ApplicationNote

High-throughput DNA sample prep using the QIAsymphony® SP instrument: An overview of workflow optimization at Bode Cellmark Forensics™

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We describe Bode Cellmark Forensics (Bode Cellmark) workflow optimization, an established best practice, in achieving the highest possible throughput, with the QIAsymphony SP platform. The final optimized workflow enables over 500 casework samples to be processed each day using two QIAsymphony SP instruments. This is achieved while maintaining the high success rates demanded of crucial casework samples.

Introduction

Bode Cellmark has grown to become one of the leading HID DNA testing laboratories in the US, with an international reputation for high-throughput, high-quality analysis of casework samples. Bode Cellmark has an average annual volume of around 50,000 casework extracts from a wide range of challenging samples, including touch DNA and mixed stains. An overview of the types of samples submitted each year to Bode Cellmark can be seen in Figure 1.

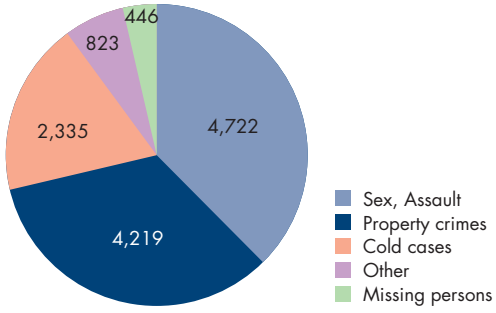

Figure 1. Casework exhibits submitted to Bode Cellmark in 2013. The 12,545 cases resulted in around 50,000 extracts requiring processing.

High-throughput DNA processing workflows for reference samples, such as buccal collection from arrestees, are now widespread. However, implementation of such high-throughput processing for casework samples entails a number of specific challenges which must be overcome. These include achieving yields of high-quality, inhibitor-free DNA, comparable with more labor-intensive manual methods. In addition, a single, robust protocol, and a workflow with a minimal number of manual handling steps, must be identified for all sample types.

Aligning increasing case work sample submissions with increasing automation

Bode Cellmark has tackled this challenge repeatedly, which has increased its annual submissions from hundreds to tens of thousands of samples, over the last twenty years. Starting with organic extraction using phenol-chloroform, Bode Cellmark has subsequently employed QIAGEN automation in three rounds of process improvement, to maintain and improve performance, as demands continue to increase. A summary of this workflow evolution is shown in Figure 2.

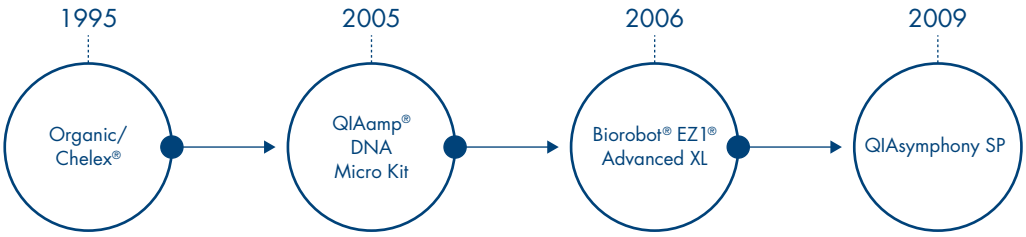

Figure 2. Bode Cellmark's workflow has progressed from organic extraction through a number of QIAGEN sample technologies, to a QIAsymphony SP instrument in 2009, which enabled high-throughput sample processing.

This application note focuses on Bode Cellmark's most recent process optimization, which comprised a detailed analysis of the QIAsymphony SP instrument workflow. The end result was a thorough understanding of this workflow, enabling a highly efficient and streamlined laboratory process to be implemented, which achieved all project objectives with regards to success rates and throughput.

Benefits of the QIAsymphony SP instrument

The QIAsymphony SP instrument was chosen by Bode Cellmark for a number of its key features and benefits:

- Deck flexibility – tube carrier, 24-well plates, 96-well plates
- Throughput – up to 96 samples per run
- Simple, robust 3-step process — bind, wash, elute
- Process integration for maximum process safety and contamination prevention
- Optimized and dedicated chemistry for maximum recovery of high-quality DNA

Project overview

Bode Cellmark's first step was to ensure overall performance of the QIAsymphony SP instrument was comparable with previous lower-throughput solutions. In order to implement a process that is as efficient as possible, Bode Cellmark characterized in detail each stage of the QIAsymphony SP instrument workflow, from sample input to elution output. This included lysis and elution conditions and a number of studies focusing on sample storage and stability.

1. DNA yield for QIAsymphony SP instrument, compared to other QIAGEN sample technologies and organic extraction.

Figure 3 shows the performance of different QIAGEN sample prep solutions with dilutions of blood. The range of dilutions was such that the series represented levels of DNA typically encountered with demanding casework samples.

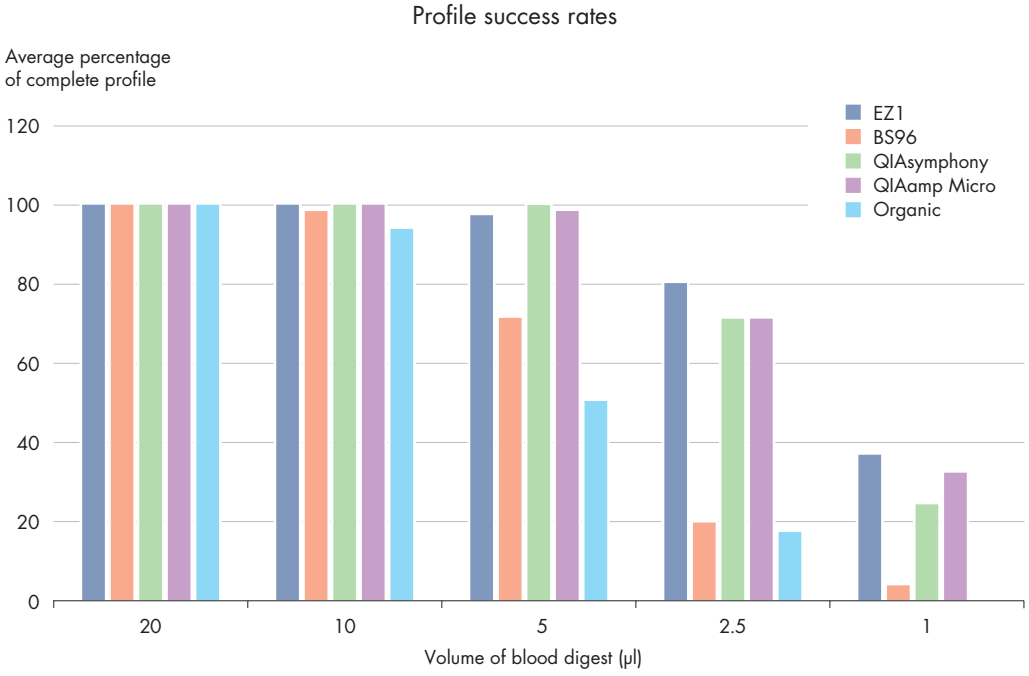

Figure 3. Performance of QIAGEN sample technologies compared to organic extraction with 5 blood dilutions extracted in triplicate. All samples were eluted into 50 µl volumes, except BS96-extracted samples, which were eluted into 75 µl volumes. All samples were processed according to Bode Cellmark's standard operating procedures: EZ1 Trace, QIAsymphony 200 µl, BioSprint® (BS) 96 reduced volume, QIAamp (QIAamp Micro) spin column purification and organic (Phenol/Chloroform/Isoamyl Alcohol [PCIA]). All extracts were quantified and amplified with commercially available kits.

All QIAGEN sample prep solutions significantly outperformed organic extraction methods. For the range of 5 µl – 1 µl blood, representing the more challenging samples containing very low levels of DNA, QIAsymphony performed comparably with EZ1 and QIAamp spin columns (the previous methods in use at Bode Cellmark). This result demonstrated that the high-throughput QIAsymphony platform could be implemented without any loss of performance, even for the most challenging samples.

2. Lysis volumes

To ensure high recovery of DNA, it is important that samples are fully immersed in buffer during sample lysis, prior to purification on the QIAsymphony SP instrument. However, this need for sufficient volume of lysis buffer must be balanced against the need to avoid excess buffer, which can result in dilution of the DNA and lower recovery levels in the final eluate. Figure 4 shows a comparison of three lysis volumes over a range of sample concentrations. The more concentrated samples, produced using the lower lysis volume of 200 µl, were demonstrated to produce better results, as measured by DNA profile peak heights. Based on these results, Bode Cellmark established a lysis volume of 200 µl as part of its QIAsymphony SP instrument workflow.

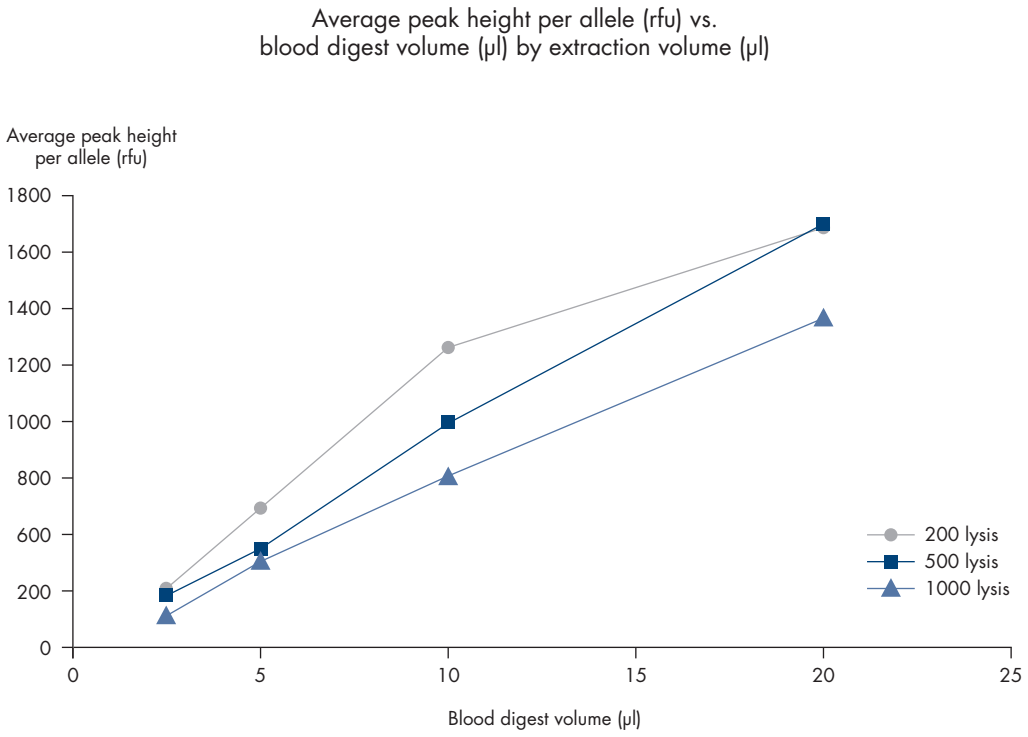

Figure 4. Peak heights of DNA profiles produced from DNA samples lysed using three lysis volumes. Blood dilution series were extracted in triplicate then quantified and amplified with commercially available kits. It was determined that 200 µl was the most effective lysis volume for blood.

3. Elution conditions

As with lysis conditions, the volume of elution buffer must be carefully optimized to ensure the best possible results. Excess elution buffer will dilute the DNA extract, reducing the concentration of DNA available for PCR. Conversely, insufficient elution buffer will result in poor recovery of DNA in the elution column.

Bode Cellmark also reviewed the impact of storing elution plates on the QIAsymphony SP platform overnight, to aid in subsequent process optimization. If plates could be left on the platform overnight once a run was complete, this would enable runs to be initiated at the end of a working day, and the samples to be removed the next morning.

Figure 5 compares 50 µl and 30 µl elution volumes across three 'on-instrument' storage scenarios: (1) plates removed immediately after each run; (2) plates left overnight on the instrument, in a cooling block at 4°C; (3) plates left overnight on the instrument, at room temperature. The impact of these storage conditions on eluate evaporation was evaluated. Results demonstrated that significant evaporation occurred in plates left overnight at room temperature, but that storage overnight using a cooling block significantly reduced this evaporation. Removal of the plates immediately upon run completion was found to be optimal.

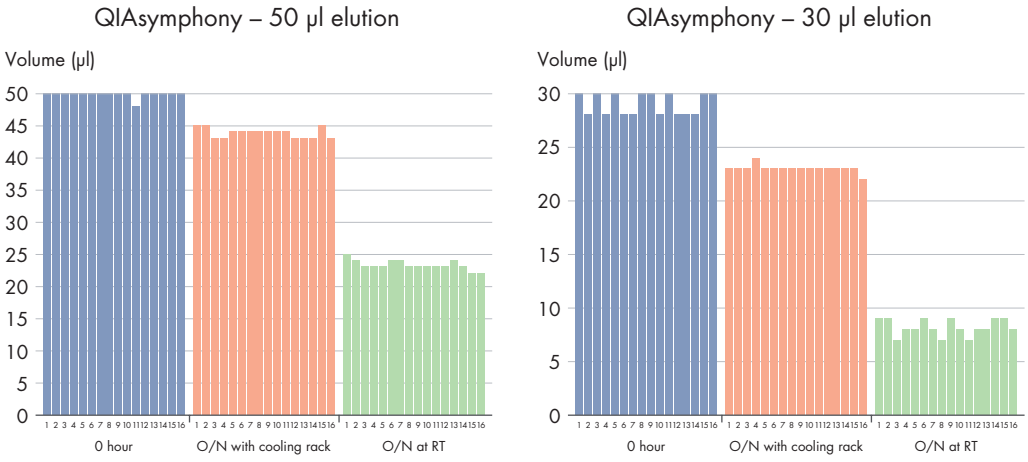

Figure 5. Impact of overnight storage in the instrument on eluate evaporation. The volume (µl) of sixteen samples eluted in 50 µl and 30 µl were measured by a pipet immediately following elution, >12 hours on a cooling rack post-elution, and >12 hours post-elution without a cooling rack. Significant evaporation was experienced with both 50 µl and 30 µl eluates when the elution plates were left on the instrument >12 hours post-elution. Evaporation decreased with the use of a cooling rack.

Figure 6 compares the performance of 50 µl and 30 µl elution volumes with respect to overall yield and profile peak height of DNA profiles. The DNA concentration and profile signal strength produced from samples eluted in 30 µl was higher than the same sample eluted in 50 µl, although the overall yield was generally lower for the 30 µl elution.

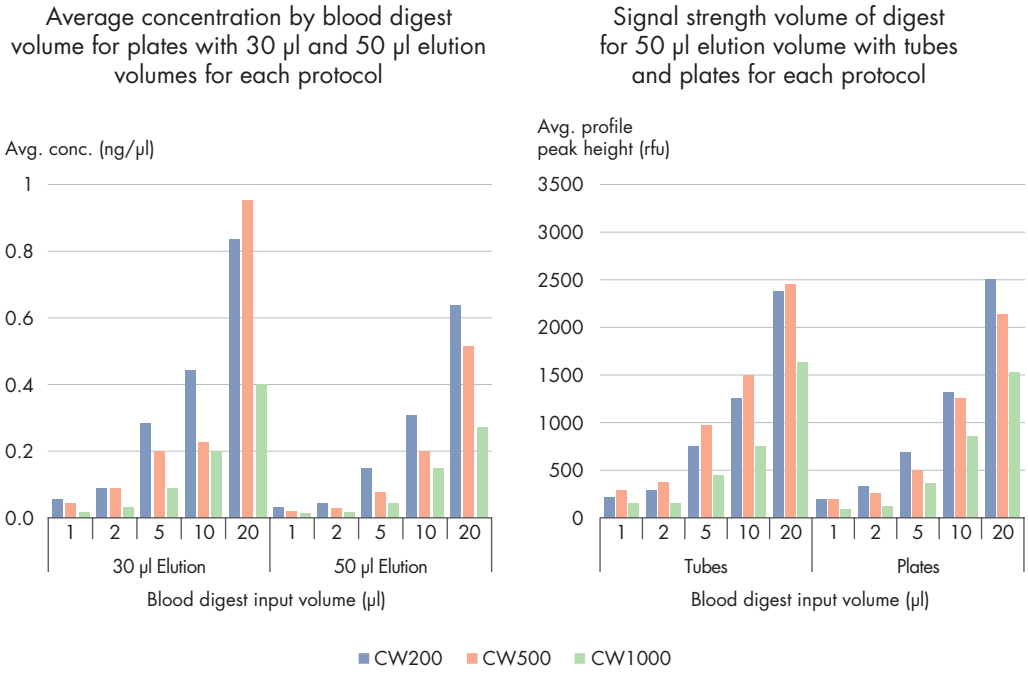

Figure 6. Comparison of 50 µl and 30 µl elution volumes on average concentration and average profile peak height. Replicates of each lysate volume dilution (20 µl, 10 µl, 5 µl, 2 µl, 1 µl and 0 µl) were extracted with high-efficiency (HE) protocols in 200 µl, 500 µl and 1000 µl lysis. Samples were quantified with Bode Cellmark's internally developed BodeQuant LCN and amplified with a commercially available kit.

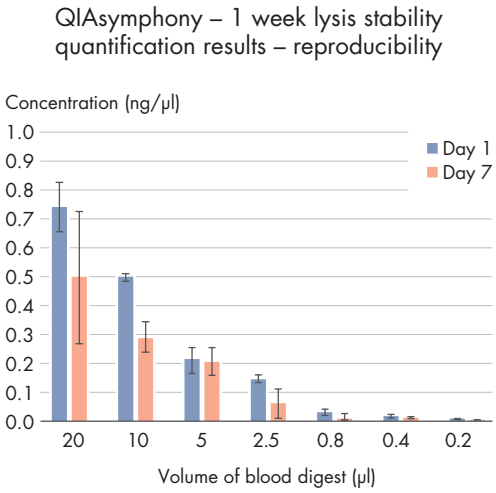

Figure 7. Concentration of DNA recovered from samples stored for 1 day and 7 days at 4°C.

4. Stability studies

A final study was conducted to determine the impact of storing sample lysate prior to processing on the QIAsymphony SP instrument. The ability to store lysed samples would enable a more streamlined process and more flexibility with regards to sample batching, however, prolonged storage in lysis buffer can potentially damage DNA. Figure 7 shows the impact of prolonged storage of DNA lysate at 4°C, and demonstrates the significant reduction in amplifiable DNA recovered. As a result, storage of lysates was restricted to no more than one to three days at Bode Cellmark, as this duration showed a minimal impact on the quality of DNA recovered (data not shown). If unavoidable, samples that have been stored for up to one week at 4°C after lysis can still be extracted and used to generate accurate profile results.

Workflow optimization summary

In the Bode Cellmark laboratory, the following conditions gave good results and enabled the subsequent introduction of a streamlined workflow:

- 200 µl lysis volume
- 30 µl elution volume
- Lysed samples can be kept at 4°C for up to 3 days
- Remove samples from instrument shortly after run completion to prevent volume loss
- Use cooling block to reduce evaporation, if samples are left on the instrument overnight

Bode Cellmark's optimized QIAsymphony SP instrument workflow

Applying results from the validation studies, Bode Cellmark introduced an optimized QIAsymphony SP instrument workflow for high-throughput processing of casework samples in 2009. This workflow, which is shown in Figure 8, enables 528 samples to be processed over six 96-sample trays each day, using two QIAsymphony SP instruments. This requires only two operators and a 14-hour overlapping working day. Compared to previous methods to achieve the same sample volume throughput, 9 scientists were each required to extract 60 samples/day with the QIAamp kit, and 38 BioRobot EZ1 Advanced XL runs would need to be completed.

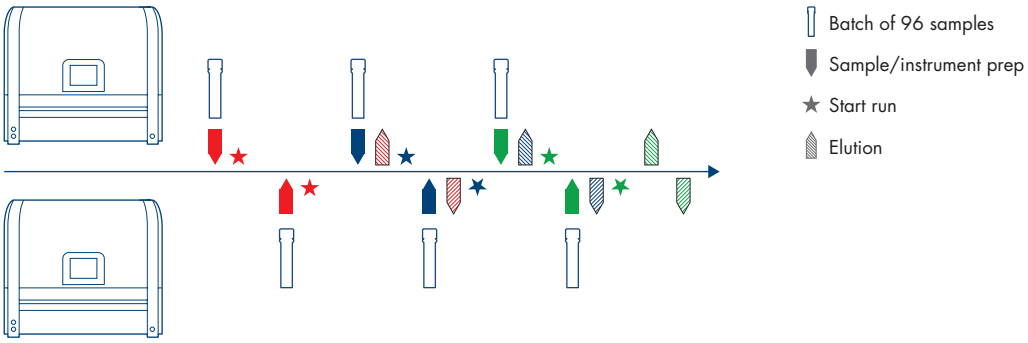

Figure 8. Bode Cellmark's optimized QIAsymphony SP instrument workflow for high-throughput processing of casework samples. In a 14-hour working day, over two shifts, 6 runs with 96 samples each are achieved.

Case success

Bode Cellmark has been using the QIAsymphony SP instrument since 2009 and continues to optimize methods for an enhanced workflow for challenging samples to include differentially extracted samples and low-yield samples.

Based on a random sampling of 1,175 sexual assault kits, 67% produced DNA results whereas 33% screened negative for human DNA and/or male DNA using commercially available quantification and amplification kits (Figure 9).

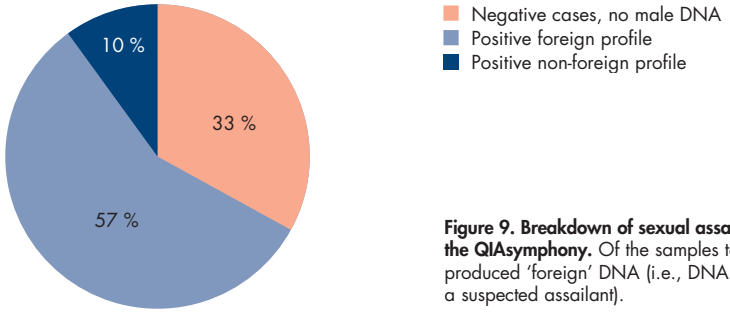

Figure 9. Breakdown of sexual assault cases processed on the QIAsymphony. Of the samples tested, 57% of samples produced 'foreign' DNA (i.e., DNA that may be attributed to a suspected assailant).

For property crime samples, based on a random sampling of 1,450 samples collected from property crimes (90% classified as 'touch' collections and 10% from blood/saliva), 60% of samples produced profiles with 6 or more loci, using commercially available amplification kits (Figure 10).

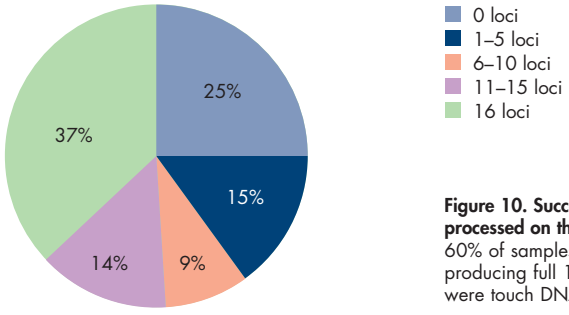

Figure 10. Success rates for property crime samples processed on the QIAsymphony. Of the samples tested, 60% of samples produced 6 or more loci with 37% producing full 16-locus profiles. 90% of the samples were touch DNA.

Conclusions

Bode Cellmark approached the challenge of introducing a new high-throughput line for casework samples by first fully characterizing their chosen instrument – the QIAsymphony SP. This enabled Bode Cellmark to understand how to achieve the best possible results with the platform, while also integrating the instrument into their overall laboratory workflow for maximum efficiency.

This approach enabled Bode Cellmark to achieve:

- Throughput of 528 samples/day using two QIAsymphony SP systems
- Need for only two employees and a 14-hour working day, over two shifts
- Process-safety from the fully enclosed QIAsymphony SP instrument design
- Comparable success rates for all sample types previously processed using the EZ1 Advanced XL

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