Validation of the Teknova Organic Sperm Wash Buffer

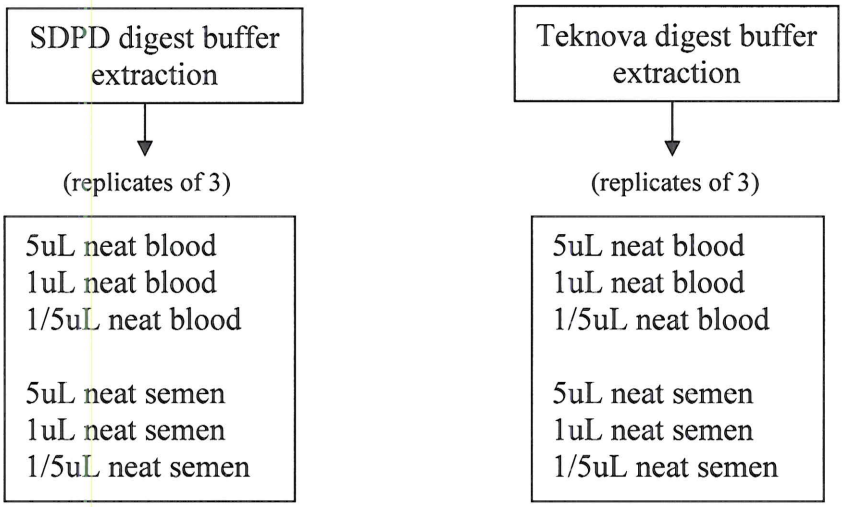
January 22, 2015

Purpose

The purpose of this study was to compare SDPD digest buffer with Teknova Organic Sperm Wash buffer with the intention of replacing SDPD digest buffer that is prepared in-house annually with the Teknova buffer.

Experimental Procedure

Liquid blood and semen were extracted with both SDPD digest buffer and Teknova buffer. Three replicates of the following volumes were extracted.



Results

Independent t-tests were conducted to compare the DNA yields from replicate samples extracted with PD digest buffer and Teknova digest buffer:

t-test blood= 0.21147 (no difference)

t-test semen (non-sperm fraction)= 0.424424 (no difference)

t-test semen (sperm fraction)= 0.044205 (significant difference) with Teknova having higher on average quantitation values

The peak heights between the samples extracted with the PD digest buffer versus the Teknova digest buffer were comparable.

Conclusions

These results suggest that there is no significant difference between DNA yields when samples and were extracted with PD digest buffer versus Teknova digest buffer. The in-house prepared SDPD digest buffer will be replaced with the Teknova Organic Sperm Wash buffer.

Coral Luce, Criminalist

Shawn Montpetit, DNA Technical Manager

THE CITY OF SAN DIEGO

MEMORANDUM

DATE: 02-03-2014

TO: John Simms, Quality Assurance Manager

cc: Patrick O'Donnell, Supervising Criminalist
Frank Healy, Supervising Criminalist

FROM: Shawn Montpetit, DNA Technical Manager

SUBJECT: Material Modification of the Eppendorf ThermoMixers

The San Diego Police Department (SDPD) Crime Laboratory's Forensic Biology Section currently uses static electric heat blocks for incubating sample during the lysing part of the DNA purification protocol. Previous experimentation [see the Prepfiler/Automate Express evaluation] indicated an improved yield by employing heater/shakers instead of the static heaters. Based on that information, the forensic biology unit acquired several Eppendorf ThermoMixers. The following is a summary of the experiments conducted to bring the thermomixers into use for casework.

The data included within indicates that higher yields are obtained when using the ThermoMixers during the incubation period of the sample lysis. As such, it is recommended that the forensic biology unit incorporate the Eppendorf ThermoMixers into casework as soon as possible.

Shawn Montpetit
DNA Technical Manager

JMS 2/4/14

February 3, 2014

Material Modification

Eppendorf ThermoMixers

The San Diego Police Department (SDPD) Crime Laboratory's Forensic Biology Section currently uses static electric heat blocks for incubating sample during the lysing part of the DNA purification protocol. Previous experimentation [see the Prepfiler/Automate Express evaluation] indicated an improved yield by employing heater/shakers instead of the static heaters. Based on that information, the forensic biology unit acquired several Eppendorf ThermoMixers. The following is a summary of the experiments conducted to bring the thermomixers into use for casework.

Original examination of the thermomixers

In September 2011 [see the Prepfiler/Automate Express evaluation], the laboratory conducted an assessment of the Prepfiler DNA purification chemistry on the AutoMate Express instrument. The Prepfiler protocol included incubation on thermomixers during the initial sample lysis step. As part of the assessment five cigarette butts were extracted using the standard SDPD protocol, however all lysis steps were performed on a thermomixer. These samples were compared to the other extraction methods using the thermomixer. The results are presented in Figure 1.

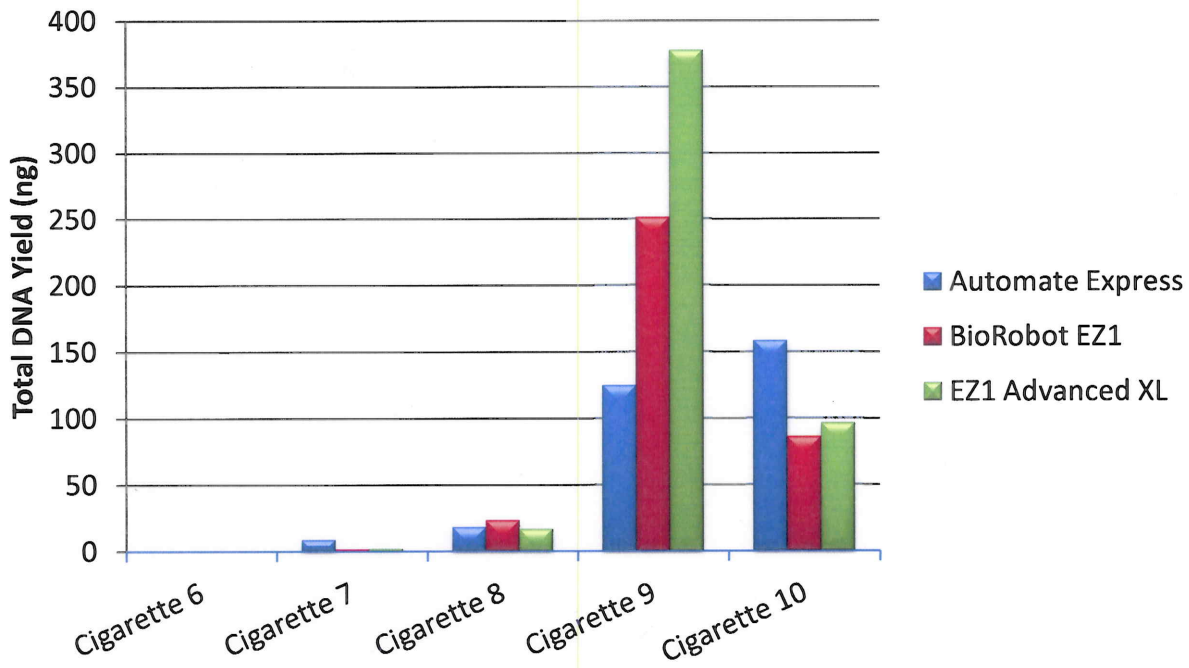


FIG. 1 – Cigarette butts with thermomixer usage. Total DNA yields for the three instruments, all using the thermomixer during the lysis step. Automate Express: ABI buffers, 40 min at 70°C with 750rpm. BioRobot EZ1: SDPD buffers, 2 hours at 56°C with 750rpm. EZ1 Advanced XL: Qiagen buffers, 2 hours at 56°C with 750rpm.

Based on this experiment, there were indications that using a thermomixer during the lysis step could have a dramatic effect on the DNA yield.

Current examination of the Eppendorf Thermomixers.

The evaluation of the ThermoMixers was accomplished as part of the validation of the QiaSymphony.

Material and Methods

4 sets of samples of samples were prepared with 6 replicates each. The samples were created from a pool of diluted blood placed onto swabs. 140µL blood in 2660µL PBS split into 24 tubes of 100µL per tube, then absorbed onto individual swabs. The 4 sets of samples were treated as follows: 6 samples lysed using SDPD reagents incubated on the ThermoMixers, 6 samples using SDPD reagents with the standard static heaters, 6 samples using the QiaSymphony reagents incubated on the ThermoMixers, and 6 samples using the QiaSymphony reagents with the static heaters. The lysis of all samples was for 2hours. The QiaSymphony was used for the extraction of the samples using the CW 500µL ADV HE protocol with 40µL elution volume.

The chemistry of the QiaSymphony DNA Investigator kit is nearly identical to the chemistry employed by the EZ1s. The QiaSymphony DNA Investigator kit uses a guanidine thiocyanate/guanidine hydrochloride magnetic particle -silica bead based purification just like the EZ1, however, there are some minor differences between the chemistry of these two instruments. The QiaSymphony DNA Investigator kit has a slightly different concentration of SDS in the lysis component of the kit, and the magnetic silica beads are of a different size and porosity than the EZ1 DNA Investigator kits. The differences were incorporated into the kit for the QiaSymphony due to the different mechanism of action between the instruments. In the EZ1s, the entire purification reaction occurs within the pipette tip, whereas the reaction on the QiaSymphony occurs within a sample well. This difference required some optimization to produce similar yields between the two instrument platforms. Based on the similarities of the extraction processes, the results of this experiment should transfer directly to the EZ1 extractions.

The purified extracts were quantified using the Applied Biosystems Quantifiler Duo kit.

Results

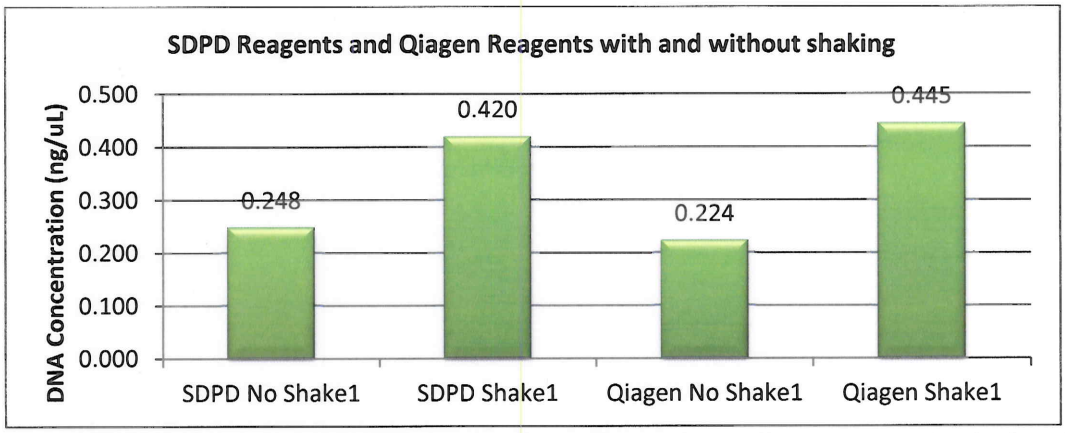


Figure 2: Average yield of each of the 4 sample sets: SDPD reagents with and without shaking and Qiagen reagents with and without shaking

Sample	Quant Value	Average	Standard Deviation
SDPD No Shake1	0.171	0.248	0.071
SDPD No Shake2	0.189		
SDPD No Shake3	0.322		
SDPD No Shake4	0.267		
SDPD No Shake5	0.337		
SDPD No Shake6	0.202		
SDPD Shake1	0.364	0.420	0.144
SDPD Shake2	0.406		
SDPD Shake3	0.353		
SDPD Shake4	0.261		
SDPD Shake5	0.685		
SDPD Shake6	0.449		
Qiagen No Shake1	0.199	0.224	0.075
Qiagen No Shake2	0.235		
Qiagen No Shake3	0.158		
Qiagen No Shake4	0.368		
Qiagen No Shake5	0.18		
Qiagen No Shake6	0.202		
Qiagen Shake1	0.412	0.445	0.091
Qiagen Shake2	0.582		
Qiagen Shake3	0.505		
Qiagen Shake4	0.465		
Qiagen Shake5	0.345		
Qiagen Shake6	0.358		

Figure 3: Data of each of the 4 sample sets: SDPD reagents with and without shaking and Qiagen reagents with and without shaking

A Student t-test was performed on the data comparing the DNA yields for the replicates incubated on the ThermoMixers to those incubated on the static heaters. The t-test tested the hypothesis that there was a difference between the two data sets against the null hypothesis that there was no difference between the data sets. A p-value of 0.000088 was obtained which is far less than 0.01, indicating that with 99% confidence the values obtained from the ThermoMixers samples were significantly higher than those that used the static shakers.

Discussion and Conclusions

The above data indicates that higher yields are obtained when using the ThermoMixers during the incubation period of the sample lysis. As such, it is recommended that the forensic biology unit incorporate the Eppendorf ThermoMixers into casework as soon as possible.

DNA Technical Manager