CASEWORK VALIDATION OF THE QIASYMPHONY AUTOMATED EXTRACTION SYSTEM

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Qiagen's EZ1 robots have been widely accepted in the forensic science community as a means to automate extraction of 14 samples or less. For forensic laboratories requiring higher throughput but wishing to use the same extraction chemistry, the QiaSymphony SP provides a solution. This large liquid handler is specifically designed to process 1-96 extractions in batches of 24 using the same extraction chemistry as the EZ1. An internal validation of the QiaSymphony DNA Investigator Kit on the QiaSymphony SP was performed for forensic casework at the Harris County Institute of Forensic Sciences.

The purpose of this study was to evaluate whether this automated extraction system produced DNA quantity and quality that was comparable to manual methods. Reference and mock-evidence samples were tested and compared to the laboratory's currently validated extraction procedures (Chelex methods for routine samples and organic extraction for differentially extracted samples). The manufacturer's recommended methods were followed with the exception of adjustments made to the initial incubation time for the differential digest (reduced from 2 h to 1.5 h) and the amount of digest buffer for evidence samples (increased to 500 uL from the suggested 200 uL to fully submerge the sample in the tube). Various sample types including blood, saliva, sperm, and epithelial cells recovered from a variety of substrates (swabs, cigarette butts, and blood stain cards) were extracted using the QiaSymphony and compared to current methods. The quantity of recovered DNA, the completeness of the resulting DNA profiles, the efficiency of recovery from low level samples, the effects of PCR inhibitors on recovery, avoidance of sample-to-sample contamination, and other measures of quality and effectiveness were evaluated.

To compare DNA yield and profile recovery, degraded and pristine samples were extracted using the Qiagen, Chelex, and organic methods. Twenty-one samples were heated to 121°C in an autoclave for various times and extracted by the three different methods. Additionally, thirty mock-evidence samples were extracted using the Qiagen and Chelex methods. For both the degraded and mock-evidence samples, the Qiagen system provided DNA yields and DNA profiles that were comparable to Chelex.

As a test of efficiency of recovery, twenty-two diluted samples of saliva, ranging from 1:100 to 1:1000, were extracted and compared. The QiaSymphony yielded as much or more DNA than extraction using Chelex, the laboratory's preferred extraction method for samples anticipated to contain small amounts of DNA. Precision and reproducibility were tested by preparing and extracting ten replicates of diluted saliva in two separate extraction batches. These batches were quantified three times each on one 96-well plate, normalized, and amplified. An average was taken of the human, male, and IPC values before normalization and amplification of the samples. Comparable DNA quantities were obtained from all samples and all samples produced the same expected profile.

The ability of each extraction method to remove the PCR inhibitors, heme and humic acid, was determined following extraction and amplification of extracted samples. For heme inhibition, concentrated bloodstains were extracted with the QiaSymphony and compared to manual Chelex extraction. The QiaSymphony produced higher DNA yields and better, more complete profiles than Chelex extracted samples. Saliva samples spiked with humic acid were extracted with the QiaSymphony,

organic extraction, and Chelex extraction to determine how well each extraction method removed the inhibitor. Both the organic and Chelex samples were inhibited by humic acid during amplification, but the QiaSymphony samples were unaffected. While the organic samples produced the highest quantity of DNA, none of the amplified samples generated a full profile. QiaSymphony extraction outperformed our current methods of extraction by producing full profiles in the presence of inhibition.

To study contamination, a total of 164 known and blank samples were extracted on the Qiasymphony using a checkerboard pattern. All samples produced the expected DNA profiles; none of the blanks contained DNA. The QiaSymphony was able to extract large batches without cross-contamination of samples.

The QiaSymphony SP performs as well or better than current manual extraction methods without introducing contamination. It is robust and reliable with the added benefit of removing inhibitors such as heme and humic acid prior to downstream amplification.