Application Note

QIAGEN's DNA Investigator® chemistry on the Hamilton® Microlab® STAR™ Liquid Handling Workstation

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Here, we describe the successful application of DNA Investigator chemistry for use with the Hamilton Microlab STAR, as part of an improved automated crime stain workflow. Data from the analysis of live crime samples demonstrates that a highly efficient workflow can be implemented using this application, enabling a decrease in turnaround time from 7 to 1.5 days and an increase in capacity from 224 to 760 samples per month.

Introduction

QIAGEN® magnetic bead chemistry has been established as a gold standard for reproducible purification of high-quality DNA from challenging forensic samples, and is validated on the QIAsymphony® SP (sample purification). The corresponding DNA Investigator Kit is dedicated to forensic samples and has been adapted for use on the Hamilton Microlab STAR. Here we describe the successful application of the DNA Investigator Kit to the Hamilton Microlab STAR at Forensic Science Ireland (FSI). FSI is the national forensic DNA profiling laboratory for the Republic of Ireland, and typically processes around 5,600 crime samples annually, which are divided equally into volume crime and serious crime. Prior to the implementation of the process described, volume and serious crime samples were processed together, with volume reporting scientists rotating in a "processing team" for 7 days and manually processing (extraction, amplification, and CE injection) all volume and serious crime stain samples. Capacity limits on the processing team caused a 3.5 day backlog before sample processing began, with processing taking an average of 3.5 days. Results were therefore typically available 7 days after submission to the processing team. In May 2014, FSI implemented an automated Core Processing Facility for volume (V) and serious crime (SC) samples. This facility provides extraction (V), quantification setup (V & SC), amplification setup (V & SC), and CE injection setup (V & SC) of crime stain samples with volume and serious crime samples being automatically separated at the CE injection setup stage, allowing run analysis to be carried out by separate teams. ▷

The objectives for this change in process were to enable volume and serious crime samples to be processed in parallel, increase efficiency, decrease turnaround times, reduce delays, and release reporting scientists from manual processing by transferring the operation of the automated systems to analysts. The Core Processing Facility, which uses QIAGEN chemistry to extract all volume samples on the Hamilton Microlab STAR, was integrated with the semi-automated EZ1® Advanced XL instrument for extraction of serious crime samples. The Investigator Lyse&Spin Basket Kit was used to streamline the process further.

Process overview prior to the Core Processing Facility

Prior to implementation of the Core Processing Facility, volume and serious crime samples were processed together at all stages using QIAamp® DNA Investigator Kit spin columns on the QIAcube® (Figure 1).

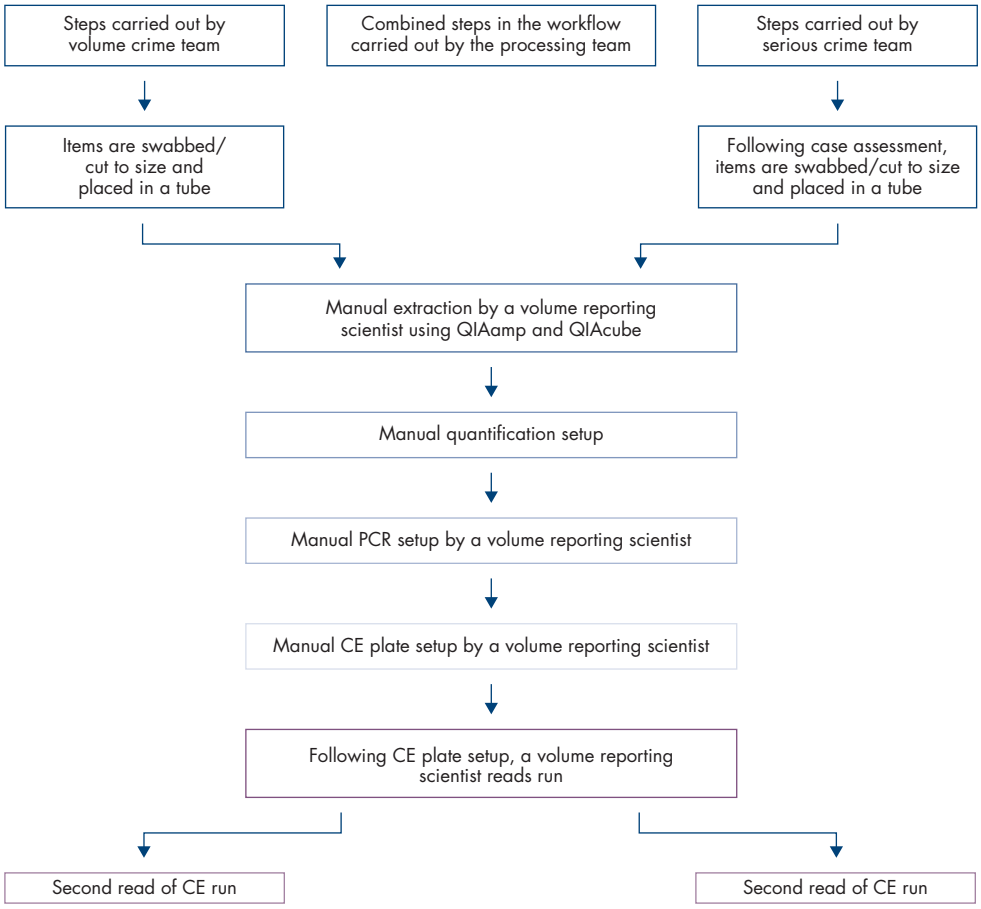

Figure 1. DNA workflow for serious and volume crime samples at FSI, prior to the implementation of the Core Processing Facility.

Reporting scientists completed all manual processing steps with the exception of quantification, which was carried out manually by DNA analysts. To achieve this, volume scientists were scheduled to work 7 consecutive days on the processing team every 8 weeks. Outside of the processing team rotation, volume scientists prepared and submitted their casework samples to the processing team and issued casework reports. Serious crime scientists processed all other serious crime samples not carried out by the processing team due to capacity limits. The processing team could handle approximately half of all crime stain samples in the laboratory.

Overall, the rota maximized the use of laboratory space and allowed regular assignment of caseloads to volume scientists. This allowed urgent cases to be assigned immediately to individuals as part of a batch, minimizing disruptions. The implementation of the rota also led to the removal of 1.4 scientists from reporting duties over 1 working week, and on average 56 samples were processed (approximately 2800 samples annually). The time from submission of a sample to a DNA profile result was 7 days (Table 1).

Table 1. The 7-day processing rota for two reporting scientists and the first 2 days for a third scientist

		Volume scientist 1	Volume scientist 2	Volume scientist 3
Mon	AM	Extraction (2 x QIAcube)		
	PM	Quantification (analyst)		
Tues	AM	Amplification		
	PM	CE injection		
Wed	AM	Extraction (2 x QIAcube)		
	PM	Quantification (analyst)		
	PM	First analysis of CE run		
Thur	AM	Amplification		
	PM	CE injection		
Fri	AM	Extraction (2 x QIAcube)		
	PM	Quantification (analyst)		
	PM	First analysis of CE run		
Mon	AM	Amplification	Extraction (2 x QIAcube)	
	PM	CE injection	Quantification (analyst)	
Tues	AM	First analysis of CE run	Amplification	
	PM		CE injection	
Wed	AM		Extraction (2 x QIAcube)	
	PM		Quantification (analyst)	
	PM		First analysis of CE run	
Thur	AM		Amplification	
	PM		CE injection	
Fri	AM		Extraction (2 x QIAcube)	
	PM		Quantification (analyst)	
	PM		First analysis of CE run	
Mon	AM		Amplification	Extraction (2 x QIAcube)
	PM		CE injection	Quantification (analyst)
Tues	AM		First analysis of CE run	Amplification
	PM			CE injection

Process overview following implementation of the Core Processing Facility

Between August 2012 and March 2014, both the volume crime and serious crime lines were revised to increase efficiency across the laboratory.

Changes to extraction processes

EZ1 Advanced XL instruments were introduced for extraction of serious crime samples in conjunction with the Investigator Lyse&Spin Basket Kit for efficient transfer of extracts from swabs and other absorbent samples. The Hamilton Microlab STAR, using QIAGEN DNA Investigator chemistry for lysis and purification, was introduced for extraction of volume crime samples. QIAGEN DNA Investigator chemistry provides maximal recovery of high-quality DNA from challenging casework samples. Reporting scientists retain responsibility for extraction of serious crime samples using the EZ1 Advanced XL.

Automation of quantification, amplification, and CE setup

Both volume and serious crime stain samples were processed on the DNA analyst-operated Hamilton Microlab STAR (i.e., quantitative real-time PCR setup including standard curve preparation and amplification and re-amplification setup). For post-PCR liquid handling, a Hamilton Microlab STARlet performed CE setup. Volume and serious crime samples were combined into batches for quantification and amplification, but separated at the CE setup stage, with volume samples being run on separate plates to serious crime samples. In addition, FSI introduced new analysts into the DNA workflow, giving them responsibility for lysis and purification of volume crime samples using the Hamilton Microlab STAR, as well as for all subsequent steps in both volume and serious crime workflows (Figure 2).

FSI implemented an automated and highly efficient Core Processing Facility for volume crime samples, and in parallel, improved serious crime workflows using the EZ1 Advanced XL. By implementing QIAGEN DNA Investigator chemistry as part of this improved workflow, FSI have maintained the high success rates previously achieved with the QIAamp manual, and QIAcube semi-automated workflows. The revised workflow also enabled a more efficient division of labor between reporting scientists and analysts, empowering analysts to complete all laboratory steps after sample preparation for volume crime samples and the majority of steps for serious crime samples. This increased the reporting scientist's capacity for case assessment, sample preparation, and reporting, thus removing the bottlenecks at these stages. Processing times were reduced from 7 to 1.5 days with significantly reduced manual intervention. As a consequence of these improvements, the current throughput at FSI is approximately 380 volume crime samples per month, with a capacity of up to 760 samples per month. Approximately 340 serious crime samples are processed monthly with significantly more capacity available, if required. The transfer of serious crime sample extraction to the serious crime reporting scientists, using EZ1 Advanced XL instruments, introduces more flexibility when processing challenging serious crime samples.

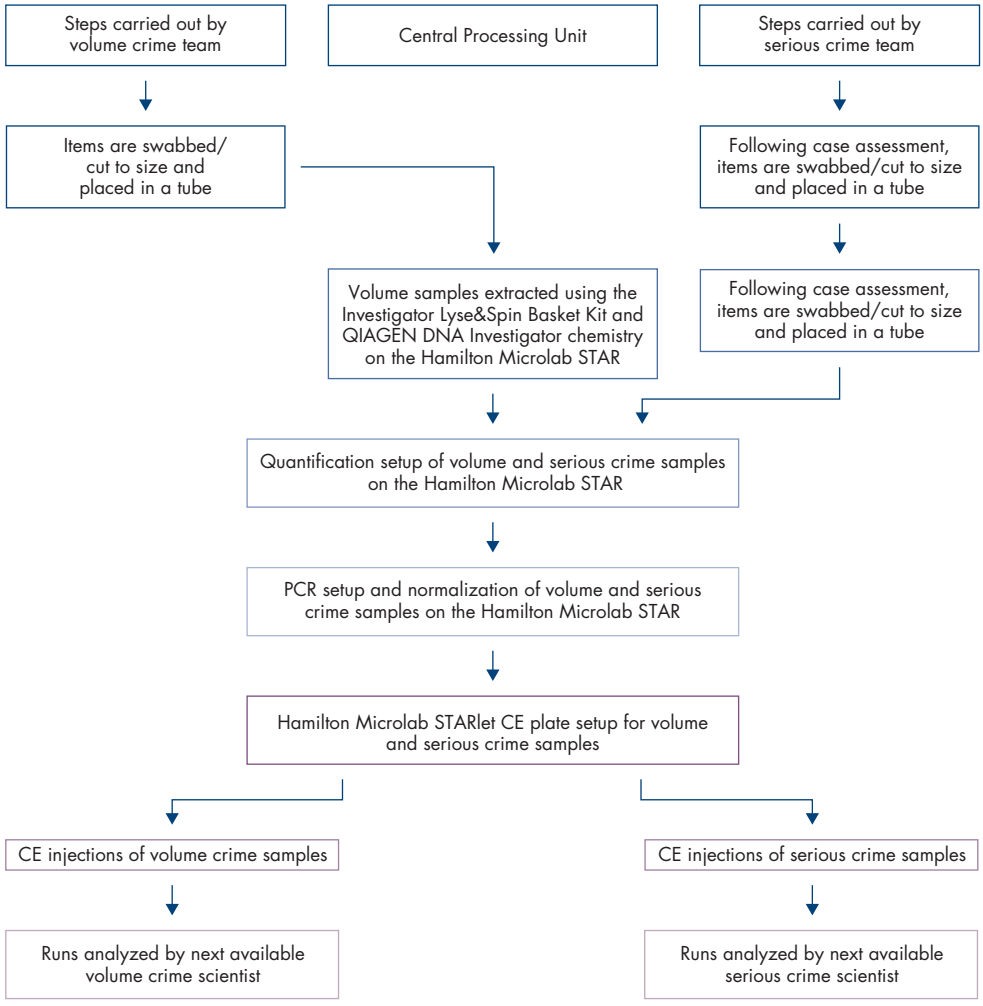

Figure 2. DNA workflow following introduction of the Core Processing Facility and EZ1 Advanced XL.

Conclusion

The introduction of the Core Processing Facility at FSI allowed significant increases in efficiency due to a number of key changes:

- Use of pre-optimized protocols for QIAGEN chemistry with the Hamilton Microlab STAR for easy development and validation
- Combined quantification and PCR setup for improved throughput and turnaround times
- Use of QIAGEN chemistry for serious and volume crime lines for improved overall laboratory workflow and downstream compatibility
- Implementation of an automated workflow that enables dedicated analysts to carry out most of the workflow steps
- Clearly defined responsibilities for effective focus of resources and improved capacity
- Improved batching of samples to allow more runs to be started (5 rather than 3 runs per week for volume crime samples)
- Implementation of the Investigator Lyse&Spin Basket Kit for a reduced number of transfer steps and associated witnessing
- 70% increase in volume crime sample throughput, with capacity increased by 340%

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