Application Note

Effects of reduced elution volumes on the QIAGEN EZ1® Advanced XL extraction platform performance

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Introduction

When processing forensic casework samples, one of the biggest challenges that laboratories face is obtaining reliable information from samples containing very little human DNA. Current DNA methods dilute DNA during the elution step of the purification process. The more dilute the DNA, the less chance there is of obtaining high-quality, reliable information. It was considered possible that results would be improved if the elution volume was reduced, thereby producing a more concentrated DNA eluate.

This study shows the effects on DNA concentration and yield when eluting into lower volumes and found that the concentration of DNA is significantly increased when using a 20 µl elution method. Although overall yield for stronger DNA templates is reduced with a lower elution volume, the yield for weaker templates is increased. This results in higher DNA inputs being possible when preparing a sample for STR amplification.

Materials and Methods

A total of 5 dilutions from an undiluted (neat) blood sample was prepared in a 10-fold dilution series. The volume was enough to cover all extraction methods used in the study to ensure consistency of results. All samples were processed in triplicate and pre-treated using the same sample lysis procedure.

A volume of 30 µl of test sample was added to 160 µl of G2 buffer and 10 µl Proteinase K, both supplied in the EZ1 DNA Investigator® Kit, creating a total sample input volume of 200 µl. Each sample was then incubated at 56°C for 20 minutes on a thermal shaker at 900 rpm, then purified using an EZ1 Advanced XL Instrument and the Trace Protocol on the EZ1 Advanced XL DNA Investigator Card (1). Samples were eluted into 40 µl, 30 µl, 25 µl or 20 µl elution volumes.

The currently validated elution volume of 40 µl was used in this experiment as a reference point for the lower elution volumes.

A total of 18 samples was processed per elution volume. With this procedure, two batches of 9 samples per elution volume were subjected to an EZ1 purification run for each. A total of 72 samples was processed using 2 x EZ1 DNA Investigator Kits of the same lot number (163038083). The same EZ1 Advanced XL instrument was used to process all samples in this study.

Purified samples were quantified using the Investigator Quantiplex® Pro RGQ Kit and a Rotor-Gene® Q real-time PCR instrument following the manufacturer's handbook and user manual guidelines (2). The quantification value of human DNA in each sample was recorded using the Quant Assay Data Handling Tool v3.4.3 for analysis and used for a ▷

comparison of the concentration of DNA within each sample for each elution volume. The actual volume of each elution was also recorded.

Results and Discussion

Table 1 shows the average concentration data obtained from each of the dilutions for all elution volumes used. For ease of readability, the human target value was used for comparison purposes for the results. As expected, the concentration of template DNA within the sample increases as the elution volume of the sample decreases. This is particularly apparent in the highest blood dilution (1:100,000) where the concentration of DNA in samples eluted in the smallest volume (20 µl) increased 4-fold over the concentration of DNA in the currently validated elution volume of 40 µl.

Table 1. Average template DNA concentration per dilution for each elution volume

Elution volume (µl)	Blood dilution	Average concentration (ng/µl)*
40	Neat	10.4274
40	1:10	1.1704
40	1:100	0.1149
40	1:1,000	0.0124
40	1:10,000	0.0011
40	1:100,000	0.0001
30	Neat	10.601
30	1:10	1.2243
30	1:100	0.1331
30	1:1,000	0.0144
30	1:10,000	0.0014
30	1:100,000	0.0001
25	Neat	11.6237
25	1:10	1.3911
25	1:100	0.1254
25	1:1,000	0.0125
25	1:10,000	0.0014
25	1:100,000	0.0002
20	Neat	13.1845
20	1:10	1.0961
20	1:100	0.1268
20	1:1,000	0.0145
20	1:10,000	0.0009
20	1:100,000	0.0004

*Average value of human DNA target

Table 2 shows the total yield obtained for each of the elution volumes for the 1:100,000 dilution. It can clearly be seen that overall yield has more than doubled when using the 20 µl elution over the 40 µl elution. This indicates that the amount of template able to be introduced to an amplification has increased from 0.002 ng to 0.006 ng, thereby increasing the possibility of obtaining more information in a DNA profile.

Table 2. Total yield from the 1:100,000 dilution for each elution volume and PCR template input per elution volume

1:100,000 dilution	20 µl	25 µl	30 µl	40 µl
Total Yield (ng)	0.009	0.006	0.004	0.004
PCR input (ng)*	0.006	0.003	0.002	0.002

*based on a 15 µl sample input volume

When programming final elution volumes for the EZ1 Advanced XL, extra volume must be added to account for factors such as dead volumes, liquid lost through pipetting steps, etc. Therefore, accurate measuring of the actual final elution during this experiment was vital to ensure correct sample volumes were obtained at the end of the protocol. Table 3 shows the average actual volume obtained for each of the elution protocols. Volumes were measured using a calibrated manual pipette.

Table 3. Average actual volume per elution protocol

Elution volume protocol (µl)	Average actual volume (µl)	% accuracy
40	39	97.5
30	34	113
25	28	112
20	25	125

Figure 1 shows a side by side comparison of concentrations of each dilution and elution volume. It can be seen that the DNA concentration of the 1:100,000 dilution in the smallest volume (20 µl) has been greatly increased over the samples eluted in a greater volume. Total yield was lost in the higher dilutions when using a lower elution volume (neat to 1:1,000). This was expected, given that the lower elution volume has reduced capacity. Nevertheless, the concentration obtained in the lower elution volumes was still at a level that would produce a strong, full DNA profile result. In the lowest elution volume, however, the reverse was observed.

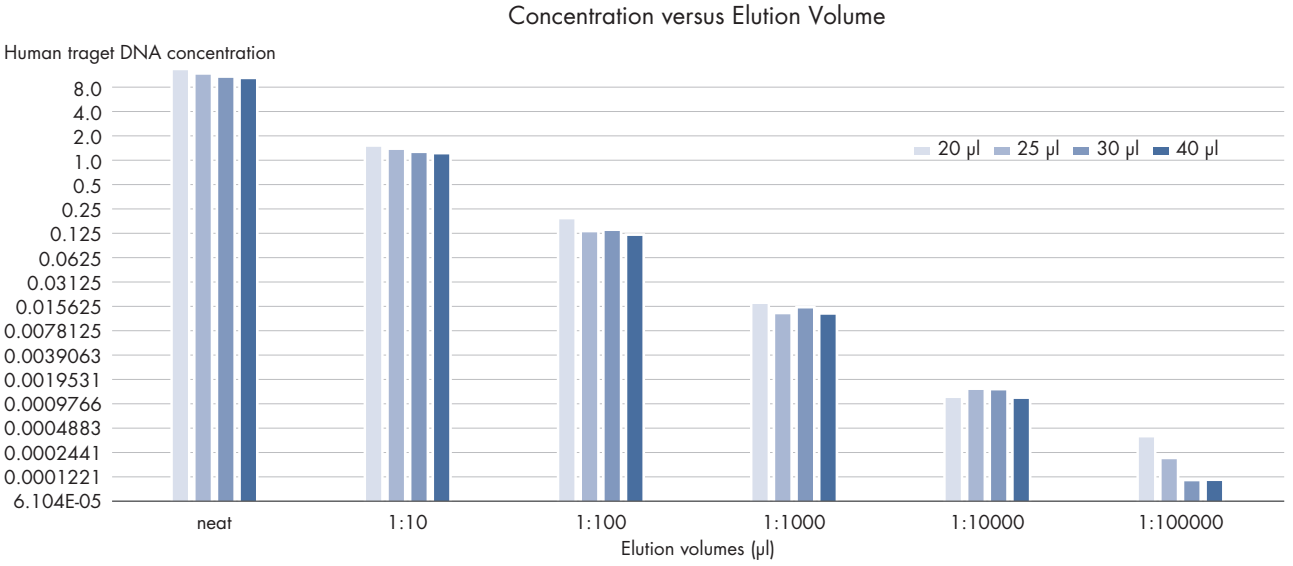

Figure 1. DNA concentration plotted against elution volume per dilution.

The actual elution volume obtained from the 20 µl protocol was 25% higher than expected. This was deemed to be too high, therefore the protocol was reprogrammed accordingly to allow for a lower elution volume closer to the expected volume. The results of a second test carried out after reducing the elution volume are shown in Table 4.

The actual volume obtained after decreasing the elution volume level was more in line with expectation. Although no further testing was performed in terms of concentration achieved with the lower elution volume, it was expected, based on previous results, that the concentrations will have increased slightly when using this protocol.

Table 4. Actual volume from 20 µl protocol after reducing elution volume

Dilution	actual vol (µl)	Dilution	actual vol (µl)
Neat	21	1:1,000	20
Neat	22	1:1,000	20
Neat	21	1:1,000	22
1:10	21	1:10,000	22
1:10	22	1:10,000	20
1:10	21	1:10,000	22
1:100	20	1:100,000	21
1:100	21	1:100,000	22
1:100	22	1:100,000	21
Average elution volume			21

Conclusion

The results show that by using a lower sample elution volume, the DNA concentration can be increased significantly. This allows for a higher physical template of DNA to be added to a PCR amplification reaction. Many forensic casework samples have low DNA templates. An increased DNA input into PCR amplification will aid in the possibility of obtaining more information within a DNA profile.

One factor to consider when using a reduced elution volume (e.g., 20 µl) is that current STR amplification kits allow for a sample input volume of up to 15 µl. When this is added to the 2 µl required for the quantification process, a lower volume of eluate means that each sample can be processed only once and that a replicate sample is not available to confirm any DNA profile obtained.

References

1. QIAGEN *EZ1 DNA Investigator® Handbook*, July 2014
2. QIAGEN *Investigator® Quantiplex Pro RGQ Handbook*, February 2018

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridge (DNA Investigator), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K, Carrier RNA	952034
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN Rotor-Gene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387316
Related Products		
EZ1 Advanced XL, System	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits: includes installation, training, 1-year warranty on parts and labor	9001874
EZ1 Advanced XL DNA Investigator Card*	Preprogrammed card for purification of DNA using the EZ1 Advanced XL.	9018699
Rotor-Gene Q 2plex Platform	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001550

* A customized EZ1 Advanced XL DNA Investigator Card with reduced elution volumes can be ordered by a special request to your local QIAGEN representative.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.