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TECHNICAL NOTE

Updated AutoMate Express™ Extraction System protocol card with variable elution volumes

The purpose of this document is to provide information regarding a new protocol card available for the AutoMate Express Forensic DNA Extraction System (PN 4441763) for use with the PrepFiler Express™ (PN 4441352, for Japan PN 4479052) and PrepFiler Express BTA™ Forensic DNA Extraction Kits (PN 4441351, for Japan PN 4479051). The AutoMate Express Forensic DNA Extraction System is a bench-top, automated nucleic acid purification instrument that utilizes the PrepFiler Express and PrepFiler Express BTA chemistries to isolate and purify DNA from up to 13 samples simultaneously. To address the emerging needs of the forensic community, a new protocol card has been developed to enable seven elution volumes ranging from 20 to 250µL.

The available elution volumes on the new protocol card are 20µL, 30µL, 40µL, 50µL, 100µL, 200µL and 250µL. The higher elution volumes (>50µL) may be considered for high quality/high quantity samples (for example, reference samples), while lower elution volumes (<50µL) may be considered for low quantity samples (for example, touch samples). The only modification to the new protocol card is the inclusion of additional elution volumes; there have been no changes made to the PrepFiler chemistry or the AutoMate Express Instrument.

The following summarizes the experiments and the analysis performed at Thermo Fisher Scientific to demonstrate the functionality of the new protocol card and the effect of different elution volumes on eluted DNA concentration, DNA recovery and STR profile quality.

Materials and Methods

Sample Types Evaluated

Liquid blood samples were taken from a single male individual (Male A). Saliva samples were collected on buccal swabs (Puritan® Standard Sterile Cotton Swabs, Ref 25-806 1WC) from a second male individual (Male B). To minimize sample input variation, after lysis incubation the lysate from the same sample and protocol type were pooled (for example, all blood lysates from the standard PrepFiler protocol). For the PrepFiler standard protocol, the pooled lysate was aliquoted into 500µL aliquots for extraction on the AutoMate Express Instrument. For the PrepFiler BTA protocol, the pooled lysate was aliquoted into 230µL aliquots for extraction on the AutoMate Express Instrument.

Summary of Studies

1. Lysate preparation

1.1 Blood lysate preparation for PrepFiler Express standard protocols

1.1.1 Prepared 15mL fresh PrepFiler lysis solution by mixing 15mL PrepFiler lysis buffer with 150µL freshly-prepared 1M DTT in a 50mL conical tube.

1.1.2 Added 30µL human blood from Male A to the above prepared 15mL PrepFiler lysis solution. Mixed by vortexing.

1.1.3 Aliquoted 1.5mL blood/PrepFiler lysis solution into ten 1.5mL low-bind tubes.
1.1.4 Carried out the lysis incubation on a thermal mixer at 750 rpm, 70^0C for 40 minutes.
1.1.5 After lysis incubation, transferred lysate from each of the 1.5mL tubes to the original 50mL conical tube. Mixed by vortexing.

1.2 Saliva lysate preparation for PrepFiler Express standard protocols

1.2.1 Prepared 15mL fresh PrepFiler lysis solution by mixing 15mL PrepFiler lysis buffer with 150µL freshly-prepared 1M DTT in a 50mL conical tube.
1.2.2 Suspended two buccal swabs heads from Male B into the above prepared 15mL PrepFiler lysis solution.
1.2.3 Removed and discarded both swab heads after re-suspension. Mixed the lysate by vortexing.
1.2.4 Aliquoted 1.5mL buccal/PrepFiler lysis solution into ten 1.5mL low-bind tubes.
1.2.5 Carried out the lysis incubation on a thermal mixer at 750 rpm, 70^0C for 40 minutes.
1.2.6 After lysis incubation, transferred lysate from each of the 1.5mL tubes to the original 50mL conical tube. Mixed by vortexing.

1.3 Blood lysate preparation for PrepFiler Express BTA protocols

1.3.1 Prepared 7mL fresh PrepFiler BTA lysis solution by mixing 6.6mL PrepFiler BTA lysis buffer with 60µL freshly-prepared 1M DTT and with 210µL Proteinase K in a 15mL conical tube.
1.3.2 Added 30µL human blood from Male A to the above prepared 7mL PrepFiler BTA lysis solution. Mixed by vortexing.
1.3.3 Aliquoted 1mL blood/PrepFiler BTA lysis solution into each of the seven 1.5mL low-bind tubes.
1.3.4 Carried out the lysis incubation on a thermal mixer at 750 rpm, 56^0C for 40 minutes.
1.3.5 After lysis incubation, transferred lysate from each of the 1.5mL tubes to the original 15mL conical tube. Mixed by vortexing.

1.4 Saliva lysate preparation for PrepFiler Express BTA protocols

1.4.1 Prepared 7mL fresh PrepFiler BTA lysis solution by mixing 6.6mL PrepFiler BTA lysis buffer with 60µL freshly-prepared 1M DTT and with 210µL Proteinase K in a 15mL conical tube.
1.4.2 Suspended two buccal swab heads from Male B into the above prepared 7mL PrepFiler BTA lysis solution.
1.4.3 Removed and discarded both swab heads after re-suspension. Mixed the lysate by vortexing.
1.4.4 Aliquoted 1mL buccal/PrepFiler lysis solution into each of the seven 1.5mL low-bind tubes.
1.4.5 Carried out the lysis incubation on a thermal mixer at 750 rpm, 56^0C for 40 minutes.
1.4.6 After lysis incubation, transferred lysate from each of the 1.5mL tubes to the original 15mL conical tube. Mixed by vortexing.

2. DNA extraction

Either 500µL (standard protocol) or 230µL (BTA protocol) of blood or saliva lysate were aliquoted into separate PrepFiler sample tubes. There were a total of 21 sample tubes for each lysate preparation described in Section 1, so that each sample was extracted in triplicate with each of the seven elution volumes. Extraction was carried out on the Automate Express Instrument using the desired protocol and different elution volumes.

3. DNA quantification with the Quantifiler™ Trio DNA Quantification Kit

Each extracted DNA sample was quantified twice using the Quantifiler Trio kit (PN 4482910) per the procedure described in the Quantifiler HP and Trio DNA Quantification Kits User Guide (PN 4485354, Rev F). Samples with a concentration greater than 0.067ng/ul were diluted to a concentration of 0.067ng/ul for amplification.

4. STR Amplification with the GlobalFiler™ PCR Amplification Kit

Three replicates for each sample were amplified using the GlobalFiler PCR Amplification kit (PN 4476609) per the GlobalFiler PCR Amplification Kit User Guide (PN 4477604, Rev E). Samples with a concentration greater than 0.067ng/ul were diluted to a concentration of 0.067ng/ul and 15ul of each sample were amplified.

5. Capillary Electrophoresis and data Analysis

Amplified products were typed on the Applied Biosystems 3500 or 3500xl Genetic Analyzer according to the conditions recommended in the GlobalFiler PCR Amplification Kit User Guide. A peak amplitude threshold (PAT) of 175 RFU was implemented for data analysis using the GeneMapper™ ID-X software.

Results of Studies

<u>Blood and saliva samples extracted and purified using PrepFiler standard protocols of seven different elution volumes.</u>

As shown in Figures 1 and 2, DNA concentration increases with decreasing elution volume; therefore, the use of a smaller elution volume may increase the DNA concentration, offering an optimal workflow for low input DNA samples.

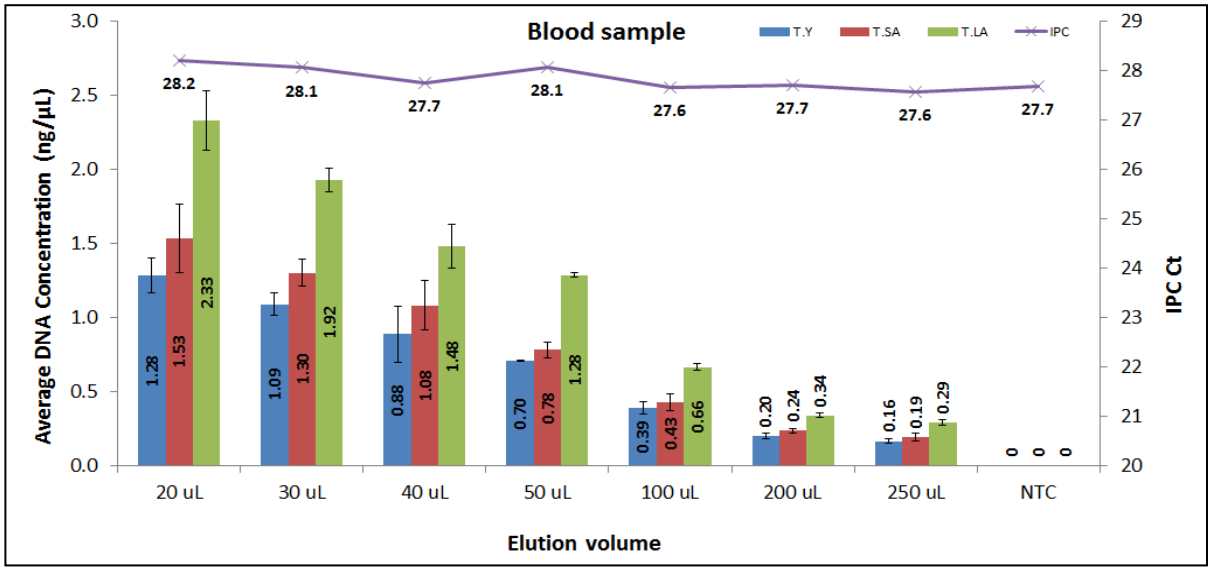

Figure 1: The effect of elution volume on DNA concentration of blood samples processed using PrepFiler standard protocols and the Quantifiler Trio kit. Samples were run in triplicate and the average concentraiton is reported. The error bars represent +/- 1SD (Standard Deviation). T.Y: male target; T.SA: small autosomal

target; T.LA: large autosomal target; IPC: internal positive control.

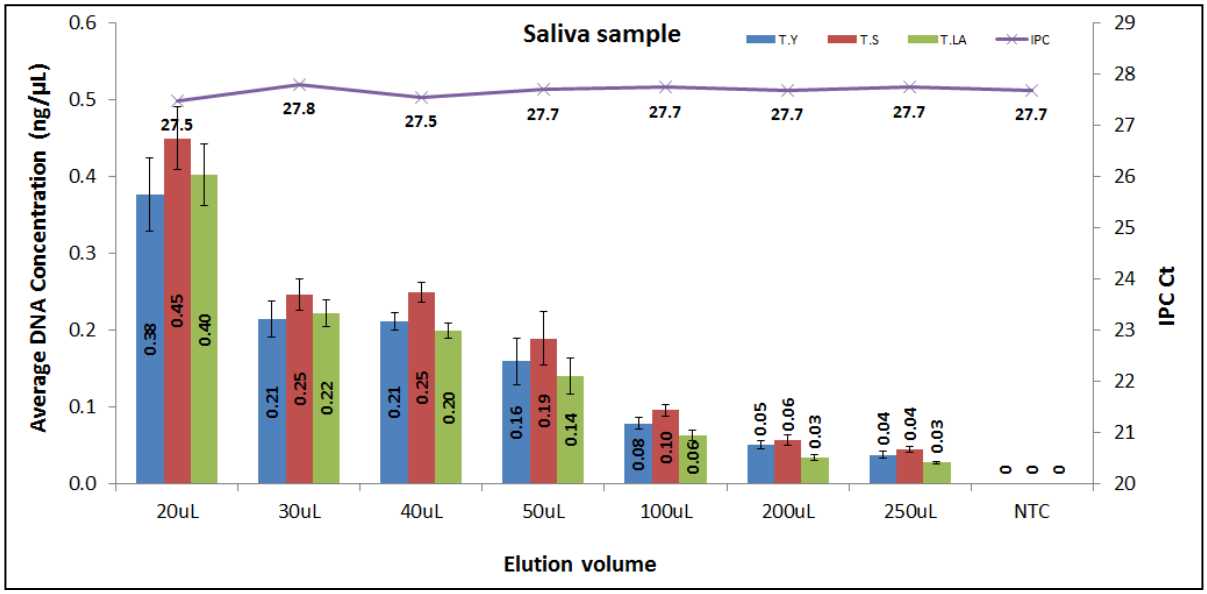

Figure 2: The effect of elution volume on DNA concentration of saliva samples processed using PrepFiler standard protocols and the Quantifiler Trio kit. Samples were run in triplicate and the average concentraiton is reported. The error bars represent +/- 1SD . T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

To calculate the total amount of DNA in the final elution, the average concentration (ng/µL) was multipled by the elution volume and is displayed in Figures 3 and 4. When the lowest (20µL) average elution volume was compared to the standard (50µL) average elution volume, the blood sample showed DNA recovery of approximately 80%, and the saliva sample had comparable recovery of approximately 95%.

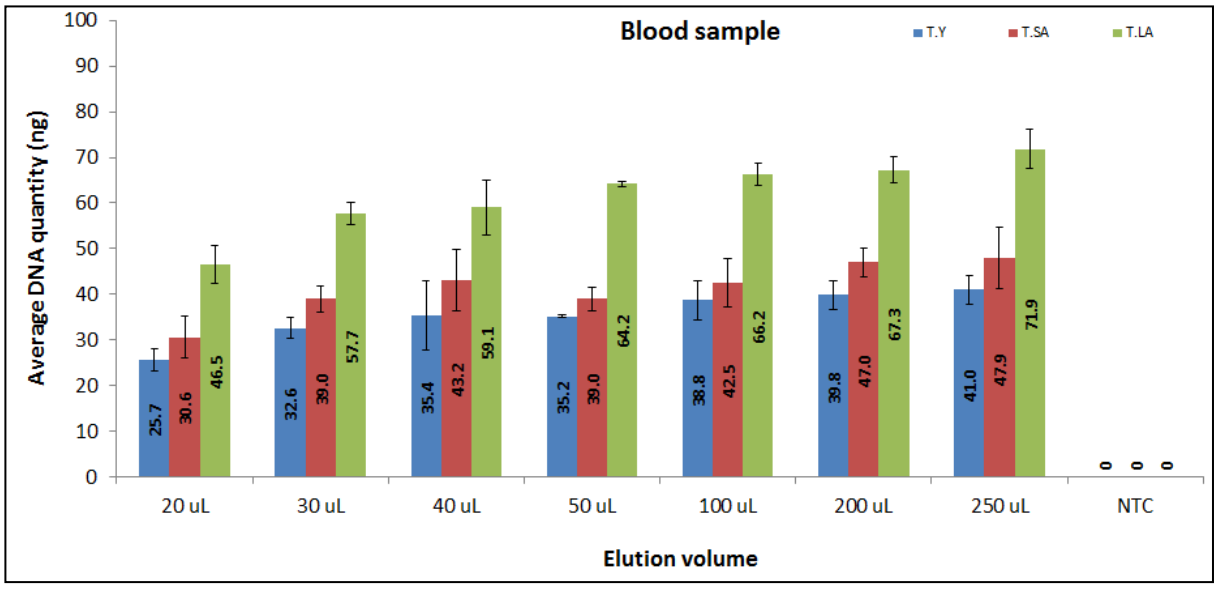

Figure 3: The effect of elution volume on DNA recovery of blood samples

processed using PrepFiler standard protocol and the Quantifiler Trio kit. Samples were run in triplicate and the average concentration is reported. The error bars represent +/- 1SD. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

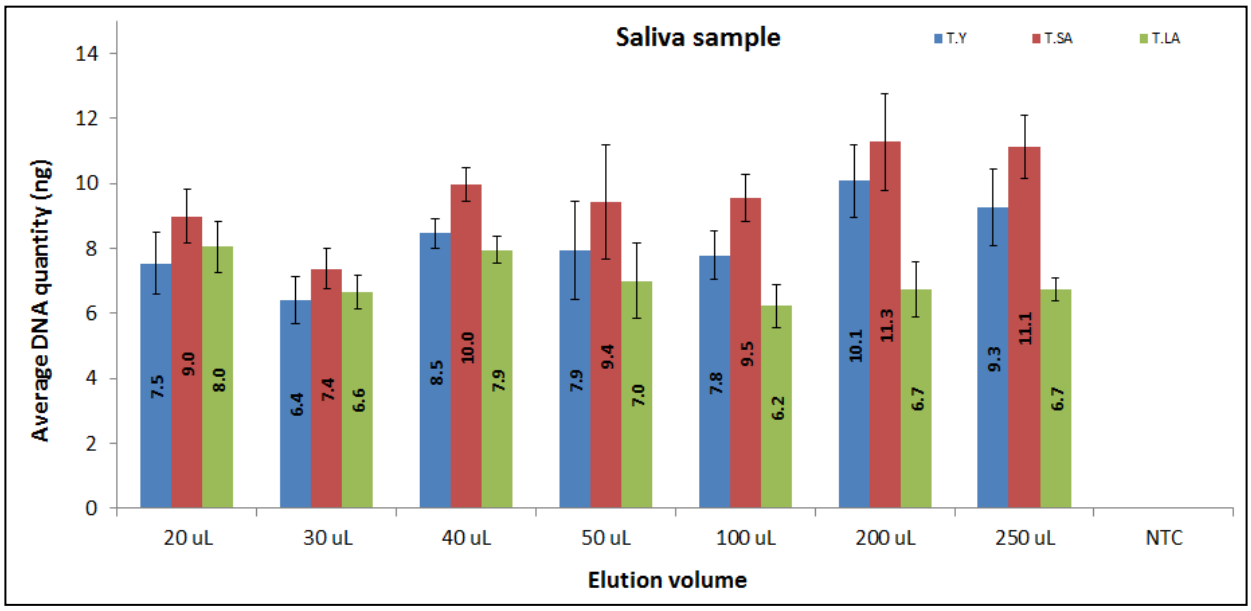

Figure 4: The effect of elution volume on DNA recovery of saliva samples processed using PrepFiler standard protocol and the Quantifiler Trio kit. Samples were run in triplicate and the average concentration is reported. The error bars represent +/- 1SD. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

Full, well-balanced profiles were generated using the GlobalFiler PCR Amplification Kit for all samples amplified (Figures 5 and 6). 1ng of total DNA was targeted for all samples, with the exception of the 200ul and 250ul saliva extracts in which 0.75ng and 0.63ng were targeted, respectively.

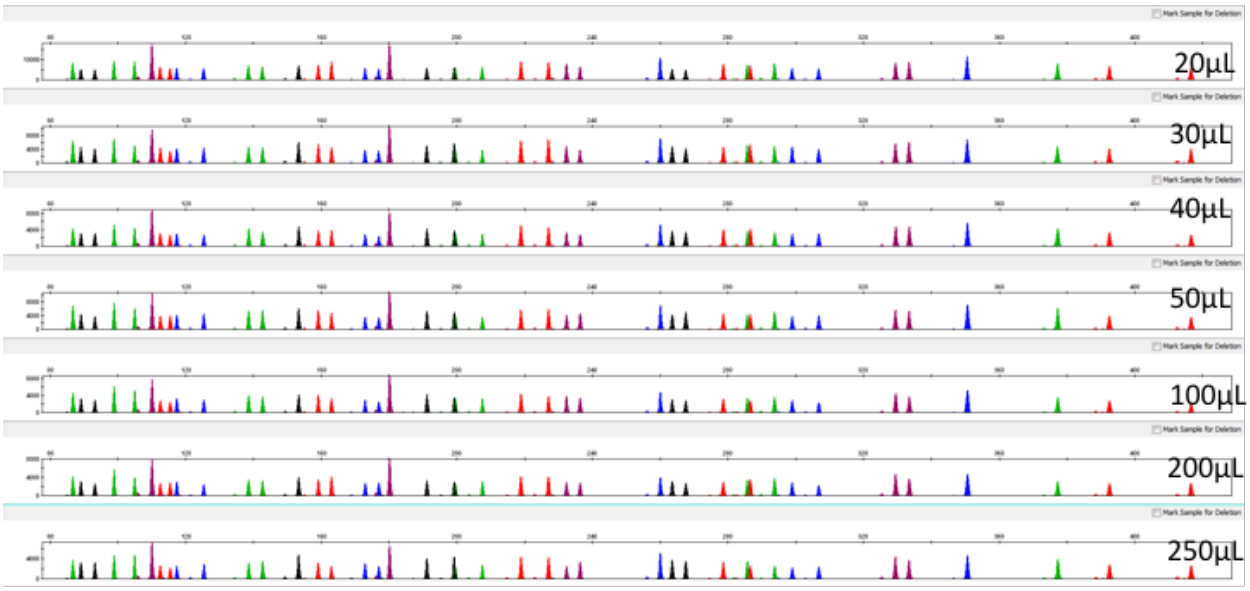

Figure 5: Overlay of all dye channels in the electropherogram for one replicate of each dilution of the blood samples tested with the standard PrepFIler protocol. The Y-axis is scaled from 7,000 RFU for the 250µL volume to 17,000 RFU for the 20µL volume.

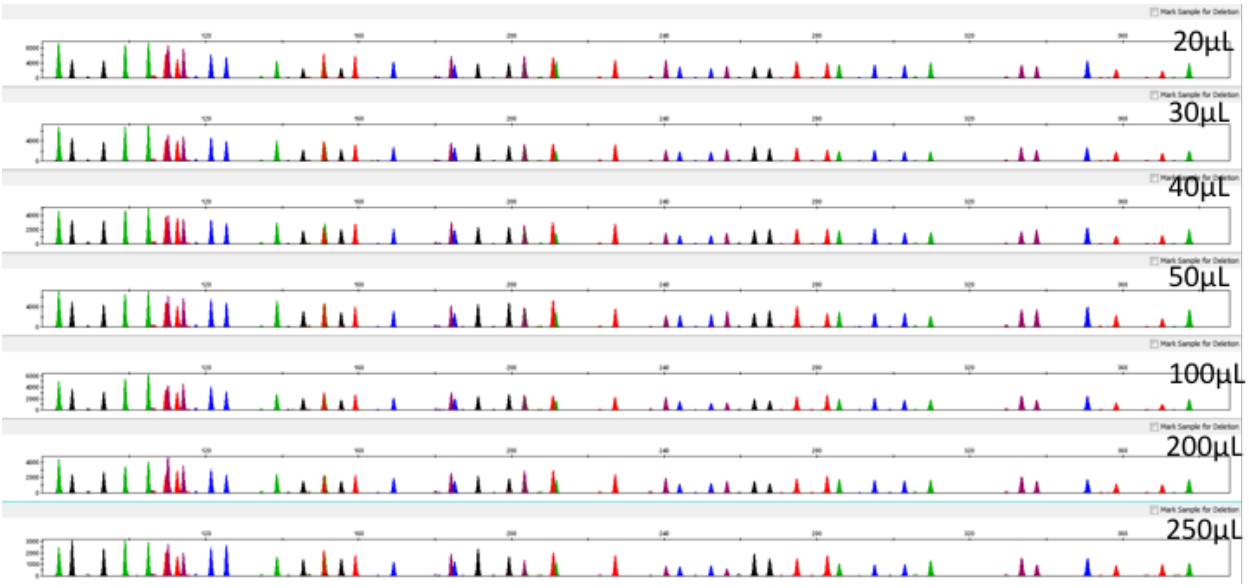

Figure 6: Overlay of all dye channels in the electropherogram for one replicate of each dilution of the saliva samples tested with the standard PrepFIler protocol. The Y-axis is scaled from 3,000 RFU for the 250µL volume to 10,000 RFU for the 20µL volume.

Blood and saliva samples extracted and purified using PrepFiler BTA protocols of seven different elution volumes.

As shown in Figures 7 and 8, DNA concentration increases with decreasing elution volume; therefore, the use of a smaller elution volume may increase the DNA concentration, offering an optimal workflow for low input DNA samples.

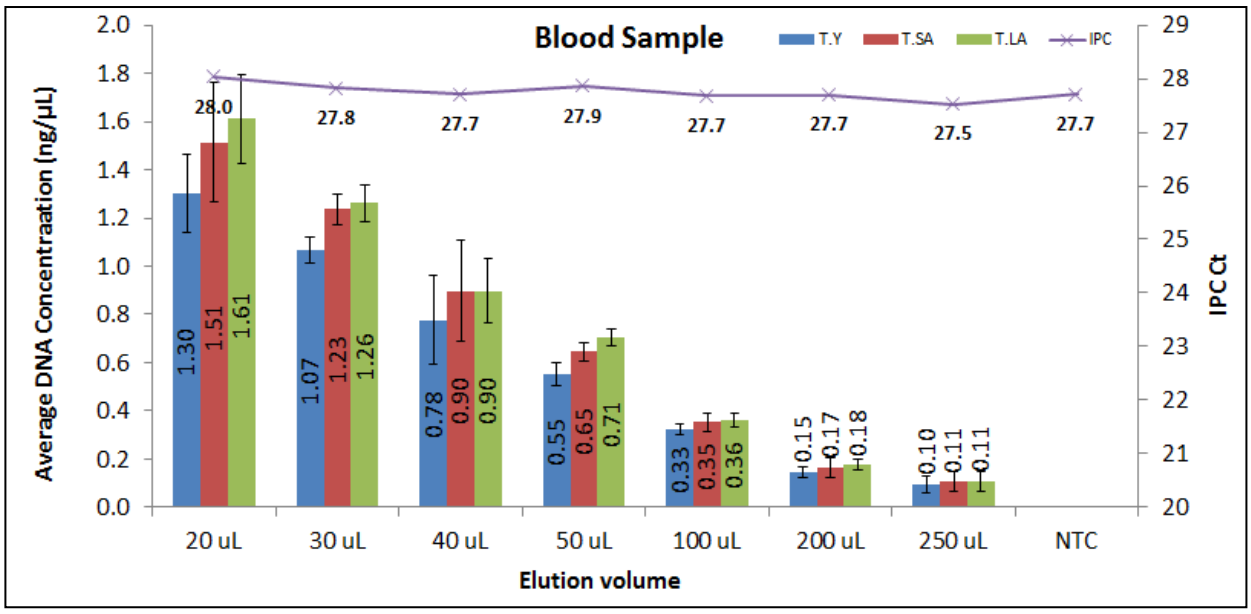

Figure 7: The effect of elution volume on DNA concentration of blood samples processed using PrepFiler BTA protocols and the Quantifiler Trio kit. Samples were run in triplicate and the average concentration is reported. The error bars represent +/- 1SD. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

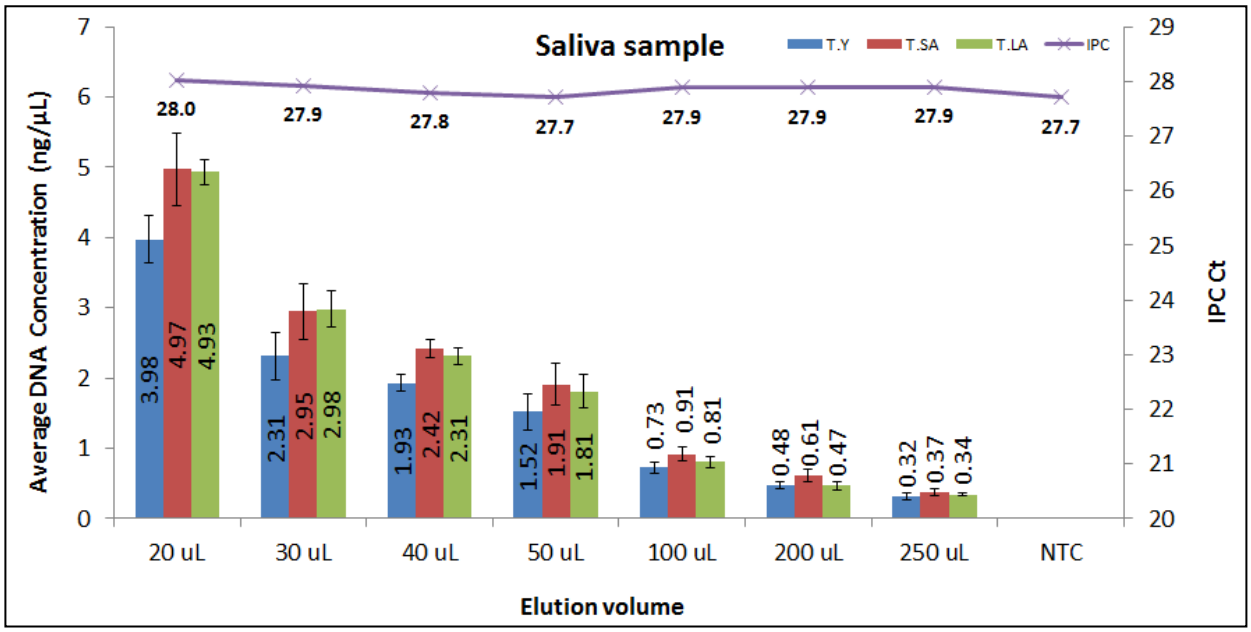

Figure 8: The effect of elution volume on DNA concentration of saliva samples processed using PrepFiler BTA protocols and the Quantifiler Trio kit. Samples were run in triplicate and the average concentration is reported. The error bars represent +/- 1SD. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

To calculate the total amount of DNA in the final elution, the average concentration (ng/µL) was multipled by the elution volume and is displayed in Figures 9 and 10. When the lowest (20µL) average elution volume was compared to the standard (50µL) average elution volume, the blood sample showed comparable DNA recovery of approximately 95%, and the saliva sample had approximately 5% more DNA recovered.

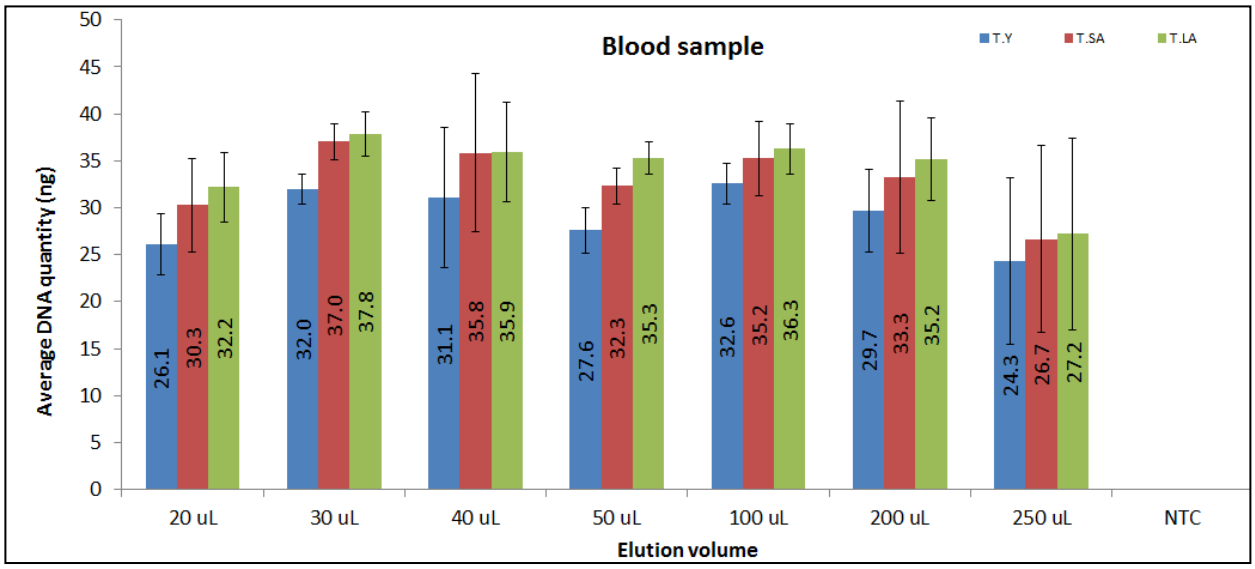

Figure 9: The effect of elution volume on DNA recovery of blood samples processed using PrepFiler BTA protocol and the Quantifiler Trio kit. Samples were run in triplicate and the average concentration is reported. The error bars represent +/- 1SD. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

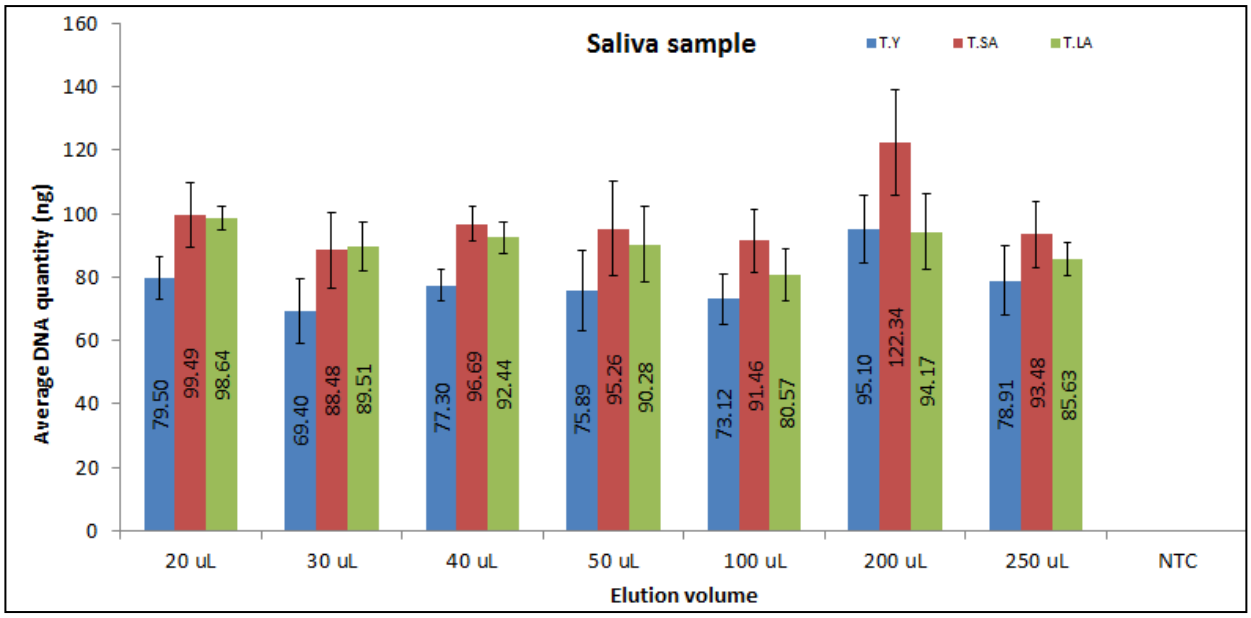

Figure 10: The effect of elution volume on DNA recovery of saliva samples processed using PrepFiler BTA protocol and the Quantifiler Trio kit. Samples were run in triplicate and the average concentration is reported. The error bars represent +/- 1SD. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

Full, well-balanced profiles were generated using the GlobalFiler PCR Amplification Kit for all samples amplified (Figures 11 and 12). 1ng of total DNA was targeted for all samples.

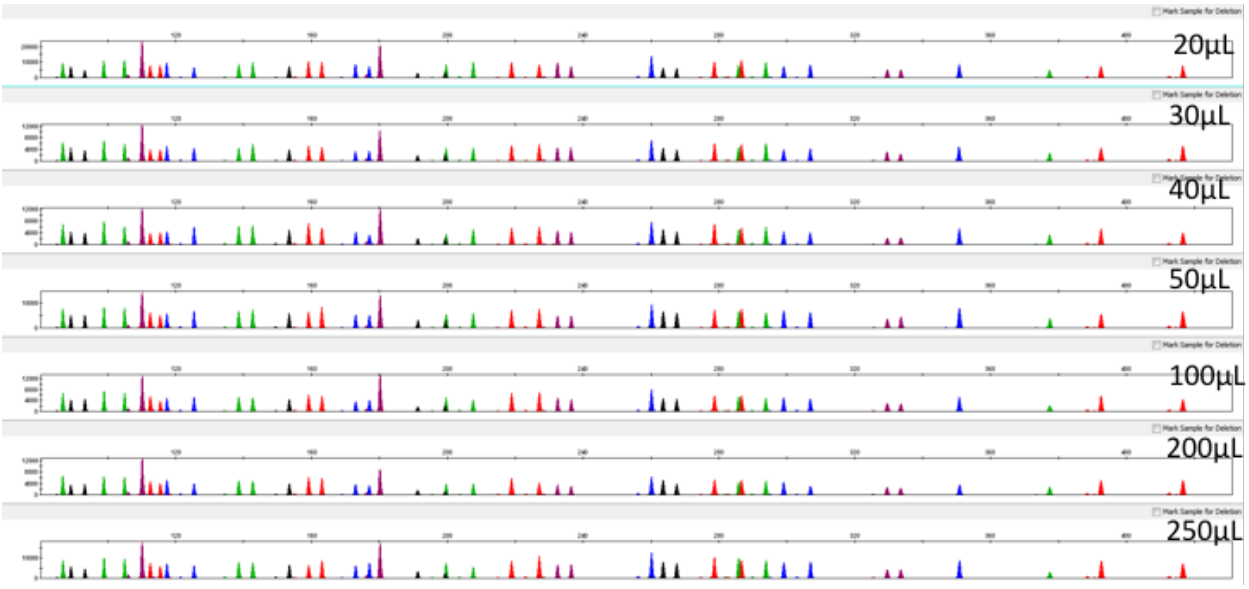

Figure 11: Overlay of all dye channels in the electropherogram for one replicate of each dilution of the blood samples tested with the PrepFIler BTA protocol. The Y-axis is scaled from 12,000 RFU for the 40µL volume to 22,000 RFU for the 20µL volume.

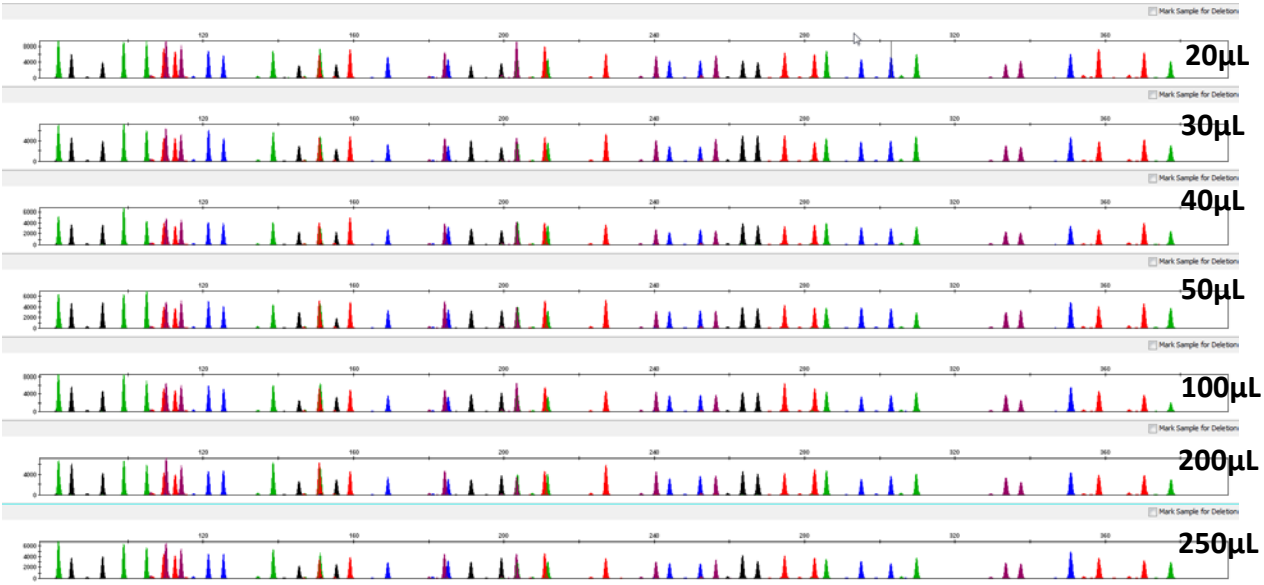

Figure 12: Overlay of all dye channels in the electropherogram for one replicate of each dilution of the saliva samples tested with the PrepFIler BTA protocol. The Y-axis is scaled from 7,000 RFU for the 30µL volume to 9,000 RFU for the 20µL volume.

Conclusions

The new protocol card enables six additional elution volumes as well as the original 50µL including 20µL, 30µL, 40µL, 100µL, 200µL and 250µL. The studies performed demonstrate that DNA concentration increases with decreasing elution volume for both blood and saliva samples. DNA recovery is relatively consistent among the seven elution volumes with saliva samples. We observed a slight reduction (approximately 20%) in DNA recovery with the 20 µL elution volume when blood samples were tested. Elution volume also has no

negative impact on GlobalFiler STR profile quality. The higher elution volumes (>50µL) may be considered for high quality/high quantity samples (for example, reference samples), while lower elution volumes (<50µL) may be considered for low quantity samples (for example, touch samples).

Applicability to Other Sample Types and Chemistries

The content of this technical note and the data generated were specific for Quantifiler Trio and GlobalFiler chemistry run on a 3500 series instrument. Given that the mechanisms of quantification and amplification after extraction are the same, the results obtained and conclusions generated are highly likely to be applicable to other HID STR chemistries and instrumentation. Laboratories should perform the necessary internal validation studies to ensure that the differences in elution volumes perform as expected in the laboratory system.

Revision History

Revision	Date	Description
A	3/1/2017	Initial publication.