

ORIGINAL ARTICLE

# Validation of a High-Throughput Automated Liquid Handling DNA Extraction System to Maximize Efficiency in Forensic Casework

Heather Sarik, MS,<sup>1</sup> Kristen Naughton, BS,<sup>1</sup> and Kevin W.P. Miller, PhD<sup>2,\*</sup>

## Abstract

**Background:** Decreasing turnaround times and reducing the backlog for criminal cases containing biological evidence remains a critical priority for public safety. In the forensic crime laboratory, one strategy to increase efficiency and capacity is to incorporate robotic instrumentation. In addition to increasing sample throughput, automation also decreases sources of variability and error compared with manual methods, while enabling analysts to focus on higher value activities. Here, we present the validation of a high throughput solution for DNA extraction and purification from forensic evidence type samples using the Hamilton Microlab<sup>®</sup> VANTAGE Liquid Handling System.<sup>®</sup>

**Methods:** Sensitivity, reproducibility, contamination, and nondifferential and differential mock evidence studies were all performed according to the SWGDAM Validation Guidelines, using both VANTAGE and QIASymphony SP liquid handling platforms. Data obtained using each automated system were compared.

**Results:** Samples (i.e. blood, saliva, semen) that ere purified on the VANTAGE generated accurate, sensitive, and reproducible quantification and profile results that were free from contamination regardless of the substrate (i.e. cotton, wood, metal, ceramic) from which the DNA was extracted. The extracted DNA yield produced by both the VANTAGE and the QIASymphony SP were comparable. However, the VANTAGE processed up to four microplates (384 samples) in approximately 1 hour and 33 minutes, executing the workflow up to 10-fold faster than the QIASymphony SP.

**Conclusions:** In current laboratory environments where the influx of DNA sample submissions often outpaces laboratory capability, the VANTAGE high-throughput solution offers the forensic analyst with a means to maximize sample processing efficiency without compromising process quality.

**Keywords:** automation, forensic validation, DNA typing, short tandem repeat, sexual assault evidence, evidence

## Introduction

The use of DNA testing in criminal cases has continuously increased over the past two decades. Recent efforts to reduce DNA testing backlogs and turnaround times for criminal cases have demonstrated that DNA testing can be used to identify repeat offenders, thereby preventing future crimes. The Bureau of Justice Assistance in the United States Department of Justice Sexual Assault Kit Initiative has inventoried >100,000 unprocessed backlog sexual assault kits since 2015.<sup>1</sup> DNA testing of these kits completed as of December 2019 resulted in >20,000 offender profiles, including 6000 profiles that were already

in the national database for serial sex crimes or other violent offenses.<sup>1</sup>

In addition, DNA technology is continually improving, and assay chemistries have advanced to detect and characterize very small amounts of DNA, including touch DNA. This has prompted law enforcement officers to submit increasing amounts of evidentiary DNA samples for testing, particularly for property crimes and, in at least one jurisdiction, has contributed to a 42% reduction in that crime classification.<sup>2</sup> Advances in DNA technology may also be an impetus to reopen unsolved cold cases and aid in exoneration cases. In the forensic crime laboratory,

<sup>1</sup>Bode Technology, Lorton, Virginia, USA.

<sup>2</sup>Government and Regulated Laboratories, Hamilton Company, Reno, Nevada, USA.

\*Address correspondence to: Kevin W.P. Miller, PhD, Government and Regulated Laboratories, Hamilton Company, 4970 Energy Way, Reno, NV, USA, Email: kevin.miller@hamiltoncompany.com

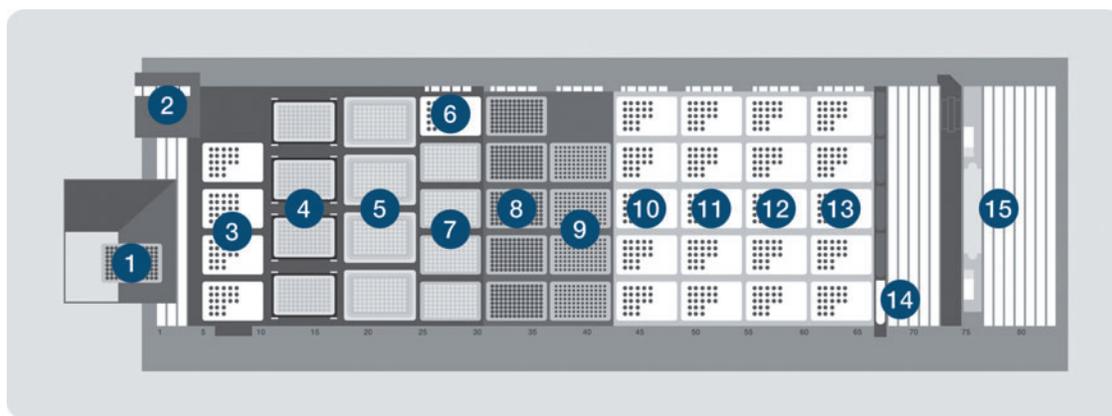
the influx of DNA sample submissions often outpaces the laboratory's resources, especially when the samples are processed manually.

New technology is available to allow laboratories to increase efficiency to keep up with law enforcement DNA testing requests. Using the same assay chemistry, the Microlab<sup>®</sup> VANTAGE Liquid Handling System<sup>®</sup> can purify up to 384 samples in ~1 h and 33 min, whereas the QIASymphony SP, which can only process 24 samples in an hour, takes 16 h to process an equivalent number of samples. Both automated DNA extraction systems require manual sampling of evidence items into processing tubes or 96-well plates, a pretreatment lysis step and subsequent removal of evidence substrate before placing the lysates onto the automated platform for purification. Both platforms fully automate DNA purification of a wide variety of forensic DNA sample types, including differentially separated samples from sexual assault kits.

The VANTAGE was scripted by the Hamilton Company to automate the Lyse&Prep kit, simultaneously purifying up to four 96-well plates at a time. The deck layout (Fig. 1) is designed to minimize overall pipette

tip consumable use as well as pipette movement over reagent troughs and sample plates, thus minimizing the potential for cross-contamination. The VANTAGE is equipped with a 2.0 m deck, eight independent single-channel pipettors, a 96-well pipetting head, four Hamilton Heater Shakers (HHS), four 96-well plate magnets, and both dry and liquid waste receptacles. A quad CORE plate gripper transports plates on the deck without the need for human intervention, and 1D/2D barcode scanning tracks samples to aid in workflow efficiency and to reduce the risk of errors.

The performance of the newly installed high-throughput VANTAGE system was compared with the previously installed QIASymphony SP robotic workstation for the extraction and purification of evidentiary and reference DNA samples using equivalent magnetic particle-based assay chemistries before downstream quantification, amplification, and capillary electrophoresis (CE) as part of an internal VANTAGE validation study. Validation criteria included sensitivity, repeatability, and reproducibility, accuracy, known samples, mock evidence, and contamination assessment in accordance



- |   |  |    |   |
|---|--|----|---|
| 1 | 96 Multi-Probe Head Solid Waste  | 8  | 300 mL Reagent Troughs                  |
| 2 | Liquid Waste   | 9  | 96-well Microplates (for Elution)       |
| 3 | 1,000 µL Filtered Tips (for Binding Buffer);<br>Tip Isolator Positions for Re-Used Tips<br>Throughout Protocol | 10 | 300 µL Filtered Tips (for Elution)      |
| 4 | Plate Magnets  | 11 | 1,000 µL Filtered Tips (for Wash 1)     |
| 5 | Hamilton Heater/Shakers (HHS)  | 12 | 1,000 µL Filtered Tips (for Wash 2)     |
| 6 | 1,000 µL Filtered Tips (for Beads)   | 13 | 1,000 µL Filtered Tips (for Water Wash) |
| 7 | 2 mL Nunc Plates   | 14 | 60 mL Trough for Beads                  |
|   |  | 15 | Independent Channel Solid Waste         |

**Fig. 1.** The Microlab<sup>®</sup> VANTAGE Liquid Handling System<sup>®</sup> 2.0 m deck layout configured for use with the Qiagen Lyse&Prep kit.

with Federal Bureau of Investigation and Scientific Working Group on DNA Analysis Methods validation recommendations.<sup>3,4</sup>

## Materials and Methods

### DNA extraction components

The QIASymphony<sup>®</sup> DNA Investigator Kit and the Qiagen Investigator<sup>®</sup> STAR Lyse&Prep Kit were obtained from Qiagen (Germantown, MD). Both DNA extraction kits included ATL, QSL3, QSW1, QSW2, and ATE buffers, bead suspension G, proteinase K, and carrier RNA. In this magnetic particle-based technology, DNA binds to the particle's silica surface in the presence of chaotropic salt. Magnets were used to immobilize the bound particles while lysates were washed out of solution. Purified DNA was eluted from the particles in TE<sup>-4</sup> buffer.

### Prerun preparation and setup

For both the VANTAGE and the QIASymphony, samples were manually lysed according to the manufacturer's protocol using ATL buffer and proteinase K. Both 200 and 300  $\mu$ L lysis volume options were scripted for the VANTAGE, and these were compared with the QIASymphony options of 200 and 500  $\mu$ L, respectively. When samples were lysed in tubes, they were transferred to 96-well deep well plates, using a separate instrument script, before DNA purification using the VANTAGE. The user dispensed buffers into reagent troughs based on the number of samples being processed for each run. After all necessary consumables were placed onto the deck, the VANTAGE scanned each carrier to ensure proper deck setup and scanned the input and output plates to verify the input file. For DNA purification using the QIASymphony, samples were lysed in either individual tubes or in a 96-well deep well plate format and were then loaded directly onto the instrument. A prefilled reagent cartridge and other consumables were loaded onto the instrument, and a manufacturer's predefined purification script was selected.

### VANTAGE purification protocol

The VANTAGE used a customized script based on the recommended procedure from Qiagen. The VANTAGE mixed and aliquoted 100  $\mu$ L of Bead Suspension G to each plate well and then added 625  $\mu$ L binding buffer to each well, using the eight individual pipetting channels and 1000  $\mu$ L nonsterile filtered conductive tips (Hamilton Company, Reno, NV). All subsequent pipetting steps within the method used the 96 multipipetting head. The quad CO-RE plate gripper transported the plate to the HHS for shaking at 700 rpm for 10 min at 56°C. After shaking incubation, the quad CO-RE plate gripper transported the plate to the magnet for a 2-min incubation at which point the supernatant was removed from each

**Table 1. Approximate timeline of sample processing using VANTAGE and the Lyse&Prep kit**

| Step                  | Time for one plate (min) | Accumulative time (min) | Time for four plates (min) | Accumulative time (min) |
|-----------------------|--------------------------|-------------------------|----------------------------|-------------------------|
| Add bead suspension G | 2.5                      | 2.5                     | 10.5                       | 10.5                    |
| Binding               | 15                       | 17.5                    | 21                         | 31.5                    |
| Wash 1                | 4                        | 21.5                    | 12.5                       | 44                      |
| Wash 2                | 4                        | 25.5                    | 12                         | 56                      |
| Water wash            | 2.5                      | 28                      | 7                          | 63                      |
| Air dry               | 10                       | 38                      | 15                         | 78                      |
| Elution               | 8                        | 46                      | 15                         | 93                      |
| Total time            | 46                       |                         | 93                         |                         |

One plate of up to 96 samples is purified in  $\sim$ 46 min, and an additional 15 min is added per additional 96-well plate such that up to four plates may be purified in  $\sim$ 1 h and 33 min. Times do not include initial lysis incubation, separation of lysates from substrates, or instrument setup.

plate. The instrument added 500  $\mu$ L QSW1 to the wells, transported the plate back to the HHS for shaking incubation at 1000 rpm for 30 s at 30°C, then placed the plate on the magnet for a 1-min incubation.

The VANTAGE removed supernatant from the wells, and repeated the wash step, using 500  $\mu$ L QSW2 along with shaking and subsequent magnetic incubations. After removing the supernatant, the VANTAGE performed a final rinse step by adding 200  $\mu$ L DNA-grade water to each well, incubating the plate for 20 s on the magnet without agitation, and then removing the supernatant. The plates remained on the magnet for a 10-min incubation at ambient temperature to allow any residual ethanol from the buffers to evaporate. Then the instrument added 50  $\mu$ L TE<sup>-4</sup> buffer to each well and transferred the plate to the HHS for a final shaking incubation at 1000 rpm for 5 min at 70°C. The VANTAGE then returned the plate to the magnet where eluant in the wells was transferred to the wells of an elution plate for off-deck downstream processing.

Additional plates were processed in the same manner. Approximate timing information for each step in the VANTAGE purification process is listed in Table 1.

### QIASymphony purification protocol

The QIASymphony used manufacturer-fixed scripts CW200ADVHE\_CR22094\_ID1376 for 200  $\mu$ L lysates and CW500ADVHE\_CR22094\_ID1386 for 500  $\mu$ L. The QIASymphony dispensed binding buffer and magnetic beads to up to three 8-well sample prep cartridges, then transferred the sample lysate to each well of a cartridge using four pipetting channels. The robotic gripper arm transferred the sample prep cartridges to the lysis station and heated the samples to 56°C for binding. The robotic gripper arm then transferred the sample prep cartridges to the conveyor belt and the samples were positioned below the magnetic head containing eight-rod covers. The covered magnetic head was submerged in

the sample prep cartridge and was shifted upward and downward to mix the samples.

Meanwhile, the instrument placed new sample prep cartridges on the conveyor belt and QSW1 was added to each well. The magnetic head containing the beads was then raised and the conveyor belt shifted so that the cartridges containing binder buffer were no longer beneath the head while the new cartridges containing QSW1 buffer were below the head. The head was then lowered into the cartridges and the samples were mixed again. Meanwhile, the instrument placed new sample prep cartridges on the conveyor belt and QSW2 was added to each well. The wash process was repeated twice more using QSW2 buffer.

The final set of sample prep cartridges contained ATE buffer (elution buffer) and Top Elute buffer (glycerol reagent to aid in elution). The head was lowered into the cartridges and mixed a final time. Then the conveyor belt positioned the cartridges to the right of the head and the four pipetting channels transferred 50  $\mu\text{L}$  of each eluant to an elution plate for off-deck downstream processing.

Additional batches of up to 24 samples were processed in the same manner, with batch processing times taking  $\sim 1$  h each.

### Sensitivity study

Human whole blood in K3 ethylenediaminetetraacetic acid (EDTA) (Cryos International, Orlando, FL) was diluted in 1 $\times$  phosphate-buffered saline (PBS) as follows: dilution A, 1:200; dilution B, 1:800; dilution C, 1:3200; and dilution D, 1:12,800. For each dilution, 20  $\mu\text{L}$  was spotted onto a cotton swab. Each blood dilution sample was lysed off deck using 180  $\mu\text{L}$  ATL buffer and 20  $\mu\text{L}$  proteinase K, for a total volume of 200  $\mu\text{L}$ , and then purified in triplicate using either a VANTAGE or a QIASymphony workstation. A total of 12 blood dilutions were processed with each instrument.

### Repeatability and reproducibility study

Buccal swabs were obtained from 11 different internal donors with appropriate informed consent and internal IRB documentation, lysed in ATL and proteinase K, and added quadruplicate 300  $\mu\text{L}$  aliquots per donor to designated wells of identical 96-well assay plates. A total of eight replicates per donor were run on each of two plates. One plate was processed in position 1 on the VANTAGE deck, whereas the second was processed in position 4 on the VANTAGE deck. The four-plate VANTAGE run included two additional plates (processed in positions 2 and 3), which contained buffer only and were not included as part of this study. A total of 88 DNA samples were processed in this

study, and the study was not performed in comparison with the QIASymphony.

### Nondifferential mock evidence study

Swabs were collected from samples that are typical of those found at crime scenes, including dried blood (Cryos International) on various substrates (i.e., cotton, blue denim, leather, wool, nylon, brick, wood, brass, and ceramic flooring), touch-based samples from nonporous substrates (such as coffee mugs), liquid saliva, cigarette butts, and chewing gum. Nonblood samples were obtained from internal donors. The cigarette butts and chewing gum had been stored for  $\sim 5$  years before sampling; all other samples were created or collected within 1 month of processing. Fabric substrates were cut and  $\sim 0.5$   $\text{cm}^2$  sample, including substrate, was lysed. Other blood samples were wet/dry swabbed and the whole swab was lysed. Nonblood samples were wet/dry swabbed, and the swab heads were cut in half so that one half of each swab was able to be purified using each instrument. Samples purified on the VANTAGE were lysed in 300  $\mu\text{L}$  digest, whereas samples purified using the QIASymphony were lysed in 500  $\mu\text{L}$  digest. A total of 17 nondifferential mock evidence samples were purified using each instrument.

### Differential mock evidence study

Semen samples from an internal donor were diluted in 1 $\times$  PBS as follows: dilution A, 1:27; dilution B, 1:81; dilution C, 1:243; dilution D, 1:729; and dilution E, 1:2187. Mock differential samples were created using 20  $\mu\text{L}$  each of dilutions A–E with 80  $\mu\text{L}$  female saliva. Mixed samples were then spotted onto clean cotton swabs and the entire swab head was lysed. Sperm cells were separated from epithelial cells using an automated nuclease enzyme procedure,<sup>5</sup> and then purified using the 200  $\mu\text{L}$  lysis protocol on both robotic platforms.

A total of 20 sperm fractions (SFs) and 10 epithelial fractions (EFs) were processed on the VANTAGE while 10 SFs and 5 EFs were processed on the QIASymphony.

### Contamination assessment

Throughout the validation, the VANTAGE processed a total of 63 reagent blanks (RBs), including a subset processed in checkerboard pattern alternated with reference-type samples. The QIASymphony processed a total of 17 RBs.

### Downstream processing

All samples were quantified using Quantifiler<sup>TM</sup> Trio DNA Quantification Kit on the Applied Biosystems<sup>TM</sup> 7500 Real-Time PCR System using HID Real-Time PCR Analysis Software v1.2, all purchased from Thermo Fisher Scientific, Waltham, MA.

In the contamination assessment, any RB from the checkerboard with a  $C_T$  value  $<40$  at one or more Quantifiler Trio targets was concentrated using a 500  $\mu\text{L}$  Microcon™ centrifugal filter (Millipore Sigma, Burlington, MA) and the maximum volume sample template of 15  $\mu\text{L}$  was added to the amplification reaction. RBs that did not generate a  $C_T$  value  $<40$  at any of the Quantifiler Trio targets were not processed further. In the other study evaluations, the maximum RB volumes of unconcentrated extract were added to the amplification reaction.

Samples were amplified using 25  $\mu\text{L}$  PowerPlex Fusion (Promega Corporation, Madison, WI) reactions with an input target of 0.5 ng and 29 cycles on the Applied Biosystems GeneAmp® PCR System 9700 thermal cycler (Thermo Fisher Scientific). In addition, a mock evidence sample subset was amplified using the Applied Biosystems GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific; 25  $\mu\text{L}$  reaction volume, 1 ng input target, and 29 cycles) and the Investigator 24plex QS Kit (Qiagen; 25  $\mu\text{L}$  reaction volume, 1 ng input target, and 29 cycles) to demonstrate chemistry compatibility with multiple vendor short tandem repeat kits.

Finally, CE was performed using the Applied Biosystems 3500xL genetic analyzer with Data Collection Software v.3.1 and analysis and interpretation were performed with the GeneMapper® ID-X (GMIDX) v1.5, all procured from Thermo Fisher Scientific. Samples were evaluated for percentage complete profile, average profile peak height, profile balance, intracolor dye balance, and heterozygote balance. Profile and intracolor dye balance values of 5 or less indicated good quality profiles, whereas higher values indicated poorer quality profiles. Additional analysis thresholds were applied as indicated hereunder.

The PowerPlex Fusion peak detection threshold, the absolute minimal level of analyte that can be expected to routinely result in a positive signal from the analytical system, was 50 relative fluorescent units (RFU) for the blue and green dye channels, and 65 RFU for the yellow and red channels. The analytical threshold, the minimum peak height in RFU that confidently ascribes a true amplicon peak, and the stochastic threshold, the minimum peak height in RFU that must be met or exceeded to have confidence that no sister alleles have failed to be detected above analytical threshold, were set at 100 and 500 RFU, respectively. Data that were at or below the analytical threshold were scrutinized for signs of contamination. Optimal heterozygote balance values were 50% or greater.

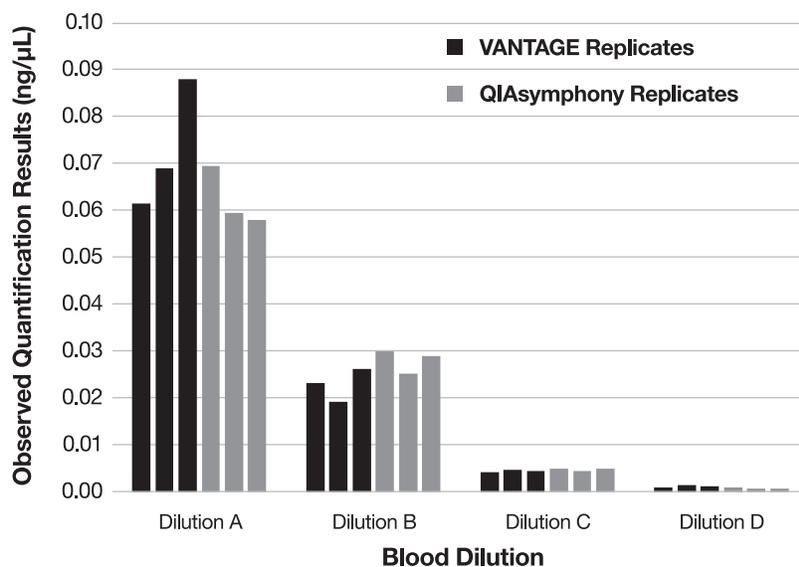
The GlobalFiler peak detection threshold per dye channel was blue, 50 RFU; green, 60 RFU; yellow, 50 RFU; red, 60 RFU; and purple, 60 RFU. The analytical and stochastic thresholds used were 125 and 600 RFU, respectively. Optimal heterozygote balance values were 60% or greater.

The Investigator 24plex peak detection, analytical, and stochastic thresholds used were 60, 100, and 600 RFU, respectively. Optimal heterozygote balance values were 60% or greater.

## Results and Discussion

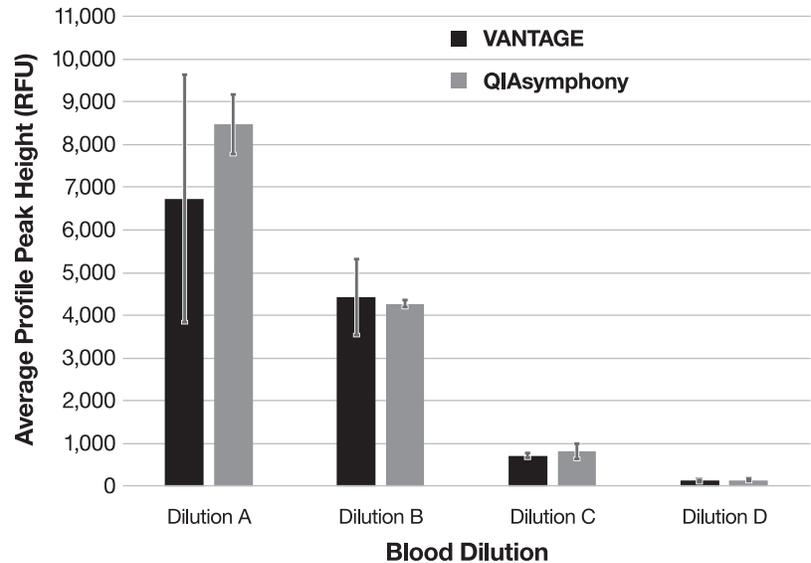
### Efficiency

On the VANTAGE, one 96-well plate was able to be purified in  $\sim 46$  min, with only an additional 15 min needed for each additional 96-well plate. The VANTAGE method allowed for up to four 96-well plates (384 samples) to be loaded simultaneously, and these could be purified in  $\sim 1$  h and 33 min. By contrast, up to four batches



**Fig. 2.** Sensitivity study. Small autosomal quantification results of 200  $\mu\text{L}$  diluted blood lysate samples purified using the Lyse&Prep extraction kit on both the VANTAGE and QIASymphony platforms. Samples were quantified using Quantifiler Trio, with DNA profiles generated using PowerPlex Fusion.

**Fig. 3.** Sensitivity study. Average profile peak height data from 200  $\mu$ L lysis samples that were purified using the VANTAGE and QIASymphony instruments and amplified using PowerPlex Fusion. Graphs represent average values across triplicate replicates in each category and error bars represent 1 standard deviation from average.



of only 24 samples each could be loaded simultaneously and processed by the QIASymphony, and these batches took  $\sim 1$  h each to be purified. Therefore, it took  $\sim 4$  h to process a set of 96 samples. The VANTAGE was 5-fold faster when processing up to 96 samples at a time, and 10-fold faster when processing 384 samples.

### Sensitivity study

Figure 2 shows the small autosomal (SA) quantification results from diluted blood samples purified using the VANTAGE and QIASymphony automated methods. Generally speaking, the eluted DNA concentration decreased as the input blood amount decreased. Samples processed on the VANTAGE generated yield comparable with corresponding samples processed on the QIASymphony.

Samples were amplified based on the observed quantification results; no samples were concentrated and, therefore, the more dilute samples were not able to reach the target input value. Figure 3 shows the average profile peak height. All blood samples generated full profiles using both purification instruments, except dilution D as the maximum input template for these samples was 0.07 ng. All samples generating full profiles had profile balance and intracolor dye balance between 2 and 5, which is indicative of good quality and balanced profiles. Samples purified on the VANTAGE had comparable heterozygote balance in relation to those purified on the QIASymphony.

### Repeatability and reproducibility study

Low standard deviations in quantification data for each donor indicate consistent purification efficiency across the full plate (Fig. 4A). Samples processed in position 4 on the deck (referred to as Plate 4) generated consistently

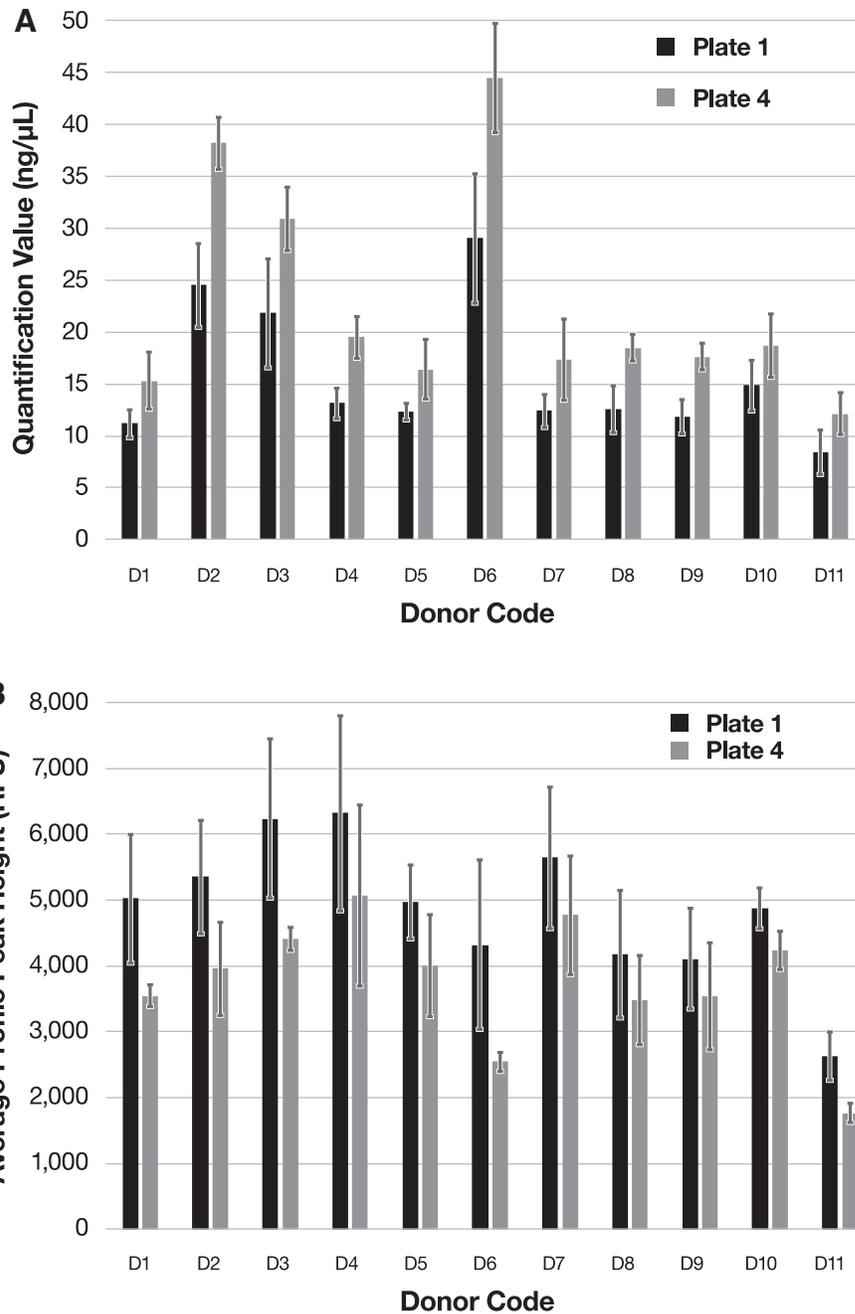
higher quantification results than those processed in position 1 on the deck (referred to as Plate 1). This is likely due to variations in real-time polymerase chain reaction. Plate 4 samples also generated consistently lower peak heights than Plate 1, indicating slightly more dilution than necessary during amplification setup due to an overestimate in concentration (Fig. 4B).

All samples generated full profiles and, with the exception of Donor 11, all samples generated 2–6.5 profile balance values, 1–6.5 intracolor balance values, and heterozygote balances  $>50\%$ . Outlier values generated from Donor 11 sample replicates were spread across different rows and columns of each plate, yet the imbalances occurred across both plates and only for this donor, so the issue is likely donor specific.

### Nondifferential mock evidence study

Nondifferential mock evidence samples yielded varied quantification results across different substrates, donors, and purification systems, including higher values for blood samples (Fig. 5A) compared with nonblood samples (Fig. 5B). Blood samples on fabric substrates and purified using the VANTAGE generated lower quantification values than those purified on the QIASymphony. A separate study not discussed in this article determined this to be due to use of commercially derived blood containing EDTA as it was not seen when testing with high levels of nonpreserved blood dried on tissue.

Profile results were comparable between samples purified using the two instruments apart from a single outlier swabbed from chewing gum stored for  $\sim 5$  years. This sample processed on the VANTAGE generated a



**Fig. 4.** Repeatability and reproducibility study. Small autosomal quantification results (A), and profile peak height results for donor replicates on each plate (B), were averaged and the standard deviation was calculated. Error bars represent 1 standard deviation from average.

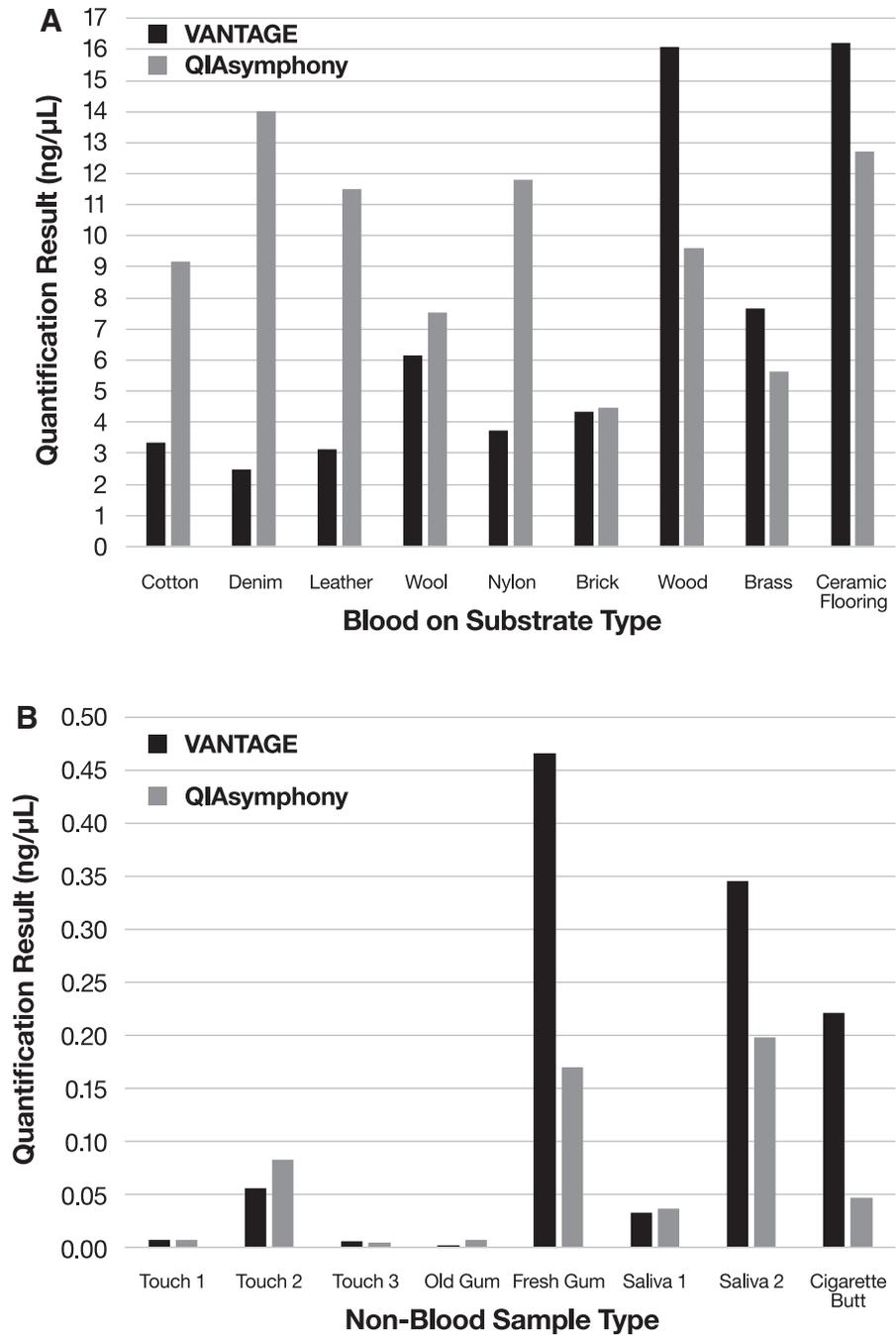
32.43% profile and average profile peak of 49 RFU compared with the sample processed on the QIASymphony that generated a 94.59% profile and average peak profile of 292 RFU. This could be a sample collection error, wherein sample was not collected evenly over the whole swab; meaning that more DNA was present on the swab half that was processed on the QIASymphony.

Percentage complete profile results for all nonblood samples were comparable between instruments and all blood samples generated 100% full profiles. Average pro-

file peak heights for blood samples are depicted in Figure 6A, whereas those for nonblood samples are depicted in Figure 6B. We found that all blood samples had profile balance values between 2 and 3.1. Although a wider balance range was seen in nonblood due to the nature of the samples themselves, results between automated methods were comparable.

All blood samples exhibited heterozygote balance >50% at all heterozygous loci. As with profile balance, an expected wider range of heterozygote balance was observed in nonblood samples; however,

**Fig. 5.** Mock evidence study. Comparative small autosomal data from nondifferential mock evidence samples including (A) blood samples and (B) touch-based samples, liquid saliva, cigarette butts, and chewing gum; quantified using Quantifiler Trio, and amplified using PowerPlex Fusion.



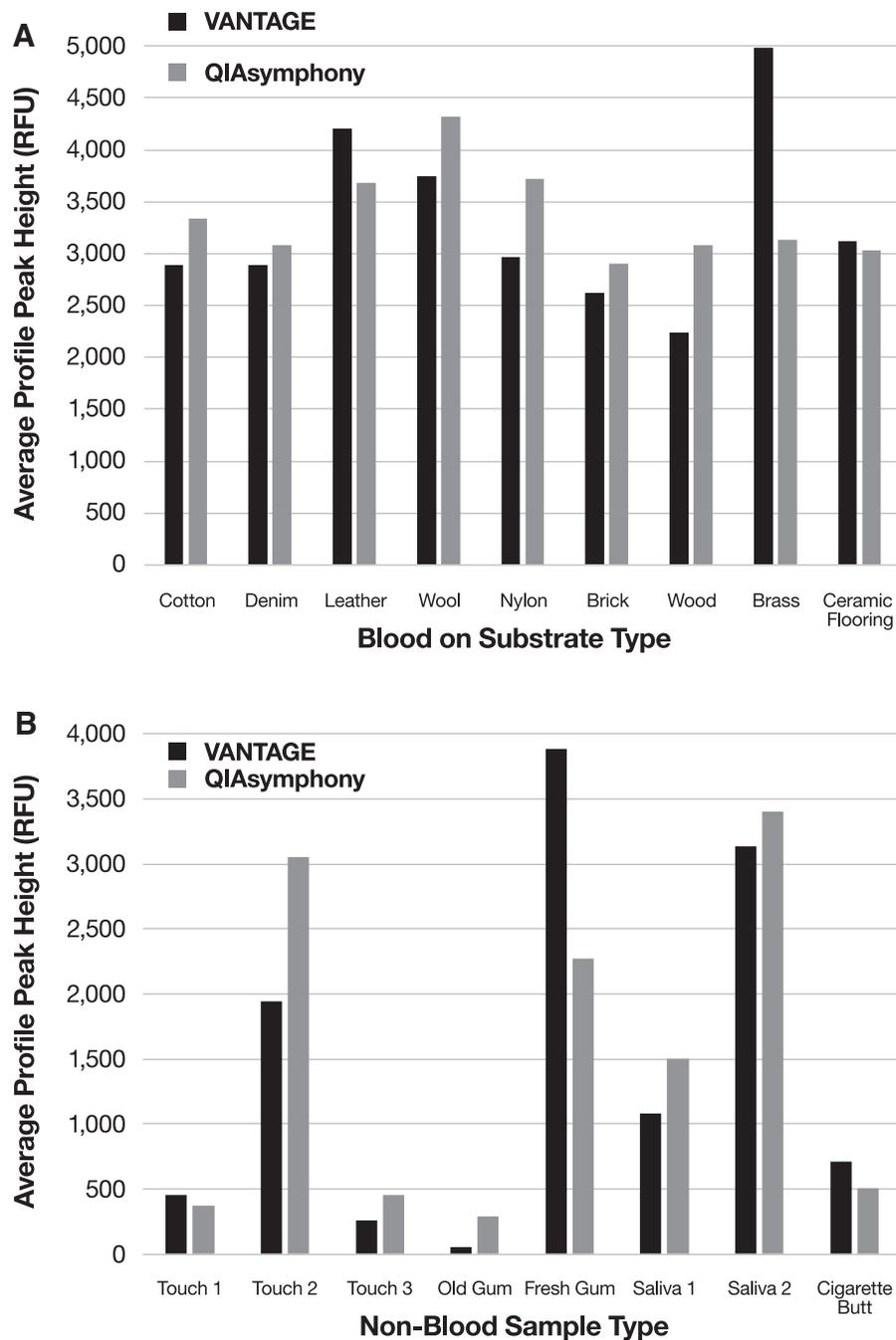
the instances of heterozygote balance <50% and 40% were comparable between the two instruments.

#### Differential mock evidence study

EF quantification results were comparable between samples processed using the VANTAGE and the QIASymphony (data not shown). All EFs produced full and balanced profiles with heterozygote balance >50% and

profile balances ranged between 2 and 5. Average profile peak heights were also comparable between the purification instruments.

When quantifying the SFs, the SA and Y target (Fig. 7) values of samples processed using VANTAGE, with the exception of dilution A, were comparable with values obtained in samples processed using QIASymphony. Quantification results from semen dilutions A–D were 97–100% male, whereas the results fluctuated in dilution



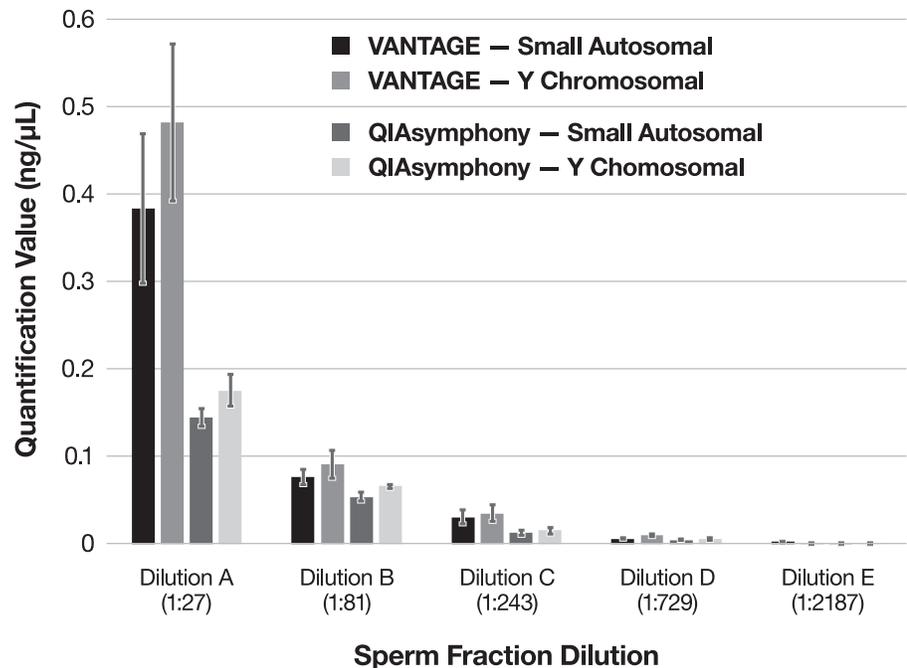
**Fig. 6.** Mock evidence study. Comparative average peak height data from nondifferential mock evidence samples including blood (A) and touch-based samples, liquid saliva, cigarette butts, and chewing gum (B); quantified using Quantifiler Trio, and amplified using PowerPlex Fusion.

E for all variables. This fluctuation is a result of the overall low quantification results from the highly diluted semen sample.

Semen dilutions A–D that were processed using VANTAGE generated full and balanced profiles. In these samples, profile balance ranged from 2 to 5. One dilution D replicate processed through QIASymphony had one allele dropout below analytical threshold and a profile balance of 5.9, indicating a slightly imbalanced profile. Dilutions A–C had zero instances of heterozygote balance

<50% when purified with either instrument. Dilution D samples processed through VANTAGE had four instances of heterozygote balance <50% across four samples, whereas the same dilution processed through QIASymphony had nine instances of heterozygote balance <50% across two samples. Regardless of instrument, dilution E samples contained allele and locus dropout and heterozygote imbalance; the percentage profile was comparable for each purification method. Finally, average profile peak heights for each dilution were

**Fig. 7.** Mock sexual assault evidence study. Comparative sperm fraction quantification results from differentially extracted mock evidence samples including small autosomal values and Y-chromosome target values; quantified using Quantifiler Trio, and amplified using PowerPlex Fusion.



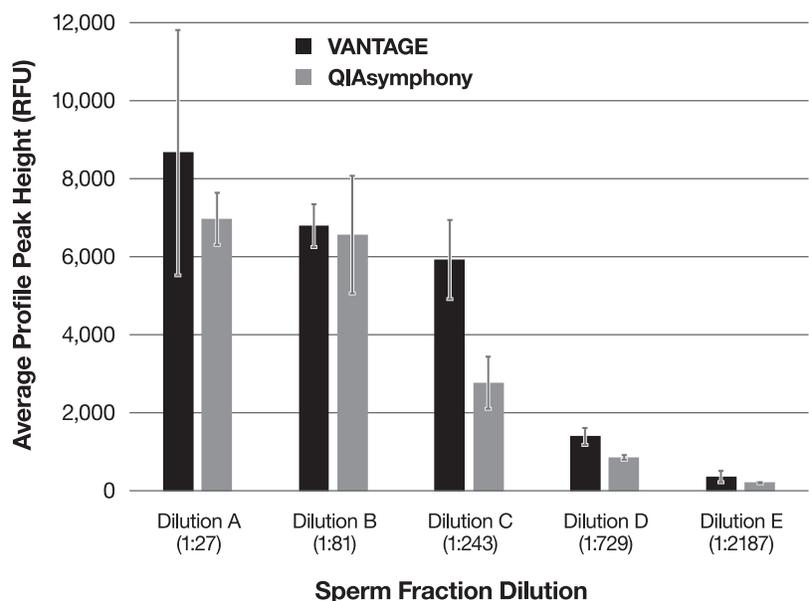
comparable among samples processed using each purification method (Fig. 8).

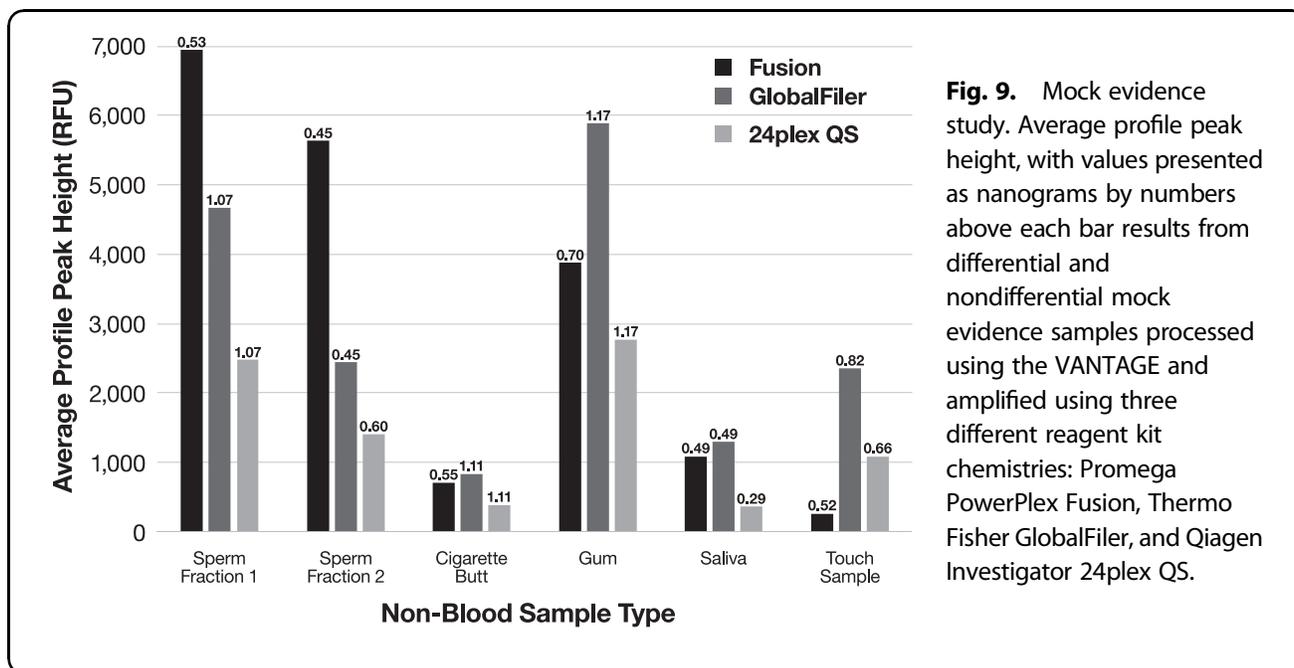
#### Amplification chemistry compatibility

To assess compatibility of DNA extracts from the VANTAGE purification method with additional STR amplification chemistries, a subset of differential and non-differential mock evidence sample extracts were tested across three different amplification chemistries (Fig. 9). The template amount in each reaction varied slightly

due to differences in optimum template amount for each chemistry and available extract volume. All samples that produced full profiles with the PowerPlex Fusion chemistry also generated full profiles with the GlobalFiler and 24plex chemistries. Dropout was observed in the cigarette sample regardless of amplification chemistries. The profile quality (e.g., heterozygote balance and profile balance) was also comparable between all amplification chemistries despite differences in average profile peak height.

**Fig. 8.** Mock sexual assault evidence study. Average profile peak height data from sperm fractions separated from mock sexual assault evidentiary samples; quantified using Quantifiler Trio, and amplified using PowerPlex Fusion.





**Fig. 9.** Mock evidence study. Average profile peak height, with values presented as nanograms by numbers above each bar results from differential and nondifferential mock evidence samples processed using the VANTAGE and amplified using three different reagent kit chemistries: Promega PowerPlex Fusion, Thermo Fisher GlobalFiler, and Qiagen Investigator 24plex QS.

### Contamination assessment study

A total of 63 RBs were processed on the VANTAGE throughout the course of the validation study, 44 of which were processed in a checkerboard pattern with buccal cell lysates. Two RBs generated  $C_T$  values  $<40$  at one of the three Quantifiler Trio targets. These RBs were concentrated, amplified, and were shown to be clean, with no allele peaks above the PowerPlex Fusion peak detection threshold. The remaining unconcentrated RBs processed through the VANTAGE were also clean, with no allele peaks above the detection threshold.

### Conclusions

It has been demonstrated that the Microlab VANTAGE Liquid Handling System for DNA purification using the Lyse&Prep chemistry is suitable for use with forensic evidence and reference samples. Validation data show that the VANTAGE produces results equivalent to those obtained using the QIASymphony workstation, while increasing the efficiency 10-fold. VANTAGE-purified DNA extracts are suitable for and compatible with downstream processing options currently available from major manufacturers (Promega, Thermo Fisher, and Qiagen). Samples purified on the VANTAGE with the Lyse&Prep chemistry generate accurate, sensitive, and reproducible quantification and profile results that are free from contamination.

### Author Disclosure Statement

The authors disclose the following potential conflicts of interest with respect to the research, authorship,

and/or publication of this article: H.S. and K.N. are full-time employees at Bode Technology in their respective positions. Bode Technology provides forensic DNA analysis services, products, and solutions. K.W.P.M. is a full-time employee at Hamilton Company. Hamilton Company manufactures and sells automated liquid handling platforms such as that discussed in this article.

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