Application Note

Improving workflow efficiency with the QIAsymphony® SP Instrument and the Investigator® Lyse&Spin Basket Kit

Markus Pirttimaa[1], Elina Hokkanen[1], Carole Peel[2], Thomas Winkler[2], Richard Newton[2], Anke Prochnow[2] and Keith Elliott[2]

[1] National Bureau of Investigation, Forensic Laboratory, Vantaa, Finland

[2] QIAGEN GmbH, Hilden, Germany

Increased sample submissions to forensic DNA processing laboratories require process optimization of the laboratory workflow to maintain the first pass success rates and turnaround times achieved in lower throughput laboratories. Here we describe how this was achieved at the national Forensic Laboratory in Finland using the QIAsymphony workflow including the Investigator Lyse&Spin Basket Kit.

Introduction

Since the receipt of its first crime samples in 1993 submissions to the National Bureau of Investigation (NBI) Forensic Laboratory for DNA analysis have increased significantly. Today the laboratory processes over 26,000 crime scene samples each year, covering a wide range of evidence types including sexual assault samples.

As throughput has increased the demand for successful analysis of DNA from even the most challenging samples has likewise grown, meaning that any improvements in workflow efficiency were required to demonstrate at least comparable success rates for casework samples.

In 2016 the laboratory implemented a new automated workflow to provide an annual capacity of up to 15,000 samples, providing the highest standardization and process safety possible for the most frequent sample types. This workflow replaced one based on the Tecan liquid handler using ChargeSwitch (CST) magnetic bead chemistry and was required to achieve equal or higher first pass success rates and to provide a simplified method for processing solid substrates (e.g., cotton swabs, cigarette butts, chewing gum) frequently submitted by the police.

Here we describe the new workflow and present validation data comparing the new QIAsymphony workflow with that previously in place using the Tecan platform with ChargeSwitch chemistry.

Investigator Lyse&Spin Basket Kit

QIAGEN's Investigator Lyse&Spin baskets are front-end devices designed to enable the combination of sample lysis and separation of cellular material from solid substrates such as cuttings of clothing, cigarette butts and swabs. The basket retains the lysis buffer during the lysis step of the forensic sample. Upon centrifugation on an ordinary benchtop centrifugation system, holes in the bottom of the basket open up and allow the sample lysate pass through. The lysate containing the nucleic acids is efficiently recovered and collected in the collection tube provided whereas the solid particles remain in the basket.

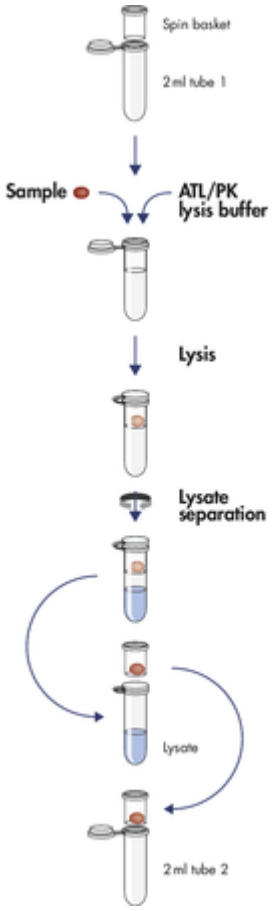

Figure 1. Schematic of the Investigator Lyse&Spin Basket Kit process. Cellular material is effectively lysed and removed from samples comprised of solid substrates, leaving a liquid-only lysate ready for downstream purification.

The Investigator Lyse&Spin baskets are particularly useful for higher throughput laboratories where repeated manual pipetting steps, otherwise required to separate lysate from solid material, are likely to result in human error such as cross-contamination between samples or unsuccessful lysate transfer. The baskets collect the sample lysate in a flip cap collection tube and therefore require a downstream workflow compatible with such tubes.

The QIAsymphony SP Instrument

The QIAsymphony SP is a unique, dedicated medium- to high-throughput sample preparation instrument and was chosen by the NBI Forensic Laboratory based on a number of its key features:

- Flexible sample loading
  - Flexible primary tubes or microplates (24- and 96-well) can be loaded
  - 1–96 samples per run with option of continuous loading in batches of up to 24 samples
- Easy process integration of flip cap tubes from the Investigator Lyse&Spin Basket Kit
- Variable elution volume and elution format (flexile tubes, 24- and 96-well plates)
- Efficient sample tracking
  - Integrated bar code reader
  - Integration with the existing LIMS system
  - Full traceability and run reports
- High process safety to prevent loading errors
  - Integrated loading check
  - Liquid level detection of consumables
- Dedicated ISO18385 Forensic DNA Grade QIAsymphony DNA Investigator Kit consumables
- Seamless workflow: Hardware, chemistry and application support from a single provider

The QIAsymphony workflow

The new workflow implemented at the NBI Forensic Laboratory benefits from streamlined sample introduction on account of the use of QIAGEN's Investigator Lyse&Spin Basket Kit with the flip cap tube insert on the QIAsymphony SP and the QIAsymphony DNA Investigator sample prep chemistry. Downstream processing has remained unchanged.

Through the use of the Investigator Lyse&Spin Basket Kit, a single lysis volume of 450 µl is achieved for most sample types, enabling the lab to streamline the lysis stage of the process into a single method. This method allows batches of 80 samples (including 5 controls) to be used for >95% of samples received (excluding sexual assault samples).

The QIAsymphony Investigator 500 ADV/HE protocol was implemented to enable purification from 450 µl lysate recovered from the Investigator Lyse&Spin baskets and to ensure maximum purification and elution efficiency for challenging casework samples with low levels of DNA. This sample prep protocol includes the heated and prolonged binding of magnetic beads (ADV) and the addition of TOPE fluid (HE) for improved DNA recovery.

The workflow in use at the laboratory includes the EZ1® Advanced XL and Maxwell 16 instruments (Promega Corporation) for serious crime samples (e.g., homicide and assault samples). This enables the laboratory to batch their major crime samples separately, achieving faster turnaround times and greater flexibility with regards to sample pretreatment and batch size for these sample types. The availability of the EZ1 Advanced XL and Maxwell 16 instruments also provides the capability to respond to more urgent samples without disrupting the QIAsymphony line used for the majority of samples.

A custom-made data handling tool was also created for NBI to integrate the new workflow with the existing Lab Vantage LIMS system. This tool ensures that process and sample related data are managed throughout the workflow without manipulation by the end user. The data is available in specified storage locations for integration into the LIMS system. The tool was included within the validation studies performed.

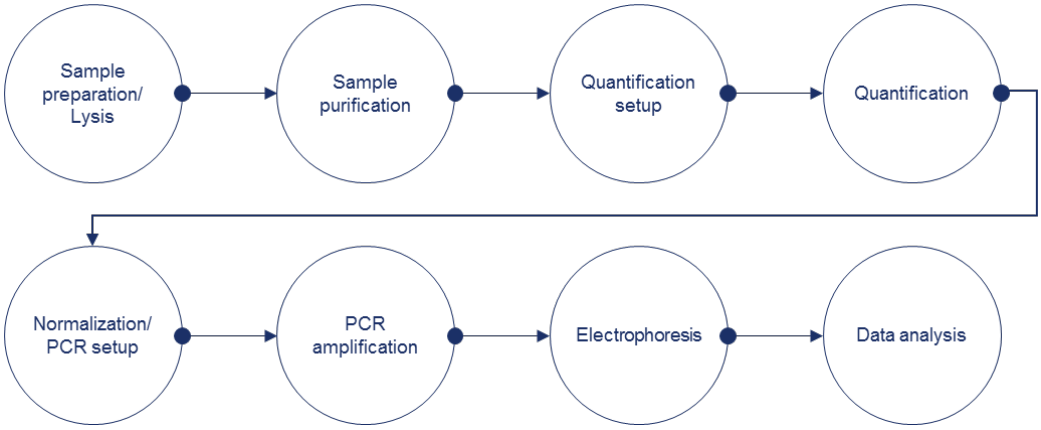

Figure 2. Overview of the crime stain DNA workflow at NBI. The new workflow is unchanged relative to the previous method, with the exception of sample pretreatment and sample purification, which have been streamlined and improved with the introduction of the Investigator Lyse&Spin Basket Kit, QIAsymphony SP Instrument and its dedicated sample prep chemistry (QIAsymphony DNA Investigator Kit). The EZ1 Advanced XL is used for major crime and urgent samples only.

Validation of the new workflow

Table 1 shows an outline of the validation studies conducted by NBI on the new Investigator Lyse&Spin Basket Kit and QIAsymphony SP workflow. Results were compared with the

Tecan/ChargeSwitch and Maxwell 16 workflows to ensure equal or improved performance over the two incumbent methods. In addition results for some studies were compared with the EZ1 Advanced XL platform where this system was intended to be used on relevant casework samples (e.g., casework sample comparison study).

Table 1. Summary of samples analyzed during the validation

Validation study	Study description	Sample type
Sensitivity, repeatability and reproducibility	Blood – 5 dilution steps x 6 replicates x 2 days (total 60 samples)	Blood on swabs
	Saliva – 5 dilution steps x 6 replicates x 2 days (total 60 samples)	Saliva on swabs
Contamination	Checkerboard – 48 bloods and 48 negative controls in alternating positions x 2 replicate plates (total 192 samples)	Blood/NTCs
Inhibition	30 blood samples spiked with hematin/humic acid	Blood on swabs
Casework-type samples	10 casework sample types (total 76 samples, no replicates)	• 10 contact trace swab samples • 10 blood stains on fabric • 10 cigarette butts • 10 saliva swab samples from bottles • 10 hair roots • 10 semen samples on swabs / fabric • 5 chewing gum samples with xylitol • 5 chewing gum samples without xylitol • 3 pools nail scrapings • 3 pools cut nails

Results

Sensitivity and linearity

The sensitivity study assessed the range of sample inputs able to produce reliable genotyping results. Linearity assessed the correlation of DNA recovery (yield) to sample input. Blood and saliva samples were prepared in fivefold dilution series. Replicates of each dilution were pipetted onto cotton swabs and allowed to dry. Six replicates of each dilution were extracted twice on different days by different operators. The Investigator Lyse&Spin Basket Kit handbook describes a range of vortex speed from 750 to 900 rpm. In this study 900 rpm (Protocol A) as well as 750 rpm (Protocol B) were tested.

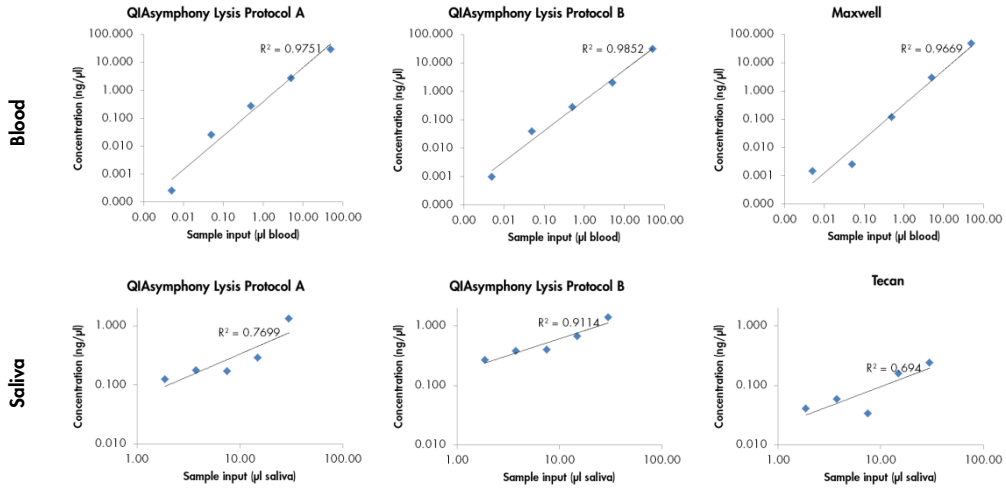

Figure 3. Blood and saliva dilution series. Quantification results are shown using QIAsymphony sample preparation with either lysis protocol A or protocol B, or an existing method (Maxwell for blood samples and CST extraction on Tecan platform for saliva samples). Each dilution point shows an average quantification result from six replicates.

Figure 3 shows results for linearity. Both shaking conditions (Protocol A and Protocol B) showed good correlation between DNA input and DNA yields, and higher than for the previous extraction methods (Maxwell and CST/Tecan extraction). When the DNA amount of the extracted sample was high enough for optimum DNA input to PCR reaction (350 pg of DNA at 35 pg/µl concentration), all alleles were typically recovered. This was true down to 0.05 µl of blood per sample. With the Maxwell and CST/Tecan extraction method, samples with 7.5 µl or less of saliva occasionally produced partial profiles, but with both QIAsymphony protocols, samples down to 1.875 µl of saliva produced full profiles.

Repeatability and reproducibility

The repeatability study assessed the variation in measurements when one person processed the same sample with the same equipment. Six sample replicates from the sensitivity study were extracted to assess repeatability, with the resulting DNA yield from each sample compared.

The QIAsymphony workflow demonstrated relative standard deviation (%CV) within ±35% with a sample input of ≥0.5 µl of blood and with all saliva inputs between 1.875 µl and 15 µl (Table 2). This compares favorably with the incumbent Tecan or Maxwell methods for which the standard deviation was within ±35% for blood samples with 50 µl input (Maxwell extraction) and for saliva samples with 15–30 µl (Table 2). These results demonstrate that a higher level of reproducibility is achieved with the new QIAsymphony workflow.

Table 2. Repeatability and reproducibility for QIAsymphony and Tecan/Maxwell workflows

		QIAsymphony/Investigator Lyse&Spin			Maxwell (blood)/Tecan (saliva)		
Sample	**Input (µl)**	**Mean (ng/µl)**	**SD**	**%CV**	**Mean (ng/µl)**	**SD**	**%CV**
Blood	50	30.5441	3.0315	9.9	47.4269	10.6411	22.4
Blood	5	2.0254	0.3218	15.9	2.9774	1.8844	63.3
Blood	0.5	0.2740	0.0579	21.1	0.1201	0.0657	54.7
Blood	0.05	0.0395	0.0239	60.5	0.0026	0.0017	64.8
Blood	0.005	0.0010	0.0011	120	0.0015	0.0026	175.1
Saliva	30	1.3859	0.5129	37	0.2404	0.0494	20.6
Saliva	15	0.6664	0.1034	15.5	0.1574	0.0236	15
Saliva	7.5	0.4007	0.0893	22.3	0.0338	0.0166	49.2
Saliva	3.75	0.3755	0.0216	5.7	0.0591	0.0249	42.2
Saliva	1.875	0.2673	0.0383	14.3	0.0408	0.0178	43.7

Reproducibility (comparison of results from different operators) was also evaluated in the study. The difference between mean yields was <50% on batches run on different days with sample input of ≥0.005 µl of blood and ≥6.25 µl of saliva (data not shown).

Contamination

To demonstrate the absence of contamination events that would compromise the integrity of results a contamination study was done. Two full batches (2 x 96) of 50 µl neat blood samples (from six known donors) and negative controls (empty tubes) were processed in a checkerboard pattern using Protocol A (higher vortex speeds than Protocol B and therefore potentially more susceptible to tube leakage). Sample positions were reversed on the second plate. In addition one full batch (96 samples) of blood samples (from six known donors, cotton swabs dipped on blood and dried, later placed on Investigator Lyse&Spin baskets) and negative controls (clean cotton swabs placed

on Investigator Lyse&Spin baskets) were processed in a checkerboard pattern. All samples and controls were processed through to quantification and STR result.

All negative controls from the three batches (3 x 48 samples) gave zero quantification results, meaning that the C_T values were not below 40. For STR results, some rare instances of alleles in negative controls were observed but these were neither above the analytical threshold in the read region nor replicable and were found to be more likely to have occurred due to contamination of capillary electrophoresis. There was no indication of cross contamination between any samples processed on the QIAsymphony SP Instrument.

Inhibition

Inhibition was evaluated to determine the effectiveness of the extraction system in removing inhibitors typically found in forensic exhibits from the sample material. 30 µl of 1:10 diluted blood sample from a known donor was pipetted onto several cotton swabs. These were spiked with hematin (3 replicates each: 500 µM, 250 µM, and 125 µM) or with humic acid (3 replicates each: 20, 50, or 100 ng/µl). After purification, inhibitor removal was assessed by visual inspection of the eluates and by quantification and STR analysis.

All extracted samples were clear, and no visible inhibitor was detected. C_T values were comparable with the negative controls and quantification standards (Table 3). Also STR profiles gave balanced results without reduction in peak heights at the high molecular weight markers (data not shown). The QIAsymphony chemistry is therefore effective at the removal of commonly encountered PCR inhibitors.

Table 3. C_T values for samples spiked with known PCR inhibitors: C_T values are similar to the negative control (sample with no inhibitor added)

Sample		Average C_T	Maximum C_T
Standards		32.15	33.01
Humic acid	0 ng/µl [NO INHIBITOR]	32.65	33.50
	20 ng/µl	32.18	32.47
	50 ng/µl	32.19	32.29
	100 ng/µl	32.28	32.37
Hematin	0 µM [NO INHIBITOR]	31.90	32.03
	125 µM	32.07	32.31
	250 µM	32.01	32.12
	500 µM	32.24	32.52
Maximum C_T for samples/standards without inhibitor:			**33.50**
Maximum C_T for samples with inhibitor:			**32.52**

Casework samples

To ensure that the installed protocols would be effective on real casework samples, the influence of different cell types and carrier substrates on the quality and quantity of recovered DNA were evaluated. The samples described in Table 1 were processed using all four methods under consideration (the Tecan and Maxwell incumbent methods and the QIAsymphony and EZ1 methods). Quantification and STR results for all methods were compared.

Overall all four methods performed comparably, with the QIAsymphony typically in the top two methods for all sample types and conditions tested. A typical result is shown for the epithelial cell samples (contact trace swabs) analyzed in Figure 4.

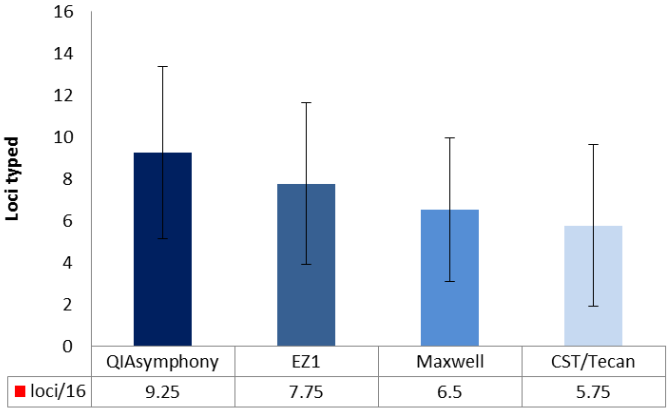

Figure 4. Comparison of STR profiling results for the four sample preparation methods evaluated. Results are shown for contact trace swabs (n = 10 for QIAsymphony, 6 for EZ1, 6 for Maxwell and 6 for CST/Tecan) with the number of loci typed for the major profile shown.

Discussion and conclusion

In the NBI laboratory, changes to the DNA casework workflow enabled throughput to be doubled from 7,500 to 15,000 samples per year, due primarily to the hands-free nature of QIAsymphony sample preparation. The QIAsymphony SP Instrument, together with QIAsymphony DNA Investigator chemistry and Investigator Lyse&Spin baskets, has been shown to be an effective, reliable and safe extraction system for various types of crime scene samples. Extraction efficiency is at least comparable with the methods used in lower throughput workflows (e.g., EZ1 Advanced XL), enabling higher throughput to be achieved with no compromise on DNA recovery or quality. Furthermore, the Investigator Lyse&Spin Basket Kit enables the contamination-prone removal of solid substrates to be avoided after cell lysis.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAsymphony SP System	QIAsymphony sample prep module: includes installation and training, 1-year warranty on parts and labor	9001751
QIAsymphony Cabinet SP	Accessory for correct positioning of the QIAsymphony SP instrument	9020244
QIAsymphony DNA Investigator Kit (192)	For 192 preps of 200 µl each from casework and reference samples: includes 2 reagent cartridges and enzyme racks and accessories	931436
Investigator Lyse&Spin Basket Kit (50)	50 pouches containing 50 baskets and 100 collection tubes	19597
Investigator Lyse&Spin Basket Kit (250)	10 pouches containing 5 x 50 baskets and 5 x 50 collection tubes	19598
EZ1 Advanced XL, System	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits: includes installation, training, 1-year warranty on parts and labor	9001874
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridge (DNA Investigator), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K, Carrier RNA	952034

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Learn more about our human identity and forensic testing solutions for confidence in your evidence at www.qiagen.com/forensics

1106233 02/2017