Internal Validation:
QIAsymphony® SP Instrument
using the
QIAsymphony® DNA Investigator® Kit

This internal validation has been reviewed and approved by:

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This validation was conducted in collaboration with the Marshall University Forensic Science Center (MUFSC) Technical Assistance Program. Studies were also completed and reported by Katherine Taylor, MUFSC graduate student and DC DFS FBU Intern.

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I. Introduction

Extraction is the critical first step in the development of a DNA profile from an evidence sample. Unlike most biology applications where samples are tested in high quantity and quality replicates, forensic biology applications oftentimes only have one chance to extract the DNA from a sample. For this reason, an optimized DNA extraction method must not only efficiently isolate the DNA and remove potential inhibitors, but also be robust and flexible enough to be effective for all sample types, especially those which are low in quantity, poor in quality, or located on difficult substrates.

Previous validation has demonstrated the effectiveness of the QIAGEN EZ1 DNA Investigator Kit's silica-coated, magnetic-bead based extraction method on the EZ1 Advanced XL instrument [1]. However, analysts are limited to 14 samples per run with required manual loading and unloading of samples, reagents, and consumables after each run. Each run takes approximately 20 minutes to complete. The QIAsymphony SP instrument uses the same technology in its associated kit, the QIAsymphony DNA Investigator Kit, with the added benefit of higher throughput capabilities. Four sets of up to 24 samples each (96 total samples) may be loaded for a single run. Additionally, the instrument simultaneously purifies each set of 24, allowing the processing of up to 96 samples in approximately three hours without the need for analyst intervention to load or unload.

The QIAsymphony system also provides additional options for lysis and elution that are not a part of the currently validated EZ1 Advanced XL system. Both systems include a protocol for lysis in 200 microliter (µL) and 500 µL; however, the QIAsymphony system also includes the capability to lyse using 1000 µL for larger sample cuttings. The QIAsymphony system has been developmentally validated to elute samples in 40 µL instead of 50 µL, which would result in less casework samples requiring concentration prior to amplification. Finally, the QIAsymphony system can accommodate different adapters and labware for elution including individual tubes or 96-well plates. These options all have the potential to improve the laboratory's overall sample process.

For all these reasons, an internal validation of the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument was conducted by the District of Columbia Department of Forensic Sciences (DC DFS) Forensic Biology Unit (FBU) in accordance with the following requirements and guidelines:

- FBI Quality Assurance Standards for Forensic DNA Testing Laboratories [2]
- SWGDAM Validation Guidelines for DNA Analysis Methods [3]
- ANSI/ASB Standard 038, First Edition 2020, Standard for Internal Validation of Forensic DNA Analysis Methods [4]
- DC DFS Department Operation Manual (DOM) – Procedures for Validating Technical Procedures (DOM04) [5]
- DC DFS Forensic Science Laboratory Quality Assurance Manual [6]
- DC DFS Forensic Biology Unit Quality Assurance Manual [7]

Studies included the following: sensitivity, accuracy, precision and reproducibility, mixtures, mock casework, EZ1 comparison, plate elution, performance/quality control check, and contamination assessment. Stochastic studies are not applicable to the internal validation of a DNA extraction system.

The QIAsymphony Operating System is developed exclusively for use with the QIAsymphony SP/AS instruments and is required for operating the instrument. Therefore, functional testing of the QIAsymphony Operating Software (version 5.0) was conducted throughout the entire internal validation. Instrument protocol issues were encountered during the Plate Elution Study (Section VIII) and remediated by obtaining and testing custom protocols from the manufacturer. Reliability of the software was successfully demonstrated by two different operators during the Precision and Reproducibility Study (Section IV).

The QIAsymphony SP Protocol Sheets [8, 9, 10], QIAsymphony DNA Investigator Handbook [11] and the QIAsymphony SP/AS User Manual [12] were referenced for all sample preparation and instrument protocols. Details regarding the specific materials and methods used for each study are in the descriptions below.

The following methods were applied to all studies:

- After sample preparation, all QIAsymphony samples received the corresponding lysis reagent volumes as indicated in Table I-1:

Cutting Type	Protocol	Buffer ATL (µL)	Proteinase K (µL)	1 M DTT (µL)
Liquid samples, small cuttings (~3 mm x 3 mm), ¼ swab cuttings	Casework_200_ADV_HE_V10 CW200 ADV HE CR23916_ID4944	180	20	N/A
¼ to full swab cuttings and a full-size hole punch (6-8 mm)	Casework_500_ADV_HE_V10	475	25	N/A
Full swab cuttings	Casework_1000_ADV_HE_V10	960	40	N/A
Sexual assault specimens (initial incubation) – ¼ swab and ~5mm x 5mm cuttings	Casework_200_ADV_HE_V10	475	25	N/A
Sexual assault specimens – Sperm fractions	Casework_200_ADV_HE_V10	160	20	20

Table I-1: Lysis reagent volumes and Casework ADV HE extraction and purification protocols used

- Lysis was performed for all QIAsymphony samples in cross-linked, 2 mL tubes (Sarstedt Screw Cap Micro Tubes, NONSKIRTED, Cat. No. 72.693) or 2 mL snap cap tubes. Prior to loading into the sample carrier, any lysates in 2 mL snap cap tubes were transferred to the 2 mL tubes (Sarstedt 72.693).
- Non-differential QIAsymphony samples were incubated for two hours to overnight at 56°C on a thermomixer shaking at 900 rpm.
- Differential QIAsymphony samples were incubated for one hour at 56°C on a thermomixer shaking at 900 rpm. TE Buffer was used for sperm washes instead of Buffer ATL. The sperm fraction was then incubated overnight at 56°C on a thermomixer shaking at 900 rpm.
- All QIAsymphony samples were run following the manufacturer's Casework ADV HE protocols for DNA extraction and purification.
- All QIAsymphony samples were eluted with 40 µl TE Buffer in cross-linked, 2 mL tubes (Sarstedt 72.693) except for those samples in the plate elution study which were eluted with 40 µL TE Buffer in a 96-well plate (MicroAmp™ Optical 96-Well Reaction Plate, Cat. No. N8010560).
- All extracted samples were quantitated using the Plexor HY System and analyzed using Plexor Analysis Software v1.6.0 [13]. Samples which were taken forward for detection were amplified with the GlobalFiler PCR Amplification Kit [14], typed by the Applied Biosystems 3500xL Genetic Analyzer [15], and analyzed with GeneMapper ID-X v1.5 [16,17]. After initial analysis with the previously listed software, the data was exported and evaluated using Microsoft Excel. Active DC DFS standard operating procedures were followed for each step.

II. Sensitivity

a. Objective

A sensitivity study was conducted to demonstrate the capability of the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument to extract and isolate DNA from low to high level samples.

b. Materials and Methods

Sensitivity was evaluated by diluting a neat saliva sample with Phosphate Buffered Saline (1X PBS) to create five dilutions: 1:5, 1:10, 1:20, 1:40, and 1:80 (Table II.b-1). 5 µL of the neat saliva sample and 5 µL of each of the five dilutions were aliquoted in triplicate to tubes and extracted with a reagent blank on the QIAsymphony using the Casework_200_ADV_HE_V10 protocol. All samples were quantified, amplified, and typed. The total number of samples included in this study was 18.

Sample Name	Comments
SALAN1	5 µL of neat Saliva A in each replicate
SALAN2	
SALAN3	
SALAa1	5 µL of 1:5 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 80 µL 1 X PBS.)
SALAa2	
SALAa3	
SALAb1	5 µL of 1:10 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 180 µL 1 X PBS.)
SALAb2	
SALAb3	
SALAc1	5 µL of 1:20 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 380 µL 1 X PBS.)
SALAc2	
SALAc3	
SALAd1	5 µL of 1:40 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 780 µL 1 X PBS.)
SALAd2	
SALAd3	
SALAe1	5 µL of 1:80 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 1580 µL 1 X PBS.)
SALAe2	
SALAe3	

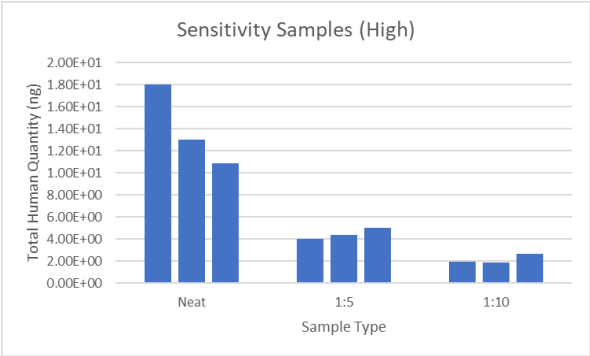
Table II.b-1: Sensitivity study sample descriptions

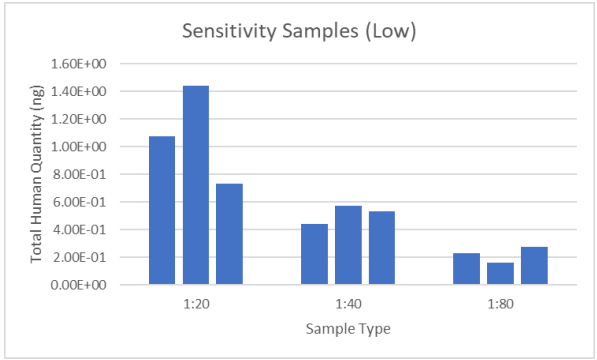
Average quantities, average peak heights and standard deviations were calculated using Microsoft Excel. DNA types and quality of the STR profile were manually evaluated using electropherograms by two different individuals.

c. Results

Sample Type	Total Human (ng)	Average Total Human (ng)	Standard Deviation	Standard Deviation %
neat	1.80E+01	1.40E+01	3.68E+00	26%
	1.30E+01			
	1.09E+01			
1:5	4.04E+00	4.49E+00	5.06E-01	11%
	4.40E+00			
	5.04E+00			
1:10	1.94E+00	2.17E+00	4.24E-01	20%
	1.91E+00			
	2.66E+00			
1:20	1.08E+00	1.09E+00	3.54E-01	33%
	1.44E+00			
	7.36E-01			
1:40	4.40E-01	5.17E-01	6.99E-02	14%
	5.76E-01			
	5.36E-01			
1:80	2.33E-01	2.23E-01	5.75E-02	26%
	1.61E-01			
	2.75E-01			

Table II.c-1: Average Total Human DNA (ng) obtained from the sensitivity samples.





Figures II.c-1 and II.c-2: Total Human DNA (ng) obtained from each sensitivity sample.

The results shown in Table II.c-1 demonstrated that there was an expected reduction in yield as the dilution of the saliva increased. All replicates produced similar quantitation yields to each other (Figures II.c-1 and II.c-2) as well with a standard deviation of 26% or less of the average quantity (Table II.c-1).

QIAsymphony Sensitivity Samples			
Sample	Average Amplification Target (ng)	Average Peak Height (RFU)	Standard Deviation
Neat	0.60	1310	100
1:5	0.60	1694	212
1:10	0.60	1592	146
1:20	0.41	1283	405
1:40	0.19	512	153
1:80	0.08	262	93

From Sensitivity Study in GlobalFiler Validation		
Amp Target (ng)	Average Peak Height (RFU)	Standard Deviation
0.0625	210	95
0.09375	361	161
0.125	533	257
0.1875	626	266
0.25	1192	457
0.375	1673	794
0.5	2091	814
0.75	3981	1426

Table II.c-2: Comparison of average peak heights obtained from samples amplified using QIAsymphony DNA extracts (left) and samples amplified in the GlobalFiler validation (right). Similar amplification targets are highlighted in similar colors (orange, green, blue, and yellow).

All replicates produced sample average peak heights (Table II.c-2, left side) that were consistent with the average peak heights obtained for similar amplification targets from the GlobalFiler kit validation (Table II.c-2, right side) [18]. Full, concordant profiles were produced for all samples except for the 1:80 dilution samples (≤ 100 pg total human DNA) which generated partial, concordant profiles. This was also consistent with the dropout observed at similar amplification targets in the GlobalFiler kit validation. Visual inspection of the profiles did not show issues with interlocus or intralocus balance, significant degradation or inhibition patterns, or random locus dropout.

Overall, these results indicated that the QIAsymphony system successfully extracted DNA from low to high level samples. These extracts were then able to be amplified into DNA profiles which were consistent with expectations based on their amplification targets.

III. Accuracy

a. Objective

The accuracy study was conducted to demonstrate that the samples extracted by the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument produced concordant genotype results from samples with known DNA profiles and an appropriate certified reference material (NIST SRM 2391d, component punch E).

b. Materials and Methods

Accuracy was evaluated by using ¼ swab cuttings from five known buccal samples from different individuals and one NIST-E component punch from NIST SRM 2391d [19]. The samples were run on the QIAsymphony using the Casework_200_ADV_HE_V10 protocol. All samples were quantified, amplified, and typed. A total of 6 samples were included in the accuracy study (Table III.b-1).

Sample Name	Comments
BUCC	1/4 swab Buccal C
BUCD	1/4 swab Buccal D
BUCE	1/4 swab Buccal E
BUCF	1/4 swab Buccal F
BUCG	1/4 swab Buccal G
NISTE	one punch

Table III.b-1: Accuracy study sample descriptions

Accuracy was also verified for all samples run throughout the validation by comparing the obtained profiles with previously run data. For the mock casework touch samples (Section VI), accuracy was verified by evaluating the profiles for the presence of the expected contributor (owner or user of the item swabbed). DNA types and quality of the STR profile were manually evaluated using electropherograms by two separate individuals.

c. Results

All buccal reference samples produced full STR profiles that accurately matched the known profiles. The component E sample from the NIST SRM 2391d kit generated a full STR profile concordant with the results published in the SRM Certificate of Analysis [19]. Visual inspection of the profiles did not show issues with interlocus or intralocus balance, significant degradation or inhibition patterns, or random locus dropout.

The mock casework touch samples contained some low-level, discordant alleles; however, the majority of the DNA detected in the profiles was concordant with the owner or user of the item swabbed. These low-level alleles were evaluated against other sample profiles in the validation and the FBU Quality Assurance Database. No specific source was able to be identified. All other samples that were run as a part of this internal validation were determined to be concordant with previous run data except for typical, identifiable amplification and detection artifacts. Based on these results, the QIAsymphony produced extracts which were subsequently amplified into high quality, accurate DNA profiles consistent with expectations based on their amplification targets.

IV. Precision and Reproducibility

a. Objective

The precision and reproducibility study was conducted to demonstrate that the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument produced consistent results within replicate sets and among different operators.

b. Materials and Methods

A neat saliva sample and a 1:10 dilution of the neat saliva sample were used for this study. 26 samples were made by spotting 5 µL of the neat saliva sample onto 13 cotton swabs and 5 µL of a 1:10 dilution of the neat saliva sample onto 13 cotton swabs. All swabs were dried overnight and then the entire swabs were cut into individual sample tubes. An additional 26 samples were prepared by pipetting 5 uL of the neat saliva sample directly into 13 individual tubes and 5 µL of the 1:10 dilution of the neat saliva sample directly into 13 individual tubes.

Sample Name	Comments	Sample Name	Comments
SWN1	5 µL of neat Saliva B spotted on individual swabs. Entire swab cut for each replicate.	SWN11	5 µL of neat Saliva B spotted on individual swabs. Entire swab cut for each replicate.
SWN2		SWN12	
SWN3		SWN13	
SWN4		SWN14	
SWN5		SWN15	
SWD1	1:10 dilution of Saliva B (500 µL Saliva B + 4500 µL 1 X PBS) 5 µL of 1:10 dilution of Saliva B spotted on individual swabs. Entire swab cut for each replicate.	SWD11	1:10 dilution of Saliva B (500 µL Saliva B + 4500 µL 1 X PBS) 5 µL of 1:10 dilution of Saliva B spotted on individual swabs. Entire swab cut for each replicate.
SWD2		SWD12	
SWD3		SWD13	
SWD4		SWD14	
SWD5		SWD15	
SALBN1	5 µL of neat Saliva B in each replicate	SALBN11	5 µL of neat Saliva B in each replicate
SALBN2		SALBN12	
SALBN3		SALBN13	
SALBN4		SALBN14	
SALBN5		SALBN15	
SALBA	1:10 dilution of Saliva B (500 µL Saliva B + 4500 µL 1 X PBS) 5 µL of 1:10 dilution of Saliva B in each replicate.	SALBK	1:10 dilution of Saliva B (500 µL Saliva B + 4500 µL 1 X PBS) 5 µL of 1:10 dilution of Saliva B in each replicate.
SALBB		SALBL	
SALBC		SALBM	
SALBD		SALBN	
SALBE		SALBO	

Table IV.b-1: Reproducibility study sample descriptions. Samples were processed on separate batches by two different operators - Operator 1 (left) and Operator 2 (right)

On the QIAsymphony, two extraction sets were processed by different operators with each set containing five replicates of each sample type (i.e., neat saliva on swab, diluted saliva on swab, neat saliva direct and diluted saliva direct) along with a reagent blank (Tables IV.b-1). The Casework_500_ADV_HE_V10 protocol was used. All samples were quantified, amplified, and typed. The total number of samples included in this study (excluding reagent blanks) was 40. The remaining 12 samples prepared for this study were used in the EZ1 comparison study (Section VII).

Average quantities and standard deviations were calculated using Microsoft Excel. DNA types and quality of the STR profile were manually evaluated using electropherograms by two different individuals.

c. Results

Table IV.c-1 shows the average quantities and standard deviations that were calculated. Standard deviations were low (28% or less of the average) for each replicate set demonstrating reproducibility between samples from the same source. Reproducibility between two different operators was demonstrated by obtaining similar quantities (within two standard deviations) (Figure IV.c-1).

Sample Type	Analyst	Average Total Human (ng)	Standard Deviation	Standard Deviation %
neat swab	Operator 1	1.39E+01	2.96E+00	21%
	Operator 2	1.17E+01	1.50E+00	13%
neat direct	Operator 1	1.57E+01	7.90E-01	5%
	Operator 2	1.66E+01	2.54E+00	15%
1:10 swab	Operator 1	9.94E-01	2.79E-01	28%
	Operator 2	1.05E+00	1.34E-01	13%
1:10 direct	Operator 1	1.98E+00	3.72E-01	19%
	Operator 2	1.69E+00	2.15E-01	13%

Table IV.c-1: Average total human quantity (ng), standard deviation, and percent standard deviation calculated for the reproducibility samples run by two different operators

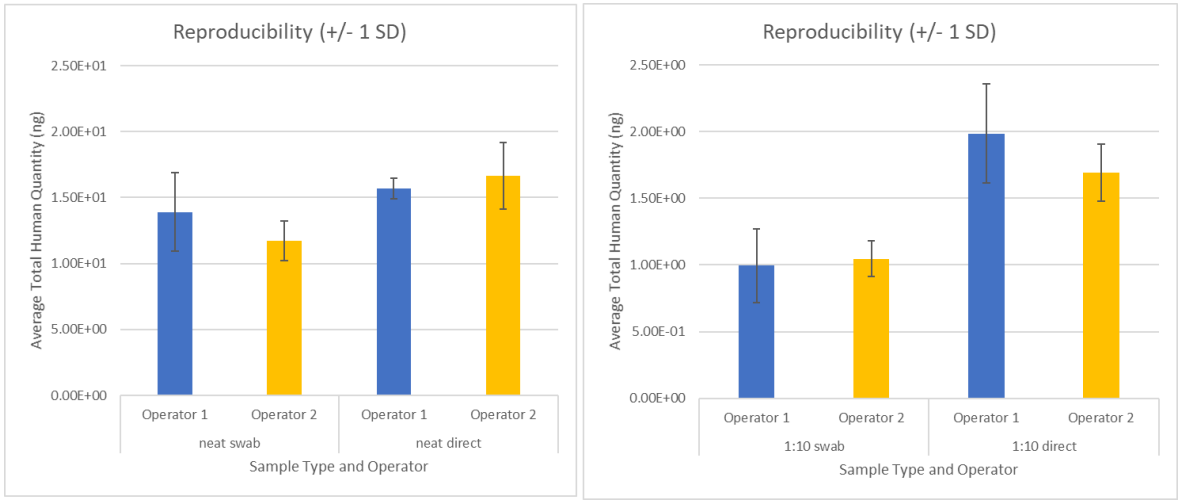


Figure IV.c-1: Comparison of average total human quantity (ng) from reproducibility samples run by two different operators

QIAsymphony Reproducibility Samples						
Sample	Operator 1			Operator 2		
	Average Amplification Target (ng)	Average Peak Height (RFU)	Standard Deviation	Average Amplification Target (ng)	Average Peak Height (RFU)	Standard Deviation
Swab Neat	0.60	2446	109	0.60	3606	524
Swab 1:10	0.37	1607	429	0.39	2197	346
Saliva Neat	0.60	2384	403	0.60	3252	448
Saliva 1:10	0.59	3095	447	0.58	3848	693

Table IV.c-2: Average amplification targets (ng) and average peak heights (RFU) between two different operators for reproducibility study samples

Genotyping results also demonstrated that average peak heights (Table IV.c-2) were consistent with expectations from similar targets in the GlobalFiler kit validation report shown previously (see Table II.c-3). Full, concordant profiles were generated for all QIAsymphony precision and reproducibility samples except for one replicate of a neat swab sample (SWN13), due to poor injection. Visual inspection of the profiles did not show issues with interlocus or intralocus balance, significant degradation or inhibition patterns, or random locus dropout.

V. Mixtures

a. Objective

The mixture study was conducted to demonstrate that the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument extracted and isolated quality DNA from samples which contained cells from more than one contributor at various ratios.

b. Materials and Methods

A series of mixture ratios were used to determine the QIAsymphony system's capability at extracting mixtures. The sample set for this study (Table V.b-1) consisted of neat saliva samples from two different DNA contributors (one male and one female). Samples were prepared at the following mixture ratios: 1:1, 1:4, 1:9, 1:19, 19:1, 9:1, and 4:1. Samples were prepared by pipetting 5 µL from each of the neat saliva samples and five microliters of each mixture ratio sample into individual tubes. The samples were run along with a reagent blank on the QIAsymphony using the Casework_200_ADV_HE_V10 protocol. All samples were quantified, amplified, and typed. A total of 9 samples were included in this study (excluding the reagent blank).

Sample Name	Comments
SHIN	5 µL Saliva H
SHIa	5 µL of a 1:1 of Saliva H (20 µL) and Saliva I (20 µL)
SHIb	5 µL of a 1:4 of Saliva H (20 µL) and Saliva I (80 µL)
SHIc	5 µL of a 1:9 of Saliva H (20 µL) and Saliva I (180 µL)
SHId	5 µL of a 1:19 of Saliva H (20 µL) and Saliva I (380 µL)
SHIe	5 µL of a 19:1 of Saliva H (380 µL) and Saliva I (20 µL)
SHIf	5 µL of a 9:1 of Saliva H (180 µL) and Saliva I (20 µL)
SHIg	5 µL of a 1:4 of Saliva H (80 µL) and Saliva I (20 µL)
SHIh	5 µL Saliva I

Table V.b-1: Mixture study sample descriptions

Female and male quantities, DNA types, contributor-specific average peak heights, and mixture ratios were evaluated/calculated using Microsoft Excel. Quality of the STR profile was manually evaluated using electropherograms by two different individuals.

c. Results

To evaluate the mixtures, the expected mixture ratio was determined for quantitation and detection results using the neat sample quantities and dilution values. All values were calculated as male DNA/total DNA. These expected mixture ratios were then compared to the observed mixture ratios calculated from the quantitation results and the genotyping results.

Table V.c-1 shows a comparison of the expected mixture ratios based on the original quantity of the neat samples used to prepare the mixture samples and the observed mixture ratios based on the quantitation and amplification results. Quantitation mixture ratios were calculated by dividing the male quantity by the human quantity for each sample. Male and female specific average peak heights were calculated by averaging the peak heights of the unique alleles for each contributor. The mixture ratio was then calculated by dividing the average peak height of the male contributor by the total average peak heights of the male and female contributor combined.

Male Neat Total Human Quantity (ng/µL) = 0.0926
Female Neat Total Human Quantity (ng/µL) = 0.0591

	Expected Quantity (based on Neat)	Observed from Quantitation			Observed from Contributor APH		
Prepared Dilution	**Male/Total**	Human Quantity	Male Quantity	**Male/Total**	Female APH	Male APH	**Male/Total**
19 to 1	**0.9675**	0.0583	0.0674	**1.1561**	208	1607	**0.8855**
9 to 1	**0.9338**	0.0329	0.0281	**0.8541**	216	874	**0.8020**
4 to 1	**0.8624**	0.0584	0.0415	**0.7106**	361	1398	**0.7947**
1 to 1	**0.6104**	0.0209	0.0038	**0.1837**	792	269	**0.2532**
1 to 4	**0.2815**	0.2720	0.0546	**0.2007**	1449	518	**0.2636**
1 to 9	**0.1483**	0.0912	0.0116	**0.1272**	1761	288	**0.1404**
1 to 19	**0.0762**	0.0592	0.0045	**0.0752**	1269	187	**0.1287**

Table V.c-1: Comparison of the expected mixture ratios based on the original quantity of the neat samples used to prepare the mixture samples and the observed mixture ratios calculated from the quantitation and amplification results. Due to a possible error during preparation, the 1 to 1 sample will be considered an outlier.

All observed mixture ratios, except for the 1:1 ratio, were comparable to the expected mixture ratios as shown in Figure V.c-1. For this sample, lower-than-expected quantities were obtained and less than half of an optimal target was amplified (310 pg). This indicated a possible error during sample preparation and resulted in stochastic effects and drop-out. This sample should be considered an outlier when evaluating the overall results of this study.

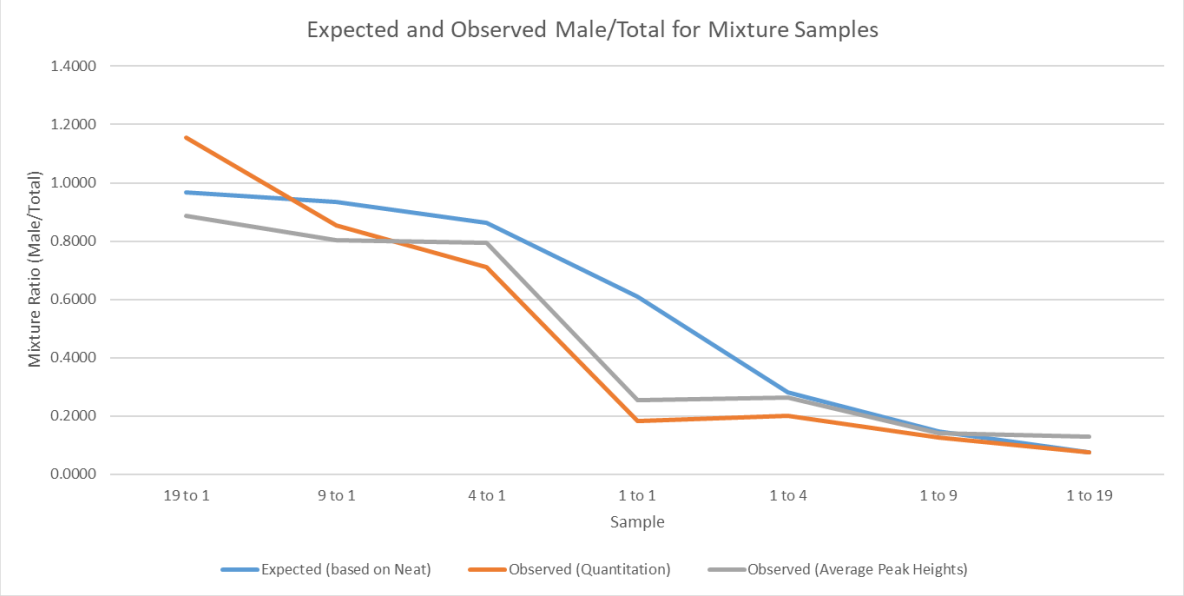


Figure V.c-1: Graphical representation of the comparisons made between expected and observed mixture ratio calculations listed in Table V.c-1.

The results of this study confirm that the QIAsymphony is capable of extracting DNA from cells at various mixture ratios without preference to the major or minor contributor (i.e., in a 1:19 or 19:1 mixture, the minor contributor does not get extracted at a lower amount than expected because there is an abundance of the major contributor).

VI. Mock Casework

a. Objective

The mock casework study was conducted to demonstrate that the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument extracted and isolated quality DNA from samples and substrates which are typically encountered in casework scenarios.

b. Materials and Methods

To determine the QIAsymphony's capability at extracting DNA from evidence, mock samples were utilized which included: differential, touch, and blood samples. Various substrate types were used, and all samples were cut based on typical casework scenarios encountered at DC DFS. There were five differential samples, five touch samples, and five blood samples with a reagent blank for each sample type. The samples used for this study are shown in Table VI.b-1.

Sample ID	Cutting Size	Source type
Diff1	1/4 swab	2017 Proficiency Test
Diff2	1/4 swab	2018 Proficiency Test
Diff3	5mm x 5mm	2012 Proficiency Test (fabric)
Diff4	5mmX 5mm	2015 Proficiency Test (fabric)
Diff5	5mm x 5mm	2015 Proficiency Test (fabric)
Touch1	whole swab	cell phone swab
Touch2	whole swab	steering wheel swab
Touch3	whole swab	computer mouse swab
Touch4	1/2 swab	chair handle swab
Touch5	whole swab	keyboard swab
Blood1	3mm x 3mm	2018 Proficiency Test (washrag)
Blood2	1/4 swab	2018 Proficiency Test (knife swab)
Blood3	3mm x 3mm	2017 Proficiency Test (FTA)
Blood4	3mm x 3mm	2016 Proficiency Test (FTA)
Blood5	3mm x 3mm	2016 Proficiency Test (FTA)

Table VI.b-1: Mock casework study sample descriptions

The protocols used for this study included the Casework_200_ADV_HE_V10 protocol for the differential and blood samples and the Casework_1000_ADV_HE_V10 protocol for the touch samples. All samples were quantified, amplified, and typed. A total of 20 samples were included in this study.

Quantities were evaluated in the Plexor HY Analysis Software. DNA types and quality of the STR profile were manually evaluated using electropherograms by two different individuals.

c. Results

All samples produced expected quantities and profiles based on their type and substrate. Blood and differential sample quantities were sufficient for amplification at an optimal target (600 pg). The resulting profiles were high quality with no signs of inhibition or degradation and all genotypes were concordant with previously run data. Blood samples produced single source profiles matching the donor. Epithelial fractions produced mixtures matching the male and female donors. And finally, sperm fractions produced single source to nearly single source profiles matching the male donor.

Touch samples produced quantities and profiles consistent with touch samples that are typically encountered in casework. Amplification target quantities from these samples varied from optimum (600 pg) to just below

the amplification cutoff (100 pg). Profiles varied from full to partial and single source to mixture. While the additional donors in the mixtures were unknown, the expected donor was still included as the majority of the DNA detected in the profile.

Sample Name	Amp target (ng)	Number of alleles expected	Number of alleles observed	Comments
BLOOD1	0.6	45	45	
BLOOD2	0.6	40	40	
BLOOD3	0.6	37	37	
BLOOD4	0.6	28*	28	
BLOOD5	0.6	28*	28	
DIFF1EF	0.6	72	71	1 SF allele dropped out
DIFF1SF	0.6	41	41	
DIFF2EF	0.6	64	64	
DIFF2SF	0.6	41	42	additional allele in stutter position and consistent with EF
DIFF3EF	0.6	48*	48	
DIFF3SF	0.6	32*	32	
DIFF4EF	0.6	52*	52	
DIFF4SF	0.6	32*	32	
DIFF5EF	0.6	49*	47	2 alleles from SF drop-out
DIFF5SF	0.6	31*	32	1 allele carryover from EF
TOUCH1	0.361	29*	31	Single source, 1 elevated stutter and 1 possible drop-in (D2S1338 has a concordant 19 allele at 7674 rfu and a 24 peak at 108 rfu)
TOUCH2	0.6	28*	37	Mixture with all donor alleles present and 9 additional low level alleles
TOUCH3	0.260	29*	52	Mixture with all donor alleles present and 23 additional low level alleles
TOUCH4	0.086	38	33	Low level profile with dropout of 7 alleles and 2 add'l alleles in stutter position
TOUCH5	0.244	38	48	Mixture with all donor alleles present and 10 add'l low level alleles

Table VI.c-1: Mock casework sample amplification targets and number of alleles expected compared to number of alleles observed. Comments were made to indicate alleles which dropped out or additional alleles that were detected. Asterisk (*) indicates samples with previous data only available for comparison to Identifiler Plus. Additional GlobalFiler loci were evaluated but not included in allele counts.

The manufacturer protocol for sexual assault samples utilizes the Casework_200_ADV_HE_V10 protocol for both the non-sperm and sperm fractions created during a differential extraction. In this protocol, the QIAsymphony instrument uses all of the sperm fraction lysate but only a portion of the non-sperm fraction lysate for isolation. While this method was demonstrated to be successful and reliable during internal validation, it will require careful evaluation of each non-sperm fraction's sample type and probative value prior to set-up on a batch for QIAsymphony extraction/isolation. Additionally, the laboratory would need to develop workflows for the long-term storage, isolation, and/or subsequent combination of the DNA in the remaining non-sperm fraction lysate. For practicality, it is recommended that the Casework_500_ADV_HE_V10 protocol be used with the entire non-sperm fraction lysate (approximately 470 µL) instead of the Casework_200_ADV_HE_V10 protocol with a portion of the non-sperm fraction lysate (200 µL). This change from the manufacturer protocol is not significant and verification of using the Casework_500_ADV_HE_V10 protocol with a 470 µL lysate instead of a 500 µL lysate was not determined to be necessary.

VII. EZ1 Comparison

a. Objective

The EZ1 comparison study was conducted to demonstrate that the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument produced similar results to the EZ1 system. Any differences in the results or procedures were evaluated to determine whether specific sample types or substrates are better suited for one method over the other.

b. Materials and Methods

For comparison to the EZ1 method for extraction, a subset of samples from the sensitivity study, reproducibility study, and mock casework study were prepared and processed on the EZ1 Advanced XL:

- From the sensitivity study: the neat, 1:10 dilution, and 1:40 dilution samples were extracted in triplicate along with a reagent blank.

Sample Name	Comments
SAN1 EZ1	5 µL of neat Saliva A in each replicate
SAN2 EZ1	
SAN3 EZ1	
SA1a EZ1	5 µL of 1:10 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 180 µL 1 X PBS.)
SA1b EZ1	
SA1c EZ1	
SA2a EZ1	5 µL of 1:40 dilution of Saliva A in each replicate (dilution prepared as 20 µL Saliva A + 780 µL 1 X PBS.)
SA2b EZ1	
SA2c EZ1	

Table VII.b-1: EZ1 sensitivity samples used for comparison to sensitivity samples run by the QIAsymphony

- From the Reproducibility study: the neat sample, 1:10 dilution, neat sample on swab, and 1:10 dilution on swab were extracted in triplicate with a reagent blank.

Sample Name	Comments
SALBEZN1	5 µL of neat Saliva B spotted on individual swabs. Entire swab cut for each replicate.
SALBEZN2	
SALBEZN3	
SALBP	1:10 dilution of Saliva B (500 µL Saliva B + 4500 µL 1 X PBS) 5 µL of 1:10 dilution of Saliva B spotted on individual swabs. Entire swab cut for each replicate.
SALBQ	
SALBR	
SWN16	5 µL of neat Saliva B in each replicate
SWN17	
SWN18	
SWD16	1:10 dilution of Saliva B (500 µL Saliva B + 4500 µL 1 X PBS) 5 µL of 1:10 dilution of Saliva B in each replicate.
SWD17	
SWD18	

Table VII.b-2: EZ1 reproducibility samples used for comparison to reproducibility samples run by the QIAsymphony

- From the mock casework study: four differential samples (two non-sperm fractions, two sperm fractions) with two reagent blanks (one non-sperm fraction, one sperm fraction), one touch sample with a reagent blank, and one blood sample with a reagent blank were extracted.

Sample Name	Comments
DIFF2EZ1 (EF, SF)	¼ swab cutting from 2018 Proficiency Test
DIFF3EZ1 (EF, SF)	5 mm x 5 mm cutting from 2012 Proficiency Test (fabric)
TOUCH4EZ1	½ swab cutting from chair handles
BLOOD3EZ1	3 mm x 3 mm cutting from 2017 Proficiency Test (FTA)

Table VII.b-3: EZ1 mock casework samples used for comparison to mock casework samples run by the QIAsymphony (Table VI.b-1)

All samples were prepared at the same time as the samples which were prepared for the QIAsymphony to help ensure uniform distribution. For the mock casework samples, cutting sizes were measured and swabs were divided as evenly as possible. All samples were extracted on an EZ1 Advanced XL using FBS20 [20]. Sensitivity samples were extracted using the 200 µL EZ1 protocol and all other samples were extracted using the 500 µL EZ1 protocol. All samples were eluted in 50 µL TE Buffer. All samples were quantified, amplified and typed. A total of 82 samples were compared for this study (27 EZ1 samples and 55 QIAsymphony samples). Reagent blank results for all EZ1 and QIAsymphony runs were evaluated in Section X.

Average quantities and standard deviations were calculated using Microsoft Excel. DNA types and quality of the STR profile were manually evaluated using electropherograms by two different individuals. For mock casework samples, quantities and profiles were evaluated to determine if they are consistent with typical casework expectations for the sample types.

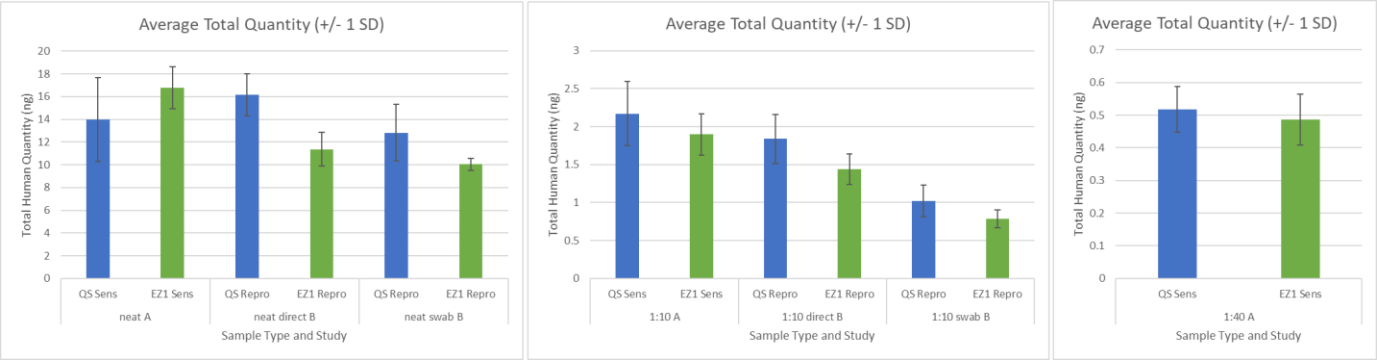
c. Results

i. Sensitivity and Reproducibility Samples

For the Sensitivity and Reproducibility samples, results were grouped by source (sample contributor A or B) and type (neat, 1:10, 1:40) and then averaged to compare the EZ1 to the QIAsymphony (Table VII.c.i-1). While the QIAsymphony produced slightly higher averages overall compared to the EZ1, all data was within three standard deviations except for neat swab B (Figures VII.c.i-1 to VII.c.i-3). This was considered an outlier due to a very low standard deviation obtained from the EZ1 replicate quantities. For this sample set, the standard deviation was only 5% of the average total quantity, while all other EZ1 sample sets had standard deviations of 11-15%.

Sample Type	Study	Average Total Quantity	Standard Deviation	EZ1 % difference
neat A	QS Sens	13.973	3.678	
	EZ1 Sens	16.767	1.832	120.0%
neat direct B	QS RR A1 tube	16.164	1.845	
	EZ1 RR	11.367	1.464	70.3%
neat swab B	QS RR A1 tube	12.816	2.491	
	EZ1 RR	10.033	0.530	78.3%
1:10 A	QS Sens	2.171	0.424	
	EZ1 Sens	1.897	0.270	87.4%
1:10 direct B	QS RR A1 tube	1.838	0.325	
	EZ1 RR	1.442	0.201	78.4%
1:10 swab B	QS RR A1 tube	1.020	0.208	
	EZ1 RR	0.787	0.119	77.1%
1:40 A	QS Sens	0.517	0.070	
	EZ1 Sens	0.486	0.078	93.8%

Table VII.c.i-1: Average total quantities and standard deviations for sensitivity and reproducibility samples extracted using the EZ1 compared to average total quantities and standard deviations for the same sensitivity and reproducibility samples extracted using the QIAsymphony.



Figures VII.c.i-1 to VII.c.i-3: Graphical representations of the average total quantities for sensitivity and reproducibility samples extracted using the EZ1 compared to average total quantities for the same sensitivity and reproducibility samples extracted using the QIAsymphony.

All samples were amplified and produced profiles consistent with expectations based on the amplification target quantity.

ii. Mock Casework Samples

For the mock casework samples, similar quantities were obtained based on expectations of these sample types (Figure VII.c.ii-1). While their values appeared to vary more than the reproducibility or sensitivity samples, these samples were not processed as replicates and were prepared from substrates or fluids with no guarantee of consistent cellular distribution.

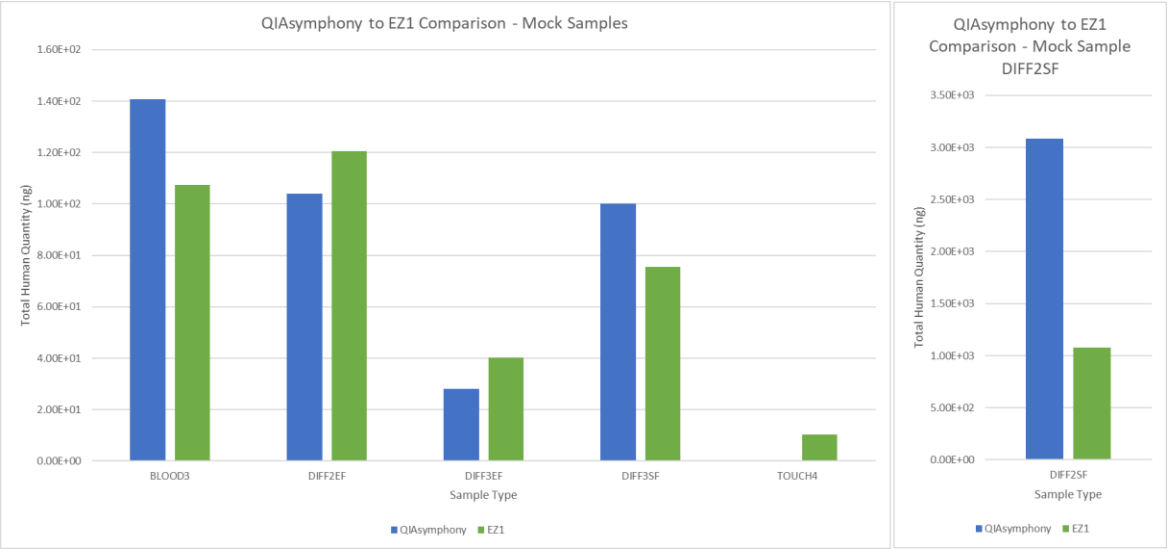


Figure VII.c.ii-1: Total human quantities (ng) for the mock casework samples extracted using the EZ1 compared to total human quantities for the mock casework samples extracted using the QIAsymphony.

All EZ1 samples were able to be amplified at optimal target and produced full, concordant profiles with no signs of inhibition or degradation. All QIAsymphony samples were also able to be amplified at optimal target and produced full, concordant profiles with no signs of inhibition or degradation except for TOUCH4. While this sample was a full profile for the EZ1 extract and partial profile for the QIAsymphony extract, both were consistent with the expected profile results based on their amplification targets. This indicates that the TOUCH4 QIAsymphony extract was low level but not poor quality.

VIII. Plate Elution

a. Objective

The manufacturer-developed instrument protocols for use with the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument included a variety of adapters and consumable types for sample elution. However, the MicroAmp Optical 96-Well Reaction Plate (Part Number N8010560) used by DC DFS FBU was not included. Therefore, a set of Advanced High Efficiency (ADV HE) custom protocols were created by QIAGEN that are identical to those currently being validated with the option to elute in these plates. The plate elution study was conducted to demonstrate comparable results were obtained when samples were eluted in tubes and plates.

b. Materials and Methods

Two initial attempts to verify elution in a MicroAmp Optical 96-Well Reaction Plate (Part Number N8010560) were made without success. The first attempt used the manufacturer developed instrument protocol included with the QIAsymphony (Casework_500_ADV_HE_V10) in combination with elution in a 96-well plate (shown as "*PCR96 Optical" in the protocol). This run resulted in an instrument error during dispensing of the eluate to the plate. A manual attempt to rescue the eluted samples from the sample prep cartridge was not successful. Customer Service was contacted, and instrument clean-up procedures were followed. A set of custom protocols were then developed by QIAGEN to allow for proper plate elution:

CW200ADVHE_CR23916_ID4944
CW500ADVHE_CR23916_ID4945
CW1000ADVHE_CR23916_ID4946

Once received, the sample set was re-prepared from the original neat saliva sample and the run was successfully repeated using the new custom protocol (CW200ADVHE_CR23916_ID4944) with elution in a 96-well plate (shown as "*custom PCR96 Optical" in the protocol).

During data analysis, it was determined that the overall sample quantities were not consistent with the same reproducibility samples prepared two months earlier and eluted in tubes. Because it was unclear if the samples were inconsistent due to the new custom plate elution protocol or the re-preparation of the samples, an entirely new study was conducted. The data associated with the samples from this run was omitted from all calculations.

A new set of 24 neat saliva, 12 diluted saliva (1:10) and eight reagent blanks were prepared. The samples were equally divided into two identical sets with batches in each of the four carriers. The samples to be eluted in tubes were run using the Casework_200_ADV_HE_V10 protocol with each batch eluted in a separate adapter. The samples to be eluted in a 96-well plate were run using the custom protocol CW200ADVHE_CR23916_ID4944 with each batch eluted as a new column in the 96-well plate. See Figures VIII.b-1 and VIII.b-2 for sample and elution set-ups.

All samples were quantified. One sample from the tube elution set (Tube07) was omitted from calculations due to a sample preparation error during set-up of the quantitation. A total of 35 samples were included in this study.

Sample Setup

Carrier #1	
Sample Name	Comments
Tube01	5ul neat saliva
Tube02	
Tube03	
RB04	

Carrier #2	
Sample Name	Comments
Tube04	5ul neat saliva
Tube05	
Tube06	
Tube07	5ul 1:10 saliva
Tube08	
Tube09	
RB05	

Carrier #3	
Sample Name	Comments
Tube10	5ul neat saliva
Tube11	
Tube12	
RB06	

Carrier #4	
Sample Name	Comments
Tube13	5ul neat saliva
Tube14	
Tube15	
Tube16	5ul 1:10 saliva
Tube17	
Tube18	
RB07	

Elution Orientation

Position 1 (cooling)					
Tube01					
Tube02					
Tube03					
RB04					

Position 3					
Tube10					
Tube11					
Tube12					
RB06					

Position 2					
Tube04	Tube08				
Tube05	Tube09				
Tube06	RB05				
Tube07					

Position 4					
Tube13	Tube17				
Tube14	Tube18				
Tube15	RB07				
Tube16					

Figure VIII.b-1: Tube-to-tube sample and elution set-up

Sample Setup

Carrier #1	
Sample Name	Comments
Plate01	5ul neat saliva
Plate02	
Plate03	
RB08	

Carrier #2	
Sample Name	Comments
Plate04	5ul neat saliva
Plate05	
Plate06	
Plate07	5ul 1:10 saliva
Plate08	
Plate09	
RB09	

Carrier #3	
Sample Name	Comments
Plate10	5ul neat saliva
Plate11	
Plate12	
RB10	

Carrier #4	
Sample Name	Comments
Plate13	5ul neat saliva
Plate14	
Plate15	
Plate16	5ul 1:10 saliva
Plate17	
Plate18	
RB11	

Elution Orientation

	1	2	3	4	5	6	7	8	9	10	11	12
A	Plate01	Plate04	Plate10	Plate13								
B	Plate02	Plate05	Plate11	Plate14								
C	Plate03	Plate06	Plate12	Plate15								
D	RB08	Plate07	RB10	Plate16								
E		Plate08		Plate17								
F		Plate09		Plate18								
G		RB09		RB11								
H												

Figure VIII.b-2: Tube-to-plate sample and elution setup

c. Results

Average quantities and standard deviations were calculated using Microsoft Excel (Figure VIII.c-1). One sample from the tube elution set (Tube07) was omitted from calculations due to a sample preparation error during set-up of the quantitation.

Neat Saliva Samples

	Sample Name	Quantity (ng/µL)	Average Quantity (ng/µL)	standard deviation
Tube Lysis, Plate Elution	Plate01	7.78E-01	6.04E-01	2.19E-01
	Plate02	5.27E-01		
	Plate03	5.21E-01		
	Plate04	4.84E-01		
	Plate05	4.16E-01		
	Plate06	3.66E-01		
	Plate10	4.46E-01		
	Plate11	4.77E-01		
	Plate12	6.50E-01		
	Plate13	9.17E-01		
	Plate14	5.75E-01		
	Plate15	1.09E+00		
Tube Lysis, Tube Elution	Tube01	5.80E-01	7.13E-01	2.00E-01
	Tube02	5.23E-01		
	Tube03	5.59E-01		
	Tube04	5.66E-01		
	Tube05	6.55E-01		
	Tube06	4.66E-01		
	Tube10	6.55E-01		
	Tube11	7.90E-01		
	Tube12	9.32E-01		
	Tube13	8.49E-01		
	Tube14	1.14E+00		
	Tube15	8.43E-01		

Dilution Saliva Samples (1:10)

	Sample Name	Quantity (ng/µL)	Average Quantity (ng/µL)	standard deviation
Tube Lysis, Plate Elution	Plate07	6.68E-02	7.78E-02	7.11E-03
	Plate08	8.00E-02		
	Plate09	8.80E-02		
	Plate16	8.09E-02		
	Plate17	7.42E-02		
	Plate18	7.69E-02		
Tube Lysis, Tube Elution	Tube08	6.56E-02	7.39E-02	5.93E-03
	Tube09	7.49E-02		
	Tube16	7.31E-02		
	Tube17	7.35E-02		
	Tube18	8.23E-02		

Figure VIII.c-1: Average quantities and standard deviations for comparison of samples eluted in tubes with samples eluted in a 96-well plate

Average quantities for the neat and dilution saliva samples eluted in the 96-well plate were within one standard deviation of the average quantities for the same samples eluted in tubes. This demonstrates similar performance between the manufacturer-supplied protocols with a tube elution and the custom protocols with a 96-well plate elution.

IX. Performance Check and Quality Control

a. Objective

A new lot of QIAsymphony DNA Investigator kits was received on 08/26/2021. While the primary use of these reagents was for training and validation, a Quality Control (QC) evaluation was used to verify the reagents. Annual Preventative Maintenance (PM) was also performed on 08/04/2021 by a QIAGEN Field Specialist. While no repairs or upgrades were made, a performance check of the instrument was required to satisfy internal and external requirements. Because the drafted procedures were the same for the QC of a new kit and performance check following a PM with no repairs or upgrades, one set of samples was run to authorize both reagents and instrument for use.

b. Materials and Methods

Three known blood sample cuttings (approximately 5 mm x 5 mm) and three reagent blanks were run using the Casework_200_ADV_HE_V10 protocol. All samples were quantified, amplified and typed. Reagent blanks were amplified at maximum volume.

In addition, the following drafted procedures were tested and followed as a part of this study: FBS41 – QIAsymphony SP – DNA Extraction, FBQ36 – Quality Control of QIAGEN DNA Investigator Kits, FBQ46 – QIAsymphony SP Maintenance. For instrument maintenance, daily, weekly, and monthly maintenance were all completed. While conducting each step, the drafted procedures were evaluated, and any needed edits were made to improve or add clarity to the written procedure.

c. Results

All samples resulted in quantities sufficient for amplification at optimal target and correct DNA genotypes with no signs of inhibition or degradation. Reagent blanks and other controls also typed accurately and showed no signs of contamination. The reagents were listed on the drafted Quality Control of QIAGEN DNA Investigator Kits Evaluation Worksheet and determined to be appropriate for use in training, validation, and/or casework procedures. The QIAsymphony SP also demonstrated acceptable performance following the annual PM.

Drafted procedures remained generally the same for FBS41 – QIAsymphony SP – DNA Extraction and FBQ36 – Quality Control of QIAGEN DNA Investigator Kits; however, a significant change was made to FBQ46 – QIAsymphony SP Maintenance. The recommended use of a quaternary ammonium salt-based disinfectant for cleaning during maintenance steps proved to be impractical and time-consuming due to the SDS requirements for collection and disposal. Because forensic biology does not include the processing of high-quantity bacterial or fungal samples, 70% ethanol was determined to be sufficient based on current practices for other similar instrumentation (e.g., EZ1 Advanced XL instruments).

X. Contamination Assessment

a. Objective

A contamination assessment is conducted during the internal validation to evaluate the detection of exogenous DNA originating from the reagents, consumables, other samples, operator, and/or laboratory environment [2]. Genotype results were previously evaluated in the accuracy study to evaluate contamination in samples. This study evaluated the obtained quantities and genotype results for the reagent blanks.

b. Materials and Methods

Quantitation and/or typing results from all samples (179) and reagent blanks (31) throughout the validation were evaluated for contamination (Table X.b-1). For reagent blanks with detected quantities, melt curves were evaluated to determine specificity. All sample and reagent blank profiles were compared to expected or previously-run data to determine whether there were indications of contamination (i.e., discordant alleles not attributable to expected amplification or detection artifacts). Mock casework touch samples did not have previously-run data available so they were evaluated for the presence of the expected contributor instead.

Study	Samples	Reagent Blanks
Sensitivity	18	1
Accuracy	6	5
Precision and Repro	40	2
Mixtures	9	1
Mock	20	4
EZ1 Comparison	27	6
Plate Elution*	56	9
Performance Check/QC	3	3
Contamination (Total)	**179**	**31**

*includes 20 samples and 1 reagent blank plate elution samples that were omitted

Table X.b-1: Total number of samples and reagent blanks evaluated for each study and for the overall internal validation

Quantity and quality of the samples and reagent blanks were evaluated using both the Plexor Analysis software and exported results documents (Microsoft Excel). DNA types were manually evaluated for samples and controls by two different individuals using electropherograms.

c. Results

All reagent blanks within this validation were determined to be free from contamination. All quantitation values were “N/A” except for three reagent blanks in the Plate Elution Study (RB06, RB08, RB09). Two of the three with resulting values displayed melt curves outside of the expected range (RB08, RB09). The third reagent blank (RB06) was determined to be a pipetting error during sample preparation. Tube07 in well D08 was “N/A” during quantitation and RB06 in well D09 had a quantity of 0.0621 ng/µL. This value was very similar to the other samples in its replicate set (Tube08 and Tube 09). The reagent blank (RB06) and sample (Tube07) were both omitted from the study. In addition, all reagent blanks that were processed through detection did not produce any alleles above analytical threshold.

There were also no samples that showed signs of contamination (discordant alleles) from previously processed or neighboring samples.

XI. Conclusion

Based on the results of the internal validation studies, the QIAsymphony DNA Investigator Kit and the QIAsymphony SP instrument demonstrated that they are acceptable for casework use by the DC DFS FBU. It reliably and reproducibly extracted and isolated DNA from low to high level samples, mixed samples, and other sample types and substrates typically encountered in forensic DNA casework. Functional testing of the QIAsymphony SP Operating System (version 5.0) was successfully completed throughout the internal validation. Reliability of the software was also successfully demonstrated by two different operators during the Precision and Reproducibility Study (Section IV).

Three different lysis volume protocols (200 µL, 500 µL, and 1000 µL), two different elution types (tubes or 96-well plates), and a 40 µL elution volume were evaluated and determined to enhance current and future laboratory capabilities. The 200 µL and 500 µL lysis volume protocols are part of the currently validated EZ1 Advanced XL system; however, the addition of the 1000 µL lysis volume protocol on the QIAsymphony allows the option to extract DNA from larger sample cuttings. The 96-well plate elution format adds to the laboratory's future capabilities by improving the sample preparation efficiency of downstream laboratory processes like quantitation and amplification. Finally, the validation of a lower-elution volume (40 µL) than the currently validated EZ1 Advanced XL elution volume (50 µL) eliminates the need for sample concentration prior to amplification.

The instrument protocols and labware have been validated for use in casework are shown in Table XI-1.

Assay Control Set	Lysis Labware	Elution Labware	Elution Rack Type
Casework_200_ADV_HE_V10	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	Tube 2.0 mL SAR #72.693 *T2.0 Screw
Casework_500_ADV_HE_V10	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	Tube 2.0 mL SAR #72.693 *T2.0 Screw
Casework_1000_ADV_HE_V10	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	Tube 2.0 mL SAR #72.693 *T2.0 Screw
CW200 ADV HE CR23916_ID4944	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	MicroAmp™ Optical 96-Well Reaction Plate (cat. No. N8010560)	PCR Plate ABI #N8010560 *custom PCR96 Optical
CW500 ADV HE CR23916_ID4945	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	MicroAmp™ Optical 96-Well Reaction Plate (cat. No. N8010560)	PCR Plate ABI #N8010560 *custom PCR96 Optical
CW1000 ADV HE CR23916_ID4946	Sarstedt Micro tube 2 mL with cap, Micro tube 2mL, PP, NONSKIRTED (cat. No. 72.693)	MicroAmp™ Optical 96-Well Reaction Plate (cat. No. N8010560)	PCR Plate ABI #N8010560 *custom PCR96 Optical

Table XI-1: Instrument protocols and labware validated for casework use by DC DFS FBU

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14. FBS28 – PCR Amplification using the GlobalFiler™ Kit
15. FBS29 – Capillary Electrophoresis using the AB 3500/3500xL Genetic Analyzer
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20. FBS20- EZ1 Advanced XL – DNA Extraction

The following documents were reviewed during the internal validation:

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Appendix

A. Validation Plan
B. Sample layout, truth profiles, lab worksheets and electropherograms
C. Miscellaneous – Reagents and supplies, PM/inspection report, request for custom protocols