Development of a new platform for fully automated purification of nucleic acids from a broad spectrum of forensic specimens

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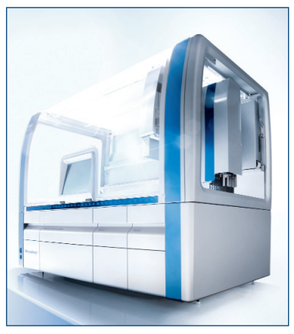
Introduction

Forensic laboratories increasingly employ automated methods for preparation of database and casework samples to increase productivity, reproducibility, and process safety and to reduce hands-on time.

The QIAsymphony® SP is a novel automated system for the purification of nucleic acids and proteins in a streamlined workflow using proven magnetic-particle technology. Optimized, ready-to-run protocols allow walk-away automation of 1 to 96 reference and casework samples per run, with flexible sample input volumes of up to 1 ml. The platform features a novel drawer concept using sealed, pre-filled reagent boxes and provides unprecedented ease of use and process safety.

We present data from the developmental validation of the instrument in the extraction of genetic evidence from forensic samples. The study shows that the extraction efficiency is comparable to well established automated methods and that the extracted DNA performs well in downstream PCR and STR analyses.

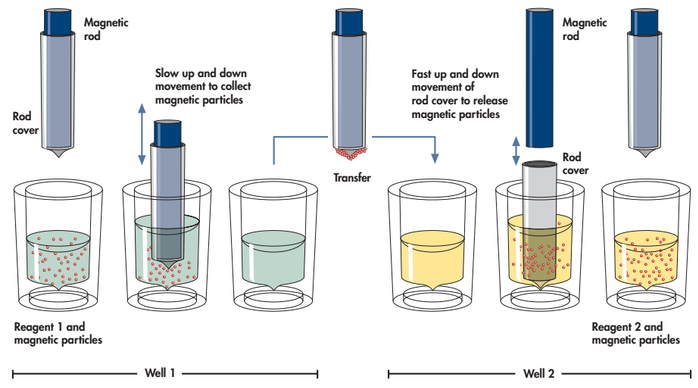


The QIAsymphony SP.

Materials and methods

Sample preparation on the QIAsymphony SP consists of 4 steps: lyse, bind, wash, and elute. Samples are lysed under denaturating conditions in the presence of proteinase K. Lysates are transferred to sample prep cartridges, and DNA binds to the silica surface of magnetic particles. Contaminants are removed by washing, and pure DNA is eluted in a user-specified volume of either modified TE buffer or water. Forensic applications use the same chemistry available in the well known EZ1 Investigator Kits. Protocols include:

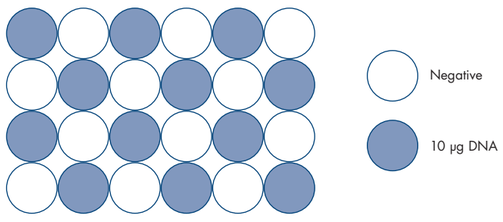
- Reference — 200 µl or 500 µl sample lysate from blood cards or buccal swabs
- Casework — 200 µl, 500 µl, or 1 ml lysate from various casework sample types



Schematic of QIAsymphony SP principle. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod with cover is positioned above another well and releases the magnetic particles. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods and can therefore process 24 samples simultaneously.

Results — exclusion of sample carryover

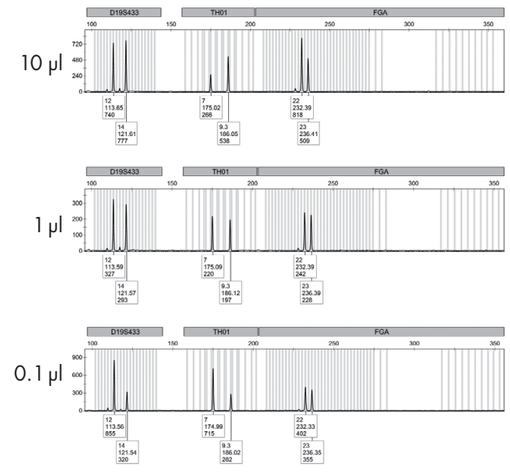
- No sample carryover was detected
- No PCR signal was detected in negative wells after 40 cycles, corresponding to <5 pg of DNA in an eluate volume of 100 µl
- Average recovery from positive samples was 4.8 µg; note that the amount of DNA input exceeds the expected binding capacity of the magnetic beads
- Difference between positive and negative samples is $>10^6$



Cross-contamination assay. Lysis buffer samples (200 µl) spiked with 10 µg DNA (pUC21 plasmid) were arranged in a checkerboard pattern alternating with samples containing buffer only. Samples were run on a QIAsymphony SP using the "Casework 200 µl" protocol and eluates were quantified with real-time PCR.

Results — linearity and sensitivity

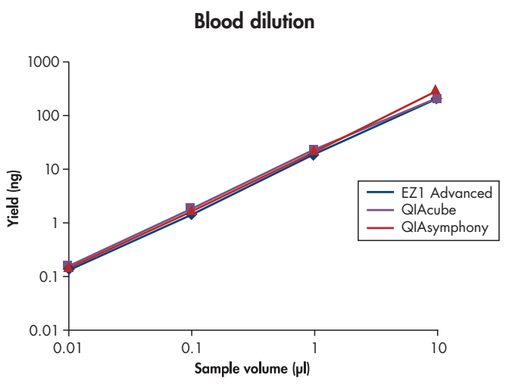
- Dilutions of saliva were processed on the QIAsymphony with the "200 µl Casework" protocol. DNA was eluted in 50 µl water.
- DNA was quantified with real-time PCR. Total yields were 210 ng, 16 ng, and 1.7 ng for 10 µl, 1 µl, and 0.1 µl saliva, respectively.
- Eluates from 10 µl, 1 µl, and 0.1 µl saliva samples were used for STR profiling. DNA (1 ng) extracted from 1 µl and 10 µl saliva was amplified in 28 PCR cycles; 150 pg of DNA extracted from 0.1 µl saliva was amplified in 32 PCR cycles.



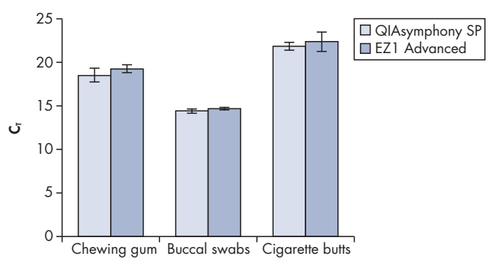
DNA profiles. DNA extracted from saliva dilutions was analyzed in 12.5 µl reactions (AmpF/str® SGMplus™). Data are shown only for the D19S433, TH01, and FGA loci.

Results — comparison of automated platforms

- EZ1® Advanced instrument using magnetic-bead technology
- QIAcube® platform using QIAamp spin columns (based on the well established manual procedures)
- QIAsymphony SP using magnetic-bead technology



Quantitative results. Serial dilutions of blood samples were extracted using the QIAsymphony SP, the EZ1 Advanced, or the QIAcube platform. Extracted DNA was quantified with real-time PCR.



Quantitative results from typical forensic sample types. Chewing gum (30 mg), one buccal swab or half of a cigarette butt filter paper were used as starting materials. Six replicate samples were extracted on a QIAsymphony SP or an EZ1 Advanced instrument following handbook instructions for the corresponding sample type. DNA was eluted in 100 µl and quantified with real-time PCR.

Conclusions

- Yield and quality of DNA extracted with the QIAsymphony SP are comparable to those obtained with the well established EZ1 platform.
- No sample carryover was detected when samples spiked with high amounts of DNA and mock samples were processed in the same run.
- Similar extraction efficiency was achieved over a wide range of sample amounts.
- STR profiles of extracted DNA show high signal-to-noise ratio.

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