The Evaluation of Multiple Commercially-Available Extraction Chemistries for Forensic Laboratory Use

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♦ Abstract

The Palm Beach County Sheriff's Office Forensic Biology Unit has observed a dramatic increase in the number of touch, environmentally challenged and inhibited evidentiary samples submitted for DNA analysis. Samples were evaluated using the *EZ1 DNA Investigator Kit* on the *EZ1 Advanced XL* (QIAGEN, Germantown, MD), *DNA IQ™ Casework Sample Kit* extracted on the *Maxwell®16* (Promega Corporation, Madison, WI) and *PrepFiler™ Forensic DNA Extraction Kit* (Applied Biosystems, Foster City, CA) extracted manually. Four comprehensive evaluations were conducted including: 1) contamination assessment study forthe EZ1 and Maxwell®16, 2) sensitivity studies comparing all three chemistries, 3) inhibition study with all three extraction protocols and 4) extraction of mock evidence prepared from "touch" samples using all three chemistries.

Although each of the chemistries and instruments provide unique advantages, results using PrepFiler and QIAGEN were consistently more sensitive. The results of this evaluation have been submitted to the Forensic Biology Unit and were used to select a chemistry that will provide an optimum extraction method that produces accurate and reliable results. The information presented in this poster may assist other laboratories in choosing an extraction method that is sensitive enough to extract low DNA template concentrations as well as remove inhibitors and avoid contamination.

♦Introduction

Currently, the Forensic Biology Unit of Palm Beach County uses *DNA IQ™ Casework Sample Kit* on the *Maxwell®16* (Promega). *DNA IQ™* uses a paramagnetic resin to isolate the DNA from the substrate. The Forensic Biology Unit constantly evaluates new technologies and methodologies introduced to the Forensic DNA community. QIAGEN products include a new, similar magnetic resin extraction chemistry, the *EZ1 DNA Investigator Kit* on the *EZ1 Advanced XL*. Both kits use reagents in pre-dispensed sealed cartridges to minimize the potential for contamination. Both kits have greatly assisted forensic labs in increasing automation for a low throughput of samples. In addition, Applied Biosystems has recently released an extraction chemistry. *PrepFiler™ Forensic DNA Extraction Kit* is a manual DNA extraction chemistry developed by Applied Biosystems that can be automated. The *PrepFiler™* system also uses magnetic resin to assist in the purification of DNA. This kit is designed for low to high throughput of samples.

This poster presents the results of an comparison between the three chemistries. This evaluation consists of 4 studies. The first is a contamination assessment evaluating the automation of Promega's and QIAGEN's instruments to detect any cross-contamination . A template concentration sensitivity study is completed to test the limits of each of the three chemistries. The third study, an inhibition study, evaluates the ability of each chemistry to remove known inhibitors of DNA. The final study mock evidence samples to further assess the sensitivity extraction chemistries.

♦Materials and Methods

- A total of 183 samples, both single source and mixed DNA samples were analyzed.
- All samples were quantified using Promega's Plexor®HY System on Applied Biosystem's 7500 Real-Time PCR System, amplified using Promega's PowerPlex™16, electrophoresed on the Applied Biosystem 3130l*xl* Genetic Analyzer using Applied Biosystem's Data Analysis Software v.3.0, and typed using the Applied Biosystem GeneMapper™ID v. 3.2.1.

Contamination Assessment

- 200 µl of liquid blood and blank reagents arranged in a checkerboard fashion
- Samples were extracted using the Maxwell®16 and EZ1 Advanced XL

Sensitivity Study

- Female blood (dried stains) dilution series from 4.5ng-1.0pg
- Samples were extracted using the Maxwell®16, EZ1 Advanced XL, and PrepFiler™

Inhibition Study

- Liquid saliva samples spiked with either 200mM tannic acid, 50mM hematin, 50mM humic acid at a 1:1 saliva inhibitor ratio. The saliva samples were dried on a sterile cotton swab prior to extraction.
- Samples were extracted using Maxwell®16, EZ1 Advanced XL, and PrepFiler™

Mock Evidence Samples

- Samples collected from soda bottle, flip flop, refrigerator door, steering wheels, front door knobs, and cell phones
- 3 swabs of each item were taken; 1 whole swab was used for extraction
- Samples were extracted using Maxwell®16, EZ1 Advanced XL, and PrepFiler™

♦ Results

All results displayed are shown as number of loci observed out of 16 possible loci needed to obtain a full profile for each sample.

A)

Contamination Study	EZ1	Maxwell
	Run 1	Run 1
Liquid Blood	0/16	0/16
Negative	0/16	0/16
Liquid Blood	11/16	16/16
Negative	0/16	0/16
Liquid Blood	16/16	16/16
Negative	0/16	0/16
Liquid Blood	14/16	16/16
Negative	0/16	0/16
Liquid Blood	16/16	16/16
Negative	0/16	0/16
Liquid Blood	15/16	15/16
Negative	0/16	0/16
Liquid Blood	16/16	16/16
Negative	0/16	0/16
Liquid Blood	N/A	0/16
Negative	N/A	0/16

B)

Contamination Study	EZ1	Maxwell
	Run 2	Run 2
Negative	0/16	0/16
Liquid Blood	15/16	15/16
Negative	0/16	0/16
Liquid Blood	14/16	16/16
Negative	0/16	0/16
Liquid Blood	16/16	16/16
Negative	0/16	0/16
Liquid Blood	11/16	16/16
Negative	0/16	0/16
Liquid Blood	14/16	16/16
Negative	0/16	0/16
Liquid Blood	16/16	16/16
Negative	0/16	0/16
Liquid Blood	15/16	16/16
Negative	N/A	0/16
Liquid Blood	N/A	16/16

Table 1 A and B: (above) shows the results of the contamination study A) is run1 of both EZ1 and Maxwell extractions. No contamination between the wells was observed. B) is run 2 of both EZ1 and Maxwell extractions. No contamination between the wells was observed

Table 2:(below) shows the results of the sensitivity study. Two replicates were ran per extraction chemistry. The samples used were ¼ " punches from a female dried blood stain dilution series. A total of 3 sensitivity studies were completed with the results from one of the studies shown.

Sensitivity Study	EZ1 Run 1	EZ1 Run 2	Maxwell Run 1	Maxwell Run 2	PrepFiler Run 1	PrepFiler Run 2
Neat	16/16	16/16	16/16	16/16	16/16	15/16
1:1	16/16	16/16	16/16	16/16	16/16	16/16
1:2	16/16	16/16	16/16	16/16	16/16	16/16
1:4	16/16	15/16	16/16	16/16	16/16	16/16
1:8	16/16	16/16	16/16	16/16	16/16	16/16
1:16	16/16	16/16	16/16	16/16	16/16	16/16
1:32	16/16	16/16	5/16	0/16	16/16	16/16
1:64	16/16	16/16	5/16	2/16	16/16	16/16
1:128	16/16	16/16	15/16	1/16	16/16	16/16
1:256	12/16	5/16	15/16	0/16	16/16	13/16
1:512	1/16	0/16	1/16	0/16	6/16	16/16
1:1024	0/16	0/16	0/16	0/16	0/16	0/16

Table 3: (right) shows the results of the inhibition study. This study was ran in triplicate with samples spiked with known DNA inhibitors (hematin, humic acid, and tannic acid).

Inhibition Study	Hematin	Humic Acid	Tannic Acid
EZ1 Plate 1	16/16	16/16	0/16
EZ1 Plate 2	16/16	16/16	16/16
EZ1 Plate 3	0/16	16/16	16/16
Maxwell Plate 1	0/16	16/16	16/16
Maxwell Plate 2	16/16	16/16	16/16
Maxwell Plate 3	16/16	0/16	16/16
PrepFiler Plate 1	16/16	0/16	0/16
PrepFiler Plate 2	16/16	0/16	4/16
PrepFiler Plate 3	16/16	0/16	3/16

Table 4: (right) shows the results of the evaluation of mock evidence samples. The samples were swabs of various "touch" samples

Mock Evidence Samples	EZ1	Maxwell	PrepFiler
Soda Bottle	16/16	16/16	16/16
Flip Flop	0/16	0/16	0/16
Refrigerator	16/16	2/16	16/16
Steering Wheel 1	0/16	0/16	1/16
Front Door Knob	1/16	0/16	16/16
Cell Phone 1	5/16	1/16	16/16
Cell Phone 2	0/16	0/16	0/16
Steering Wheel 2	16/16	12/16	15/16

♦ Conclusions and Discussion

Contamination Study

- No contamination was observed for the samples extracted with *DNA IQ™* or *EZ1 Investigator.*
- The blood samples used were from the same donor and full profiles were expected to be obtained from all blood samples. Full profiles were not obtained for 9 whole blood samples extracted on the EZ1 Advanced XL and 4 whole blood samples extracted on the Maxwell®16. This may have resulted from a failure to remove all the heme from the sample due to the high amount of liquid blood used thus causing inhibition of the PCR reaction.

Sensitivity Study

- *DNA IQ* consistently provided full profiles from concentrations down to a 1:16 dilution. Results beyond dilutions of 1:16 were inconsistent. *EZ1 Investigator* results consistently provided results down to dilutions of 1:128, with no profile seen at 1:1024 dilutions. *PrepFiler™* results provided full profiles at concentrations down to 1:256 dilutions. No profile was seen at dilutions of 1:1024. Overall, the *PrepFiler™* and QIAGEN chemistries are more sensitive than the *DNA IQ* chemistry. This conclusion is further supported by the results seen with mock evidence samples as well as additional sensitivity studies (data not shown).

Inhibition Study

- *DNA IQ* and the *EZ1 Investigator Kit* were successful in removing all inhibitors from samples in most cases. *PrepFiler™* was successful in removing hematin from samples. However, *PrepFiler™* was not able to completely remove humic acid and tannic acid. This could be due to incomplete washing of the samples during the manual extraction process. Overall, all chemistries show potential to remove inhibitors from samples.

Mock Evidence Samples

- Mock "touch" samples provided a higher yield of DNA profiles using both *PrepFiler™* and the *EZ1 Investigator Kit* extraction chemistries over the *DNA IQ* chemistry. These results are consistent with the results seen with the template concentration sensitivity study.

Following recommendations offered after the completion of this evaluation, the Palm Beach County Sheriff's Office Forensic Biology Unit chose to implement both *PrepFiler™* and *EZ1 DNA Investigator Kit* into the lab's standard operating procedures.

♦ Future Studies

- Studies evaluating the correlation between the quantification values and the typing results should be performed to aid in deciding which extraction method should be adopted.
- Studies evaluating the correlation between RFU heights of the results and the extraction chemistry used may also be performed to aid in deciding which extraction method should be adopted.
- Studies evaluating the results of a manual PrepFiler™ extraction versus the results of an automated PrepFiler™ extraction.
- Once an extraction method is selected further validation studies as per the FBI Quality Assurance Standards should be completed.

♦ References

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