Formamide Blank	Q_A1-4	M_Formamide Blank	M_A1-4						
B	C_Formamide ISS	C_A1-5	Q_Formamide ISS	Q_A1-5	M_Formamide ISS	M_A1-5						
C	C_Ladder 1	C_A2-1	Q_Ladder 1	Q_A2-1	M_Ladder 1	M_A2-1						
D	C_007_Control	C_A2-2	Q_007_Control	Q_A2-2	M_007_Control	M_A2-2						
E	C_TE_Amp_Blank	C_A2-3	Q_TE_Amp_Blank	Q_A2-3	M_TE_Amp_Blank	M_A2-3						
F	C_A1-1	C_A2-4	Q_A1-1	Q_A2-4	M_A1-1	M_A2-4						
G	C_A1-2	C_A2-5	Q_A1-2	Q_A2-5	M_A1-2	M_A2-5						
H	C_A1-3	C_Ladder 2	Q_A1-3	Q_Ladder 2	M_A1-3							

Instrument: 3500B

Sample setup: Corbett CAS-1200

QIAgility

Manual

Reagent	Lot #	vol (µl)	# of samples + 10	Total (µl)
Form.	1903619	19.2	60	1152
Int.Std.	00767052	0.8	60	48
Ladder	GF1-19	2		

Applied Biosystems
GeneMapper® ID-X 1.4

Project: 2019-12-26 QIAgility Verification AHD

AHD

	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	SQ	CGQ
1	C_Formamide Blank	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	●	NA
2	C_Formamide ISS	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
3	C_Ladder 1	Allelic Ladder	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
4	C_007_Control	Positive Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
5	C_TE_Amp_Blank	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
6	C_A1-1	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
7	C_A1-2	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
8	C_A1-3	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
9	C_A1-4	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
10	C_A1-5	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
11	C_A2-1	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
12	C_A2-2	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
13	C_A2-3	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
14	C_A2-4	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
15	C_A2-5	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
16	C_Ladder 2	Allelic Ladder	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
17	Q_Formamide Blank	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	●	NA
18	Q_Formamide ISS	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
19	Q_Ladder 1	Allelic Ladder	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
20	Q_007_Control	Positive Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
21	Q_TE_Amp_Blank	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
22	Q_A1-1	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
23	Q_A1-2	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
24	Q_A1-3	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
25	Q_A1-4	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
26	Q_A1-5	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
27	Q_A2-1	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
28	Q_A2-2	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
29	Q_A2-3	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
30	Q_A2-4	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
31	Q_A2-5	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
32	Q_Ladder 2	Allelic Ladder	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
33	M_Formamide Blank	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	●	NA
34	M_Formamide ISS	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
35	M_Ladder 1	Allelic Ladder	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
36	M_007_Control	Positive Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
37	M_TE_Amp_Blank	Negative Control	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
38	M_A1-1	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
39	M_A1-2	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
40	M_A1-3	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
41	M_A1-4	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
42	M_A1-5	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
43	M_A2-1	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
44	M_A2-2	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
45	M_A2-3	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	▲
46	M_A2-4	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■
47	M_A2-5	Sample	GF_reference	GlobalFiler_2019	GS600_LIZ	■	■

Genemapper project location :

H:\GeneMapperID projects\QA-QC for Instruments and Reagents\QIAgility\2019

SDPD Forensic Science Section – Forensic Biology Unit **March 4, 2008**

Validation of the CAS-1200 Liquid Handling System from Corbett Robotics

Validation Summary

Introduction

The CAS-1200 Liquid Handling System from Corbett Robotics is a precision liquid handling instrument originally designed for automated quantitative real-time PCR set-up. The intent of the Forensic Biology Unit is to utilize the CAS-1200 to automate the set-up of plates for capillary electrophoresis on the ABI 3130. The automation of this set-up process will increase the overall efficiency and productivity of the laboratory by saving analyst time and increasing precision. The CAS-1200 is capable of preparing an entire 96-well reaction plate for capillary electrophoresis in approximately 20 minutes, compared to an hour or longer manually. Additionally, automated set-up eliminates the possibility of analyst error which could occur if an analyst were to inadvertently skip a sample or transfer a sample to an incorrect position on the reaction plate. Pipetting precision should also be improved with the CAS-1200 compared to manual techniques due to uniformity in the parameters defining the volume the instrument pipettes versus the variability of human performance.

Throughout this validation study the protocol and instrumental settings were optimized for the desired application in the Forensic Biology Unit. The CAS-1200's ability to set up plates with precision and in a reproducible manner were examined and compared to the manual set-up procedure. The potential for cross-contamination between samples was also assessed during this study.

Precision and Reproducibility Study

Preliminary "dye" experiments allowed for the optimization of the instrumental settings. These experiments also led to the determination that it is best to either use no cover on the sample plate during the set-up process or a perforated film. Direct comparison between samples set up on the CAS-1200 and those set up manually, revealed that 1 µL pipetted manually is approximately equivalent to 1.4 µL on the CAS-1200. Furthermore, it was determined that the instrument has difficulties pipetting volumes near 1 µL. Since experimentation demonstrated that the doubling of reagent and sample volumes does not affect results, the determination was made that 2.8 µL of sample and 18 µL of master mix (17.4 µL formamide & 0.6 µL GS500 LIZ Size Standard) on the CAS-1200 is comparable to 1 µL of sample and 9 µL of master mix by hand. Using these specifications comparisons were made between automated and manual set-up. On average, the manual total peak heights were ~95% of those from the CAS-1200. Peak heights remained consistent with replicate samples set up on the CAS-1200. Furthermore, the correct DNA profiles were obtained for all samples and controls.

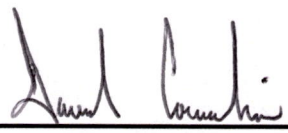
Contamination Study

Initial experiments using a "checker-board" pattern of samples and TE blanks revealed extremely low level DNA peaks in a small percentage of the TE blanks. All of these peaks were below the 75 RFU threshold and were typically less than 30 RFUs. One exception, was a TE blank that demonstrated peaks near 60 RFUs. Residual contamination in the capillary array was ruled out as a possible source of these observations. The mechanism for these observations was not isolated during this study, however, it was demonstrated that when true amplified blanks (containing primers, dNTPs, $MgCl_2$, etc) are used instead of TE blanks no peaks are observed in the blank samples. It is hypothesized that the PCR primers in the amplified blanks outcompete any such low levels of DNA, if present, during electrokinetic injection eliminating any visible peaks from possible contamination. Since only amplified samples and blanks are used in forensic DNA casework there is no cause for concern of detecting any low level types that would affect typing results. The CAS-1200 also proved that it is capable of isolating specific samples from the source plate and transferring them to the correct position on the destination plate ("cherry-picking").

Conclusion

The CAS1200 underwent rigorous inspection by the staff of the Forensic Biology Unit. The instrument was tested with regards to precision, sensitivity, and contamination. It was also subjected to tests to enable optimization of the protocol and assess the flexibility of its capabilities. The performance of the CAS-1200 in all of these facets was shown to be satisfactory. The study has demonstrated that automated set-up on the CAS-1200 is at least as good as manual set-up with regards to all areas tested.

The CAS-1200 liquid handling system from Corbett Robotics has successfully passed internal validation studies performed by the Forensic Biology Unit. Therefore, the CAS-1200 is approved for the automated preparation of amplified samples for analysis on the ABI 3130 in forensic DNA casework.

X 

David Cornacchia
Criminalist

X 

Deborah Blackwell
Criminalist

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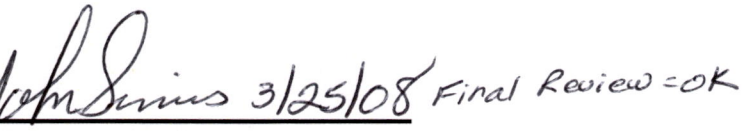
Darren Wright
Criminalist

X 

Shawn Montpetit
DNA Technical Manager

X 

Patrick O'Donnell
Supervising Criminalist

X 

John Simms
QA Manager

SDPD Forensic Science Section – Forensic Biology Unit

Validation of the CAS-1200 Liquid Handling System from Corbett Robotics

Precision and Reproducibility Study

Purpose

The CAS-1200 Liquid Handling System from Corbett Robotics is a precision liquid handling instrument originally designed for automated quantitative real-time PCR set-up. The intent of the Forensic Biology Unit is to utilize the CAS-1200 to automate the set-up of plates for capillary electrophoresis on the ABI 3130. The goal of this study is to demonstrate that the instrument can reliably set-up these plates reproducibly and with precision. This will be demonstrated by comparing the automated set-up process to the manual process currently in use. The instrument must successfully distribute the required reagents to their proper positions in the 96-well reaction plate, as well as transfer the PCR product samples to their desired location on the reaction plate. Also, the proper volumes of liquid reagents/PCR product must be transferred to the reaction plate. Samples set up using the CAS-1200 should consistently generate the correct DNA profile following capillary electrophoresis and peak heights should be comparable to those obtained following manual set-up. This study will also help determine the type of sample plate cover that is most suitable for use on the instrument, if one chooses to use a plate cover. Additionally, the experimental protocol and instrumental settings will be assessed throughout the experiments and optimized as a result of them.

Materials and Methods

"Dye" Experiments:

Multiple experiments were performed utilizing colored dyes in order to assess the CAS-1200's ability to uniformly distribute the desired volume of sample to the reaction plate. In these experiments water was used to represent the master mix and different colored dyes were used in place of samples and reagents. Reaction plates were visually inspected after set-up, checking that the correct dyes were transferred to the correct wells and that this was accomplished uniformly. Following various adjustments to the instrument/protocol and sample volumes the experiment was repeated until satisfactory results were obtained.

Sample Plate Covers:

Additional "dye" experiments were performed to determine which type of sample plate cover worked best. Foil covers, septa mat, no cover, and perforated film were all assessed during these experiments.

Manual vs. Automated Set-Up:

EMPLOYEE SAMPLES

Four previously extracted and amplified employee DNA samples were set up for capillary electrophoresis on the CAS-1200. 1 uL of each sample was transferred from the sample plate to 23 wells on the reaction plate containing 9 uL of master mix. The plate was then run on an ABI 3130. These same samples had previously been set up manually and run on an ABI 3130.

07-575 #1-4

Two 96 well plates were manually loaded with four different amplified DNA samples (07-575 #1-4) in duplicate. One plate was prepared with 1 uL of sample and 9 uL of master mix (1:9) and the other with 2 uL of sample and 18 uL of master mix (2:18). Capillary electrophoresis was performed on each of the two plates. Peak heights between the two plates were compared with each other. Additionally, the peak heights from the 1:9 plate were compared to those obtained from the CAS-1200 prepared 1:9 plate from the "employee samples" experiment (see above).

88-KH EXPERIMENT #1

The previously extracted and quantitated DNA sample, 88-KH, was amplified 60 times along with a positive and negative control and transferred to the 96-well plate format. The CAS-1200 was used to set up a reaction plate from these samples with 15 replicates of the 88-KH DNA containing 2.8 uL of sample and 18 uL of master mix. Additionally, 15 wells were set up manually with the 88-KH DNA. Capillary electrophoresis was performed on an ABI 3130 and the resultant peak heights compared between the CAS-1200 and manual samples.

88-KH EXPERIMENT #2

The previously extracted and quantitated DNA sample, 88-KH, was PCR amplified with the Identifiler kit on a 9700 thermal cycler using the Qiagen BioRobot Universal for automated sample set-up in a 96-well plate. The resultant PCR product consisted of 94 replicates of the 88-KH DNA with an input concentration of 1.3 ng, as well as a positive and negative control.

A 96-well reaction plate was prepared for capillary electrophoresis in the following manner. Automated set-up of wells A1 to H10 included: 1 formamide blank, 1 formamide/internal size standard, 2 allelic ladders, 1 positive control, 1 negative control, and 74 88-KH DNA samples. Each sample well was prepared with 17.4 uL of formamide, 0.6 uL of GS500 LIZ Size Standard, and 2.8 uL of sample or allelic ladder. Manual set-up of wells A11 to H12 included: 1 formamide blank, 1 formamide/internal size standard, 2 allelic ladders, 1 positive control, 1 negative control, and 10 88-KH DNA samples. Each sample well was prepared with 8.7 uL of formamide, 0.3 uL of GS500 LIZ Size Standard, and 1.0 uL of sample or allelic ladder. (**NOTE:** Previous work has demonstrated that 2.8 uL on the CAS-1200 is roughly equivalent to 1.0 uL manually, thereby explaining the differing volumes for the two methods.)

Capillary electrophoresis was then performed on an ABI 3130 followed by data analysis using GeneMapper ID software.

Results and Discussion

"Dye" Experiments:

Initial experiments resulted in a large number of failures demonstrated either by the lack of dye in wells or non-uniform distribution of dye between wells. The instrument had some trouble transferring volumes near 1.0 uL. After a number of adjustments to the instrument and protocol, the CAS-1200 was able to uniformly distribute 2.8 uL of dye to the correct wells containing 18 uL of water.

Sample Plate Covers:

Using the foil cover on the sample plate not all wells on the reaction plate received appropriate sample. With the septa mat the 1 uL volume did not work well and the mat was partially lifted off the sample plate during the protocol. Successful transfer of the dye occurred using no cover, as well as using the perforated film.

Manual vs. Automated Set-Up:

EMPLOYEE SAMPLES

Full DNA profiles were obtained for each of the employee samples set up on the CAS-1200. However, the peak heights were visibly lower than those obtained from those same samples using manual preparation. Additionally, it was observed that too much amplified product remained in the sample plate. The original volume of amplified product for each sample was 25 uL; therefore, after removing 1 uL a total of 23 times, there should have been approximately 2 uL remaining. Visual inspection of the product revealed that the volume remaining was in excess of 2 uL. This implies that the instrument is not transferring an entire 1 uL to the reaction plate.

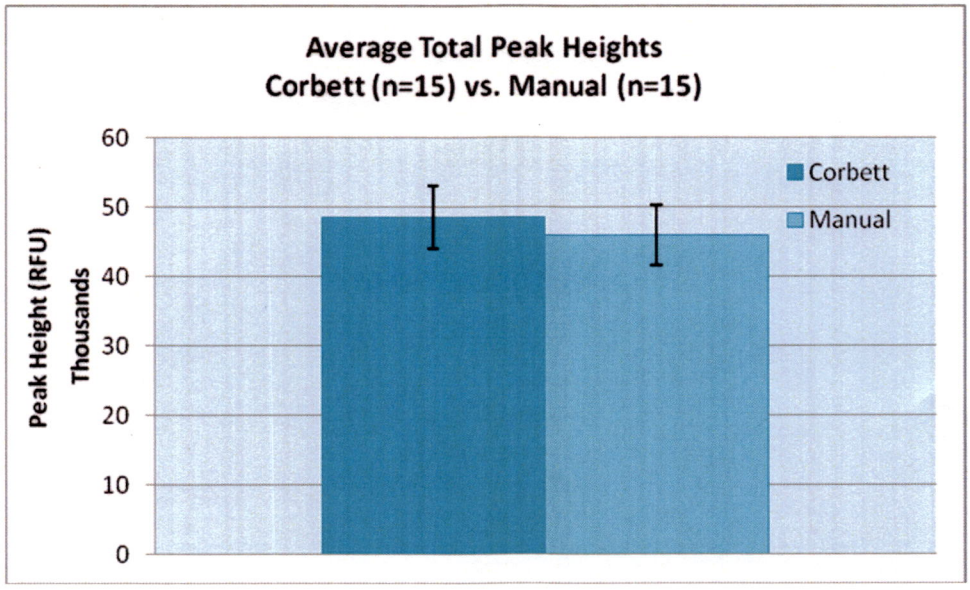
07-575 #1-4

Peak heights for the 1:9 and 2:18 manually prepared plates were similar. On average, the peak heights for the 1:9 were 1.003 times higher than the 2:18 (basically identical). Comparing the manual and CAS-1200 1:9 peak heights revealed that, on average, the manual peak heights are roughly 1.4 times greater than the automated ones. This indicates that 1 uL manually is roughly equivalent to 1.4 uL on the CAS-1200. Previous experiments (see above) demonstrated that the CAS-1200 has difficulties transferring volumes near 1 uL, however, is very successful at 2.8 uL. Since the 1:9 and 2:18 manually were equivalent, it can be inferred that 1.4:9 and 2.8:18 would be equivalent as well. Because 2.8 uL is easier for the CAS-1200 to handle than 1.4 uL, using

2.8 uL sample and 18 uL of master mix should be used in the future and yield similar results to 1uL and 9 uL manually.

88-KH EXPERIMENT #1

For each sample the peak height of each allele was added together generating a total peak height for the sample in relative fluorescence units (RFUs). The total peak heights for the samples set up manually were averaged as were those for the CAS-1200 prepared samples. On average, the manual total peak heights were ~95% of those from the CAS-1200 (see graph).



88-KH EXPERIMENT #2

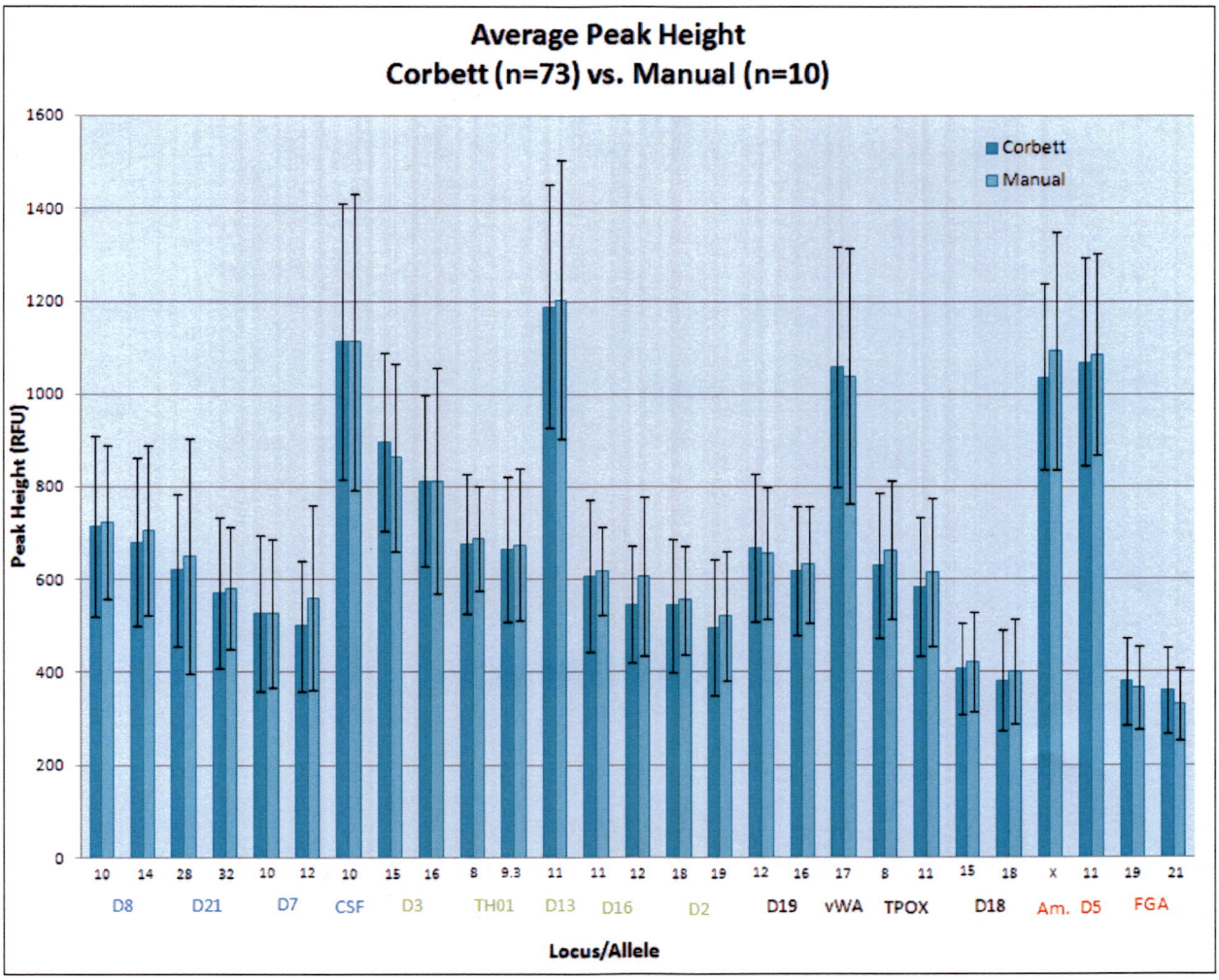
The CAS-1200 was monitored during the automated plate set-up process and was observed to correctly perform the assigned protocol with no detectable errors. Following set-up, the plate was visually inspected and each well appeared to contain the appropriate volume of liquid. Successful DNA typing results were obtained for each sample and the resultant profiles were consistent with the known DNA source. Additionally, all controls and blanks performed as expected.

The average peak height and standard deviation for all CAS-1200 prepared samples was calculated for each allele (see table). The same was done for each manually prepared sample (see table). The peak heights remained consistent for all samples prepared on the CAS-1200, with the exception of a single sample which exhibited significantly decreased peak heights across the profile. The GS500 LIZ Size Standard for this sample was also weak, suggesting an injection issue rather than a set-up problem, even though the sample was re-injected three times with similar results. Therefore, the peak heights from this sample were excluded from average

and standard deviation calculations. Peak heights were also consistent for all manually prepared samples.

Locus	Allele	Avg. PH (Corbett)	Std. Dev.	Avg. PH (Manual)	Std. Dev.
D8	10	714	211	723	165
	14	680	197	706	183
D21	28	620	178	650	253
	32	571	173	581	132
D7	10	527	177	528	160
	12	500	152	561	200
CSF	10	1114	323	1112	319
D3	15	896	217	863	202
	16	812	205	812	243
TH01	8	677	170	689	113
	9.3	665	173	674	164
D13	11	1188	295	1203	300
D16	11	608	177	618	95
	12	546	140	606	172
D2	18	544	155	556	117
	19	497	155	523	139
D19	12	668	177	656	142
	16	617	155	632	126
vWA	17	1058	285	1038	274
TPOX	8	630	172	664	150
	11	584	162	615	159
D18	15	407	107	422	107
	18	381	116	401	113
Amel.	X	1036	200	1092	257
D5	11	1068	255	1084	216
FGA	19	380	102	366	88
	21	360	99	332	78

A comparison of average peak heights with standard deviations between the samples prepared on the CAS-1200 and those prepared manually revealed no significant differences between the two methods (see graph).



Conclusions

The CAS-1200 is able to reliably transfer volumes of 2.8 uL and 18 uL. These volumes of sample and master mix, respectively, are roughly equivalent to 1 uL and 9 uL utilizing manual set-up. Peak heights resulting from manual set-up are generally around 95% of those obtained following automated set-up. The CAS-1200 is able to successfully execute the assigned protocol to set up samples for capillary electrophoresis, demonstrated by direct observation of the instrument during set-up, as well as through inspection of the prepared plate and analysis of resultant DNA typing data. 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 CAS-1200 and those prepared manually. Sample plates should be covered with perforated film, or not covered at all, during plate set-up.

The CAS-1200 Liquid Handling System from Corbett Robotics has proven it is capable of setting up plates for capillary electrophoresis on an ABI 3130 with precise and reproducible results, at least equal to those generated by manual set-up.

SDPD Forensic Science Section – Forensic Biology Unit

Validation of the CAS-1200 Liquid Handling System from Corbett Robotics

Contamination Study

Purpose

The CAS-1200 Liquid Handling System from Corbett Robotics is a precision liquid handling instrument originally designed for automated quantitative real-time PCR set-up. The intent of the Forensic Biology Unit is to utilize the CAS-1200 to automate the set-up of plates for capillary electrophoresis on the ABI 3130. The goal of this study is to demonstrate that the instrument can set up these plates without the occurrence of cross-contamination between samples on either the source plate or destination plate. Additionally, this study will further document the instrument's ability to successfully perform assigned protocols. This study will also test the instrument's ability to select specific samples from the source plate and transfer the required volume to the correct position on the destination plate ("cherry-picking").

Materials and Methods

"Checker-board" Experiments:

A source plate was prepared by placing amplified 88-KH DNA into alternating positions in a 96 well reaction plate. TE buffer was placed in the remaining wells resulting in a "checker-board" pattern. From this source plate the CAS-1200 was used to create a destination plate mimicking the "checker-board" pattern with the necessary ladders and controls added in. The plate was prepared with 18 uL of master mix and 2.8 uL of sample or ladder as previously described (see "Precision and Reproducibility Study"). Capillary electrophoresis was then performed on 3130A.

A second destination plate was prepared from the original source plate, exactly as described above, and capillary electrophoresis was performed on 3130B.

"Cherry-Picking" Experiment:

Ten samples from various positions on the original source plate were selected at random to be prepared by the CAS-1200 in a third destination plate in order to test the "cherry-picking" capabilities of the instrument. Capillary electrophoresis was performed on 3130B.

Capillary Contamination Check:

An experiment was designed to assess whether low levels of contamination observed in previous experiments (see *Results and Discussion*) could be the result of incomplete flushing of the capillary array between samples. Six samples of the 88-KH DNA were set up manually for

capillary electrophoresis with six TE blanks set up in the well positions immediately following the DNA samples. The plate was run on 3130B.

True Amplified Blanks:

Prior experiments revealed low levels of DNA contamination in multiple TE blanks (see *Results and Discussion*). It was hypothesized that the contamination was observed due to the fact that the blanks contained only TE buffer and did not represent true amplified blanks used in casework. True amplified blanks contain PCR primers that should, theoretically, out compete any low level DNA contamination during electrokinetic injection. Therefore, a plate was prepared for amplification with nine samples containing DNA and seven TE amp blanks interspersed. The amplified plate was used as the source plate for automated 3130 plate set-up on the CAS-1200. The destination plate was then run on 3130B.

Results and Discussion

"Checker-board" Experiments:

The data from the original plate run on 3130A generated the expected typing results with the exception of 12 samples. These samples had sizing quality issues likely related to electrokinetic injection during capillary electrophoresis rather than any problems with the CAS-1200's performance during set-up. All of the controls produced the expected results and, excluding these 12 samples, all of the wells that were supposed to contain the 88-KH DNA generated the correct STR profile with similar peak heights. The TE blanks generated no STR profiles and appeared clean at first glance. However, upon close inspection of the TE blanks, low level peaks were observed (none greater than ~30 RFUs) in positions corresponding to those detected in the 88-KH DNA samples (see Figure 1). The appearance of this apparent low level contamination was observed in approximately eight TE blanks, with no apparent pattern to the occurrence.

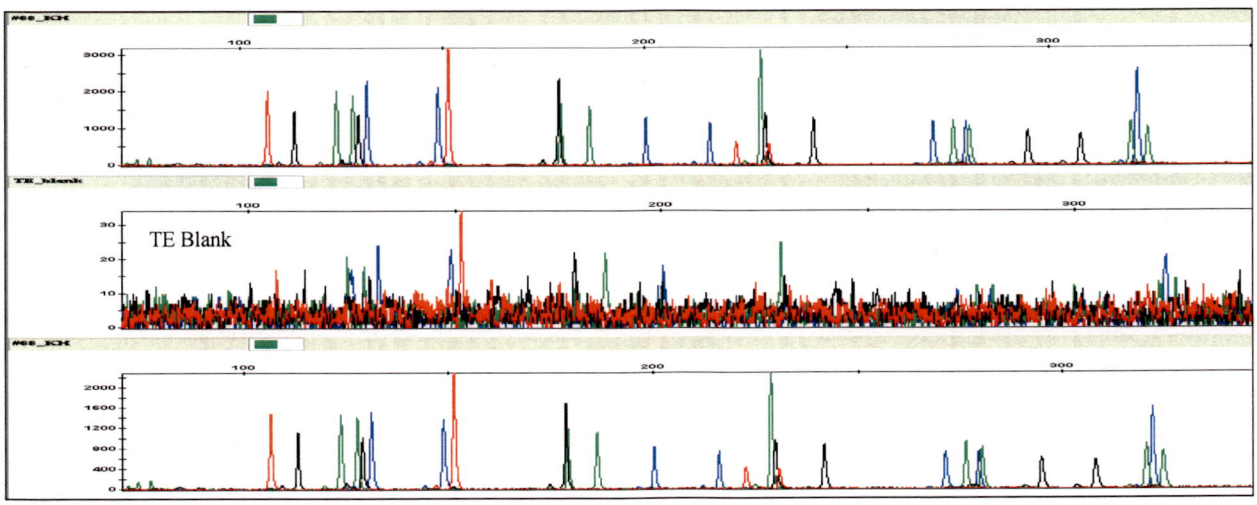


Figure 1: Electropherogram showing two 88-KH DNA samples and one TE blank from the original "checker-board" experiment.

Visual inspection of the prepared destination plate revealed that the samples and blanks were present in their correct positions as demonstrated by the presence of pinkish colored (sample) or clear (blank) liquid and uniform volumes. One would not expect to be able to visually detect any pinkish color in the blanks from the low levels of contamination described above. Of the 12 failed samples, 11 of these were TE blanks. The raw data of these 11 did not reveal any detectable signs of an STR profile. Ten of these blanks had LIZ being detected, however, with very poor resolution. The remaining blank had no LIZ or DNA types detected. The single failed sample could not be sized appropriately due to LIZ failure; however, the raw data demonstrates that a sufficient amount of amplified DNA is indeed present.

It should be noted that according to Applied Biosystems poor resolution can result if the water flush maintenance is not performed regularly. Further investigation revealed that the last water flush completed prior to this run was greater than a month earlier. While, it is unclear whether or not this is the direct cause of the observed problems, it is recommended that the water flush maintenance is performed on a weekly basis. Many of these failed samples were actually second injections of the sample (as a result of an initial run that was aborted prior to completion due to an incorrect plate record) with similar results for each injection. The Forensic Biology Unit has previously observed samples that don't work correctly during capillary electrophoresis such as these, but work fine after being set-up again. Again it is unlikely that this is a CAS-1200 issue, but rather a problem related to electrokinetic injection or electrophoresis.

The experiment was replicated in order to achieve more conclusive data about the low level contamination of blanks, and to trouble shoot the failed injections. The injection failures were not observed in the replicate experiment, however, low level DNA was observed in some of the TE blanks. In one TE blank, peaks were noted as high as ~60 RFUs (see Figure 2). This experiment was run on 3130B which has proven to be ~1.5 times as sensitive as 3130A.

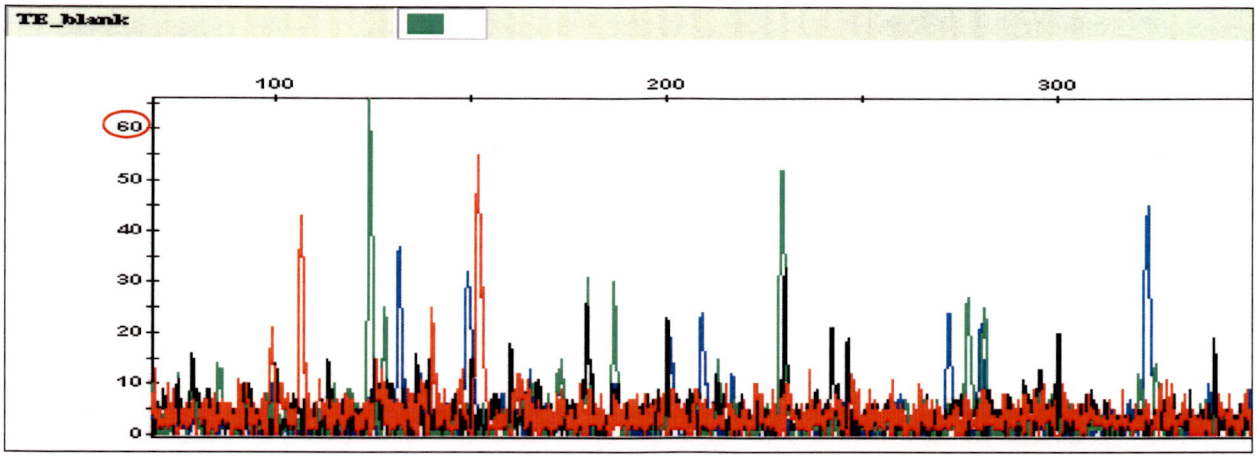


Figure 2: Electropherogram showing TE blank with peak heights reaching ~60 RFUs from the repeat of the "checker-board" experiment.

"Cherry-Picking" Experiment:

The CAS-1200 was monitored during the set up of the destination plate and it appeared to successfully transfer liquid from the defined positions on the source plate. Furthermore, all samples that were supposed to contain the 88-KH DNA generated the correct DNA profile. The TE blanks appeared to be negative based on either the electropherograms or the raw data, in the instance of two blanks with failed sizing likely due to injection issues. Those blanks that were successful, revealed no signs of the low level contamination observed previously.

Check for Capillary Contamination:

All of the 88-KH DNA samples generated the correct STR profile. None of the blanks demonstrated any low level peaks corresponding to those in the DNA samples separated immediately prior on the capillary array.

Amplified Blanks:

Each DNA sample generated the correct STR profile. No DNA profiles were generated from any of the amplified blanks. A close inspection of the electropherograms of the true amplified blanks revealed flat baselines with no visually detectable peaks (see Figure 3).

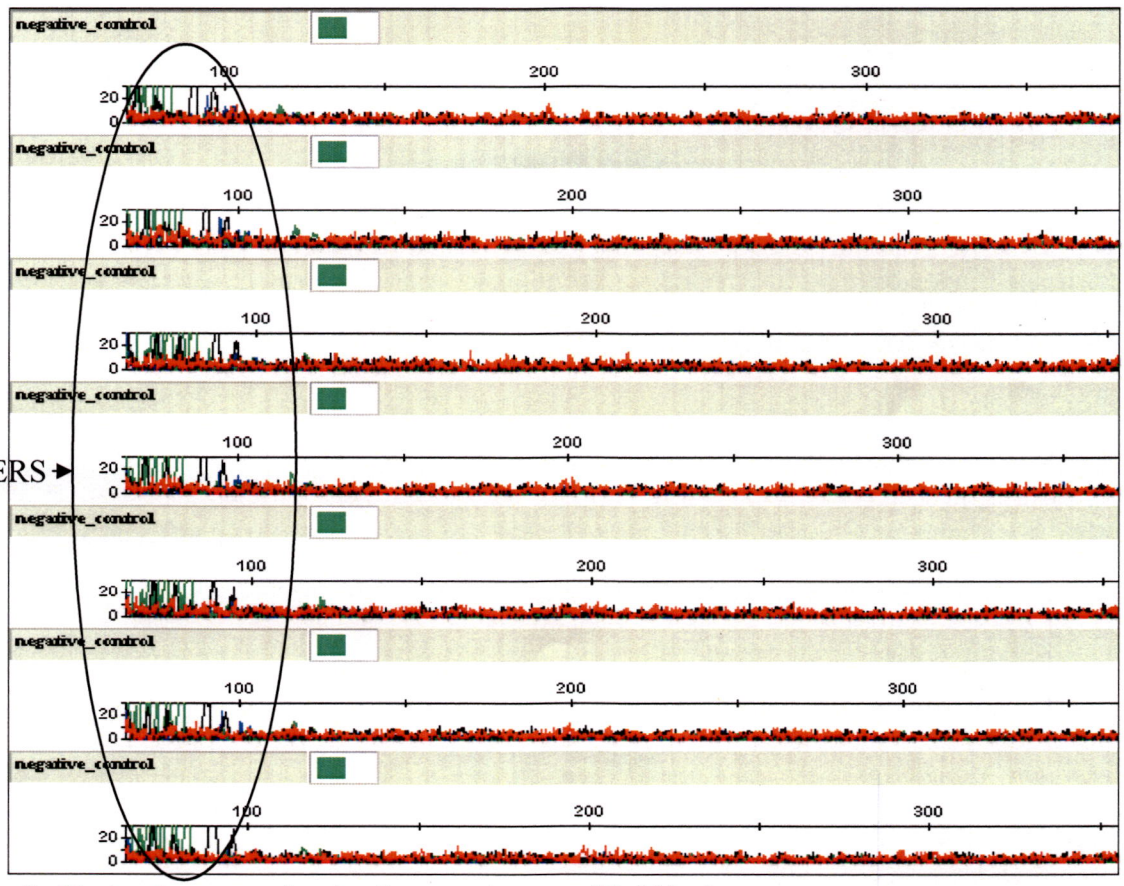


Figure 3: Electropherogram showing the seven true amplified blanks.

Conclusions

The initial "checker-board" experiments demonstrated that at some point in the process the TE blanks were being contaminated with a very low level of DNA consistent with the peaks from the 88-KH DNA profile. At no point were any of the peaks above the 75 RFU threshold utilized by the Forensic Biology Unit. However, one TE blank produced peaks that could be observed visually as high as ~60 RFUs which is uncomfortably close to the 75 RFU threshold. It was determined that the low level contamination observed in these blanks cannot be attributed to incomplete flushing of the capillary array between sample runs or capillary bleed thru. The exact mechanism by which the contamination occurred was not able to be determined. However, this study demonstrated that when true amplified blanks are used this low level contamination is eliminated. It is hypothesized that even if a minute amount of cross-contamination were to occur, that this extremely low level of DNA would be outcompeted by PCR primers, dNTPs, and $MgCl_2$ during electrokinetic injection resulting in no observable amounts of contamination in the data.

The CAS-1200 is able to successfully "cherry-pick" specific samples from the source plate and transfer them to the destination plate. This enables an analyst to choose which samples from an amplification plate will be set-up for capillary electrophoresis.

Throughout this study a number of sample failures were observed. This is not a novel observation in the Forensic Biology Unit and can be classified as a capillary electrophoresis related issue rather than a problem with the CAS-1200.

Samples mimicking those from forensic DNA casework (i.e. amplified samples and amplified blanks) exhibited no signs of contamination in this study. The CAS-1200 was observed to conduct the assigned protocols with no problems. Additionally, the instrument's ability to "cherry-pick" specific samples from the source plate was demonstrated.