Comparative Evaluation of Manual Extraction Methods for the Biology/DNA Detail of the Las Vegas Metropolitan Police Department Forensic Laboratory

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ABSTRACT

An extraction method for forensic DNA casework must produce a high quantitative yield as well as a clean STR profile free from artifacts across all sample types, including low level samples such as touch DNA. The Las Vegas Metropolitan Police Department DNA Laboratory has previously relied on phenol-chloroform organic extraction for forensic casework. In order to expedite the extraction process and foray into automation instruments, a comparison study was undertaken with Applied Biosystems' PrepFiler™ Forensic DNA Extraction Kit and Qiagen's QIAamp® DNA Investigator Kit for the purpose of determining a manual extraction chemistry to replace organic extractions and to be automated in the future. The kits were evaluated on the basis of I) contamination issues II) quantitative yield III) STR profile quality IV) future automation potential V) time consumption of the method and VI) cost of the method. Based on the results of this study, LVMPD chose to validate PrepFiler™, citing higher quantitative yields for low level samples and better detection of minor contributors in mixture samples than the other methods, along with STR profile quality comparable to an organic extraction.

INTRODUCTION

Advancing technology has allowed the area of forensic DNA to expand to serve cases where it has not traditionally been used, due to degradation or minute amounts of DNA, such as property crimes and cold cases. These new applications for DNA create a wide variety of sample substrates that are being submitted for DNA testing in addition to the traditional blood, saliva and semen samples, including body swabs, hair, bones, cigarette butts, and what is now being called "touch DNA". The challenge of obtaining full STR profiles from such samples makes the first step of DNA analysis, extraction, incredibly important. The goal of extraction is not only to produce a pure sample extract with a large amount of DNA that is free from inhibitors, but to produce one that can ultimately generate a robust STR profile as well.

Currently, the most common extraction method used in forensic laboratories is Phenol-Chloroform organic extraction, the "gold standard" method, which regularly produces very high yields from a variety of sample types. The Las Vegas Metropolitan Police Department DNA laboratory sought to move from organic extractions to a commercial kit that could take less time, have a less labor intensive technique and no hazardous components, and be automated in the future, but could still perform as well as an organic extraction. This study was designed to evaluate Applied Biosystems' PrepFiler™ DNA Extraction Kit and Qiagen's QIAamp® DNA Investigator Kit to determine the best method for extraction that could produce comparative or better results than organic extractions to be used for databasing and casework.

MATERIALS AND METHODS

To mimic casework, twelve commonly received sample types were collected in triplicate from objects and personnel at the Las Vegas Metropolitan Police Department Forensic Laboratory (Las Vegas, NV). Effort was made to uniformly sample in order to obtain approximately the same amount of DNA across three replicates for each sample type. All samples were extracted by Phenol-Chloroform Isoamyl Alcohol extraction according to LVMPD protocol, the Applied Biosystems PrepFiler™ Forensic DNA Extraction Kit, and Qiagen QIAamp® DNA Investigator Kit.

DNA was quantified with the Promega Plexor® HY System with 2 µl of extract. Cycling was performed on an Applied Biosystems 7500 Real-Time PCR System with SDS Software v1.2.3. The thermal profile consisted of an initial hold step of 95˚C for 2 minutes, thirty-eight cycles of 95˚C for five seconds and 60˚C for thirty-five seconds, and a dissociation stage of 95˚C for fifteen seconds, 60˚C for one minute and 95˚C for fifteen seconds.

Samples were then amplified on a GeneAmp 9700 Thermal Cycler targeting 1 ng of DNA using the AmpFlSTR® Identifiler® Plus PCR Amplification Kit. The amplified products were run on an Applied Biosystems 3130xl Genetic Analyzer using a 3 kV injection for 5 seconds, and analyzed with Applied Biosystems GeneMapper® ID-X Software v1.1.1 with a reporting threshold of 75 RFU and analysis threshold of 25 RFU.

Evaluation of the three extraction methods was primarily based on contamination, quantitative yield of both total nanograms of DNA and concentration of DNA, and genetic profile quality. The profile quality itself was evaluated by average RFU per sample, number of minor contributor alleles detected, and overall profile appearance. Other factors assessed included future automation potential, time of method, and cost of method.

RESULTS

Contamination Study

Reagent blanks for each extraction method, as well as quantification no template controls and amplification negatives, were processed with the samples. Negative controls for all steps showed no extraneous peaks or other signs of artificial presence, indicating no contamination in any of the three extraction methods.

Quantitative Yield Study *(yellow boxes indicate highest value per sample)*

Sample	Sample Type	Organic	PrepFiler™	QIAamp®
EC_01	Buccal Swab	11.70	**16.90**	6.21
EC_02	Buccal Swab	**28.60**	20.30	18.20
EC_03	Buccal Swab	13.00	**29.90**	18.00
EC_04	FTA Card	**10.40**	8.22	6.08
EC_05	FTA Card	**5.34**	5.07	2.44
EC_06	Cigarette Butt	0.04	**0.05**	0.02
EC_07	Clean Soda Can Swab	**3.20**	0.61	1.11
EC_08	Dirty Soda Can Swab	0.53	**2.41**	0.57
EC_09	Hand Swab	0.02	**0.04**	0.02
EC_10	Gun Swab	0.55	**1.07**	0.70
EC_11	Hair	**2.17**	1.43	1.13
EC_12	Keyboard Swab	0.02	**0.03**	0.02
EC_13	Cell Phone Swab	0.03	**0.03**	0.01
EC_14	Clothing Swab	0.08	0.20	**0.21**
EC_15	Degraded Whole Blood	17.00	**18.30**	3.45

Table 1. Concentration of human DNA extract in ng/µl. A higher concentrated DNA extract prevents the addition of a large amount of PCR inhibitors and salts to the amplification reaction.

Sample	Sample Type	Organic	PrepFiler™	QIAamp®
EC_01	Buccal Swab	**1310.40**	845.00	465.75
EC_02	Buccal Swab	**3088.80**	1015.00	1365.00
EC_03	Buccal Swab	**2171.00**	1495.00	1350.00
EC_04	FTA Card	**468.00**	411.00	456.00
EC_05	FTA Card	224.28	**253.50**	183.00
EC_06	Cigarette Butt	**7.55**	2.35	0.58
EC_07	Clean Soda Can Swab	**76.80**	30.70	44.40
EC_08	Dirty Soda Can Swab	32.09	**120.50**	22.80
EC_09	Hand Swab	**2.40**	1.91	0.64
EC_10	Gun Swab	31.90	**53.50**	28.08
EC_11	Hair	**147.56**	71.50	45.20
EC_12	Keyboard Swab	1.09	**1.58**	0.54
EC_13	Cell Phone Swab	1.00	**1.49**	0.46
EC_14	Clothing Swab	4.52	**9.90**	8.16
EC_15	Degraded Whole Blood	646.00	**915.00**	138.00

Table 2. Total human DNA yield in nanograms for each sample, calculated by multiplying concentration in ng/µl by total microliters collected during elution.

Profile Quality Study *(yellow boxes indicate highest value per sample)*

Sample	Sample Type	Organic	PrepFiler™	QIAamp®
EC_01	Buccal Swab	3146	**3481**	2337
EC_02	Buccal Swab	**2248**	1496	1711
EC_03	Buccal Swab	**1645**	1347	1568
EC_04	FTA Card	723	**1018**	953
EC_05	FTA Card	2486	2520	**2539**
EC_06	Cigarette Butt	**536**	180*	108
EC_07	Clean Soda Can Swab	**1698**	1192	1385
EC_08	Dirty Soda Can Swab	510	654	**711**
EC_09	Hand Swab	**456**	414	160
EC_10	Gun Swab	**509**	300	376
EC_11	Hair	1270	847	**1417**
EC_12	Keyboard Swab	40	**59**	52
EC_13	Cell Phone Swab	**270**	112	102
EC_14	Clothing Swab	391	255	**484**
EC_15	Degraded Whole Blood	1182	935	**1184**

Table 3. Average RFU level per sample. The PrepFiler™ extraction produced the highest average RFU height on only three samples, while the QIAamp® extraction produced the highest average RFU height on five samples.

Sample	Sample Type	Organic	PrepFiler™	QIAamp®
EC_09	Hand Swab	5	**6**	1
EC_12	Keyboard Swab	0	**12**	1
EC_13	Cell Phone Swab	**4**	0	1
EC_14	Clothing Swab	2	**6**	2

Table 4. Minor contributor alleles detected per sample from non-probative touch DNA samples. Four samples produced mixture profiles, and in three of the four cases, the PrepFiler™ extraction produced the highest number of minor alleles. The QIAamp® extraction never produced the highest amount of minor alleles.

Automation Capability Study

Extraction Method	Optimized For:	Other Amenable Platforms
Organic	Cannot be automated	N/A
PrepFiler™	Tecan Freedom EVO 150 or 200 robots; AutoMate *Express*™ Forensic DNA Extraction System	Multiple liquid handling platforms
QIAamp®	EZ1 Advanced, EZ1 Advanced XL, QIAcube, QIAsymphony	None

Table 5. Future automation options to save time spent doing manual lab work. PrepFiler™ has many robotic options, whereas QIAamp® will only work with Qiagen robotics.

Time Consumption/Cost Study

Extraction Method	Incubation (min)	Centrifugation (min)	Protocol Steps	Method Cost
Organic	60 - overnight, overnight preferred	38	14	$0.05
PrepFiler™	117	2	20	$6.15
QIAamp®	85 - overnight, depending on chosen time	9	18	$3.78

Table 6. Time of method was assessed based on lengths of incubations, centrifugations, and protocol steps. Organic takes the longest amount of time, while PrepFiler™ is the shortest extraction method. Cost per reaction revealed that the organic extraction method is the least costly, whereas the PrepFiler™ is the most expensive method.

DISCUSSION

- Extraction efficiency is increasingly more important as sample size decreases. Although, the organic extraction produced the highest quantitative yields for high level samples such as buccal swabs and FTA cards, for the majority of the more important low level samples such as touch DNA samples, PrepFiler™ produced superior yields both in total DNA and concentration in ng/µl.
- Regarding STR profiles, although QIAamp® produced more robust RFU heights, overall, PrepFiler™ profiles looked cleaner and gave better balanced heterozygote peaks than the QIAamp® profiles. The PrepFiler™ extraction profiles were comparable to the appearance of the organic extraction profiles. PrepFiler™ also detected more minor contributors in mixture samples than QIAamp®.
- *The one exception to superior PrepFiler™ profiles was the cigarette butt sample, for which the PrepFiler™ extraction showed significant PCR inhibition. After it failed to produce a profile with a 1 ng target, the extract was re-amped with a 0.5 ng target, and it produced a low level partial profile. Troubleshooting for cigarette butts was tested and designed for the PrepFiler™ extraction method during the subsequent validation.
- PrepFiler™ provided better automation options than QIAamp®, as the choice exists for labs between multiple vendors and platforms, rather than being restricted to the manufacturer of the kit.
- Organic and QIAamp® extractions both require an extensive incubation process, but with PrepFiler™'s mandatory maximum incubation time of 90 minutes, proves to be the shortest extraction method as well.
- Although PrepFiler™ was the most expensive extraction method per reaction, cost was determined to be a less important decision-making factor than superior data quality.

CONCLUSIONS

- Overall, **PrepFiler™ Forensic DNA Extraction Kit** was determined to be the best kit for the LVMPD Biology/DNA Detail.
- The decision was based on **higher quantitative yields** for low level samples, better **detection of minor contributors** in mixture samples, and an **STR profile quality** comparable to an organic extraction.
- **PrepFiler™ was validated** according to SWGDAM Guidelines for use in LVMPD DNA casework.

REFERENCES

Applied Biosystems. AmpF/STR® Identifiler® Plus PCR Amplification Kit User's Guide. Foster City, CA: Applied Biosystems, 2009; Part #4402743 Rev. A.

Applied Biosystems. PrepFiler™ Forensic DNA Extraction Kit User Guide. Foster City, CA: Applied Biosystems, 2008; Part #4390932 Rev. B.

Barbaro, A, Cormaci P, Agostino A. Validation of PrepFiler™ forensic DNA extraction kit (Applied Biosystems). Forensic Sci Int 2009; 2: 176-177.

Brevnov MG, Pawar HS, Mundt J, Calandro LM, Furtado MR, Shewale JG. Developmental Validation of the PrepFiler™ Forensic DNA Extraction Kit for Extraction of Genomic DNA from Biological Samples. J Forensic Sci 2009 May; 54(3): 599-607.

Las Vegas Metropolitan Police Department Forensic Laboratory. DNA Procedure Manual. Las Vegas: LVMPD, 2009.

Promega Corporation. Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems Technical Manual. Madison, WI: Promega Corporation, 2007; Part #TM293. Rev A.

Qiagen Incorporated. QIAamp® DNA Investigator Handbook. Valencia, CA: Qiagen Incorporated, 2007; Part #56504.

Wickenheiser RA. Trace DNA: A Review, Discussion of Theory, and Application of the Transfer of Trace Quantities of DNA Through Skin Contact. J Forensic Sci 2002; 47(3): 442-450.

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