A Comparison Study of DNA Collected Using Reference Collection Devices for Improved Lab Efficiency

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INTRODUCTION

DNA has been established as the gold standard in the field of forensic biology for over a decade. Due to this, collecting and preserving DNA is crucial to the success of forensic testing as a complete reference profile is often necessary for generating accurate statistics. Maintaining a simple, yet effective reference collection procedure is not only beneficial to the sample donor, but also the analyst processing samples in the lab. Gentueri® Inc. is a DNA preservation company with the goal of developing devices that are not only user friendly, but also convenient in design and storage. The Gentueri® products being tested in this study are the GenSwab™ generation 1 and 2 DNA collection devices. The GenSwab™ device is an oral collection tool including a foam pad that is rubbed on the inside of the donor's cheeks and then folded, allowing for the foam pad to rest on the sample transfer area of an FTA-like card. In this study, the Gentueri® products have been evaluated and compared to another reference collection device, EasiCollect.

METHODS

Sample Preparation:
- 20 Buccal samples per device were obtained (13F, 7M donor pool)
- After overnight drying, a 1.5 mm punch was taken from each device

Extraction and Quantitation Workflow:
- AutoMate *Express*™ Incubation Settings: 850 RPM for 80 minutes at 80ºC
  - PrepFiler *Express*™ DNA Extraction Kit
  - Elution Volume – 100 µl
  - N = 20 per device, 60 samples total
- EZ1® Incubation Settings: 850 RPM for 15 minutes at 56ºC
  - EZ1® DNA Investigator® Kit
  - Elution Volume - 100 µl
  - N = 5 per device, 15 samples total
  - Statistical Analyses: ANOVA ,Tukey Test, and Descriptive Statistics were generated to analyze the amount of DNA collected using each device type and method

AutoMate *Express* ™ System

EZ1® Advanced XL System

7500 RT PCR System with Quantifiler ™ Trio

DNA Profile Comparison Workflow:
- Four of each sample type with varying DNA concentrations were randomly selected for STR genotyping
- Amplification Settings: GlobalFiler™ Full Scale Reaction
- Injection Parameters: 10 second injection
- Electropherogram Software: GeneMapper® IDX

Applied Biosystems™ 9700 Thermocycler
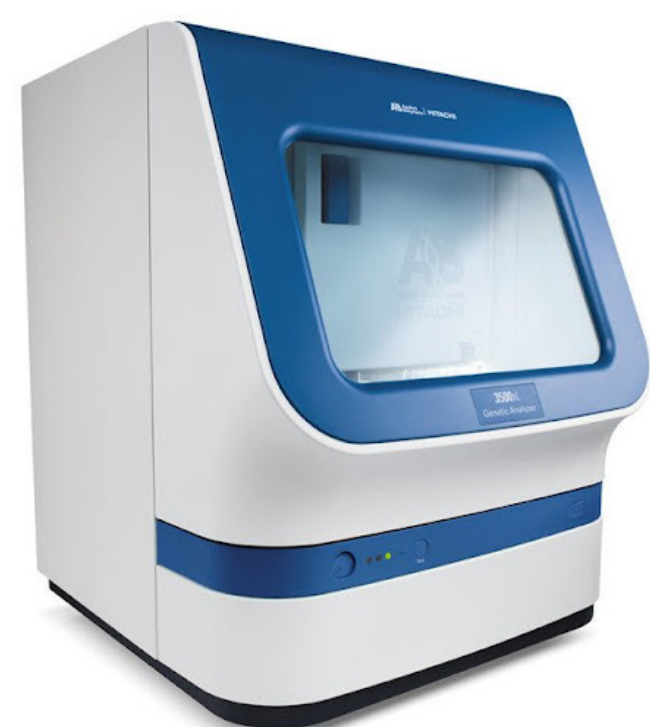
Applied Biosystems™ 3500 Genetic Analyzer

RESULTS

DNA Quantification Results

AutoMate™ Method	Avg.(ng)	N=	Min (ng)	Max (ng)	St. Dev.
GenSwab™	7.32	20	0.62	20.7	5.34
GenSwab™2	10.4	20	0.95	27.8	7.65
EasiCollect	13.6	20	4.37	23.9	5.41
EZ1® Method	Avg.(ng)	N=	Min (ng)	Max (ng)	St. Dev.
GenSwab™	1.28	5	0.88	1.80	0.47
GenSwab™2	9.24	5	1.53	23.0	9.31
EasiCollect	5.16	5	1.60	10.8	3.75

ANOVA for AutoMate™ Method

Source	DF	Sum Sq.	Mean Sq.	F Value	Pr > F
Device	2	376.9	188.45	4.86	0.0115

ANOVA for EZ1® Method

Source	DF	Sum Sq.	Mean Sq.	F Value	Pr > F
Device	2	141.58	70.79	1.93	0.1914

AutoMate™ Tukey Multiple Comparison Test
Dependent Variable: DNA (ng)

i/j	1	2	3
1	-	**0.0081**	0.2676
2	**0.0081**	-	0.2768
3	0.2676	0.2768	-

*1: EasiCollect 2: GenSwab™ 3: GenSwab™2 α=0.05

EZ1® Tukey Multiple Comparison Test
Dependent Variable: DNA (ng)

i/j	1	2	3
1	-	0.6195	0.5539
2	0.6195	-	0.169
3	0.5539	0.169	-

*1: EasiCollect 2: GenSwab™ 3: GenSwab™2 α=0.05

QUANTITATION RESULTS

Figure 1: Left – Quantification results for the AutoMate *Express*™ method. **Right** – A comparison of the quantification results using the EZ1®(green) and AutoMate *Express*™(blue) methods.
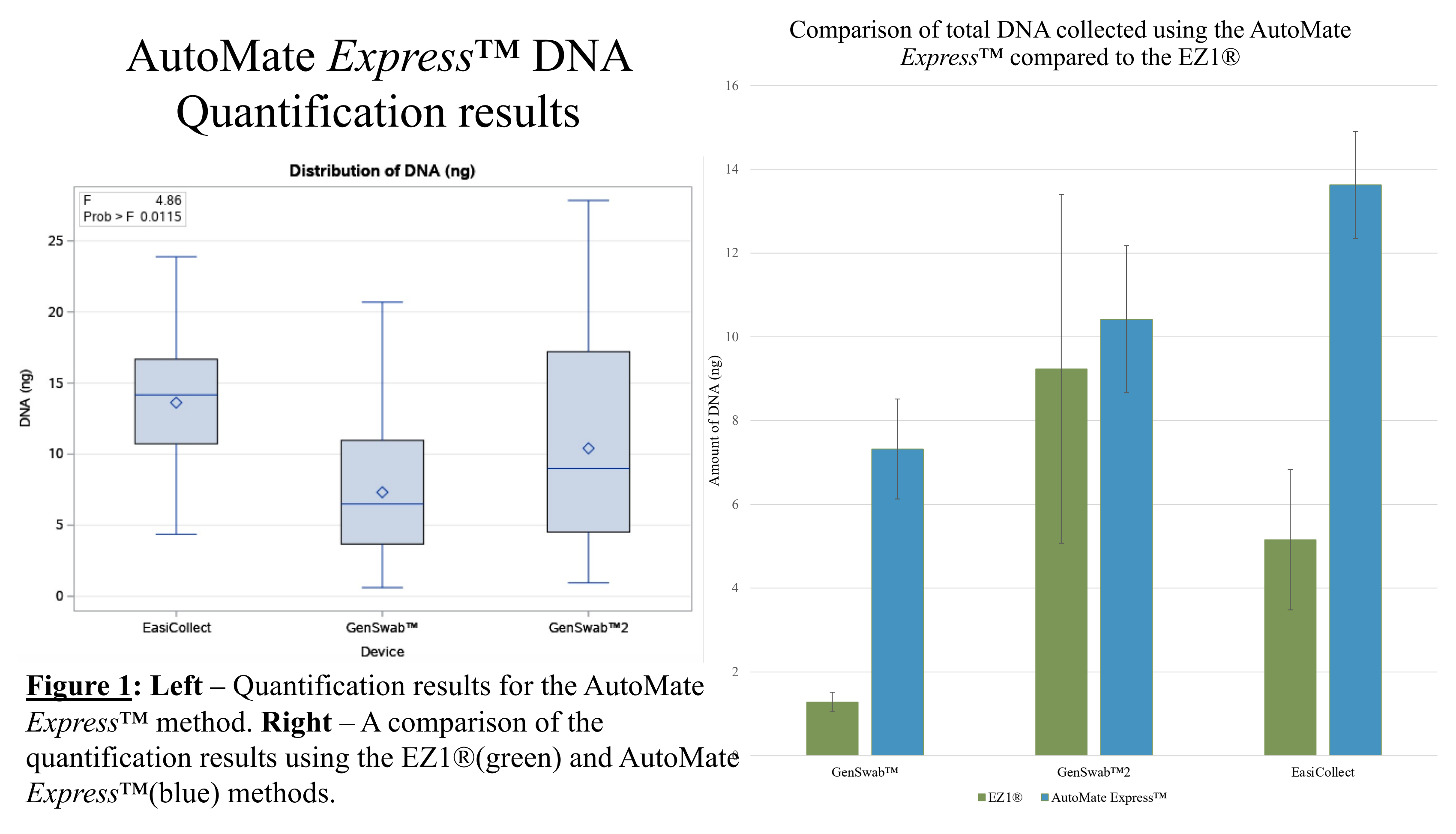
DNA PROFILE RESULTS

Sample	Avg. RFU Value
GS_2	3398
GS_5	365.4
GS_9	620.6
GS_18	651.5
Avg. RFU GS1	545.8
GS2_4	170.1
GS2_10	1034
GS2_13	679.7
GS2_19	1531
Avg. RFU GS2	853.7
EC_2	1046
EC_3	1032
EC_5	203.2
EC_14	1143
Avg. RFU EC	856.1
Overall Avg. RFU	751.9

Figure 2: RFU value comparison between devices of the 12 samples selected for STR analysis.

DNA PROFILE RESULTS

Figure 3: Electropherograms from samples GS_5, GS2_10, and EC_2.
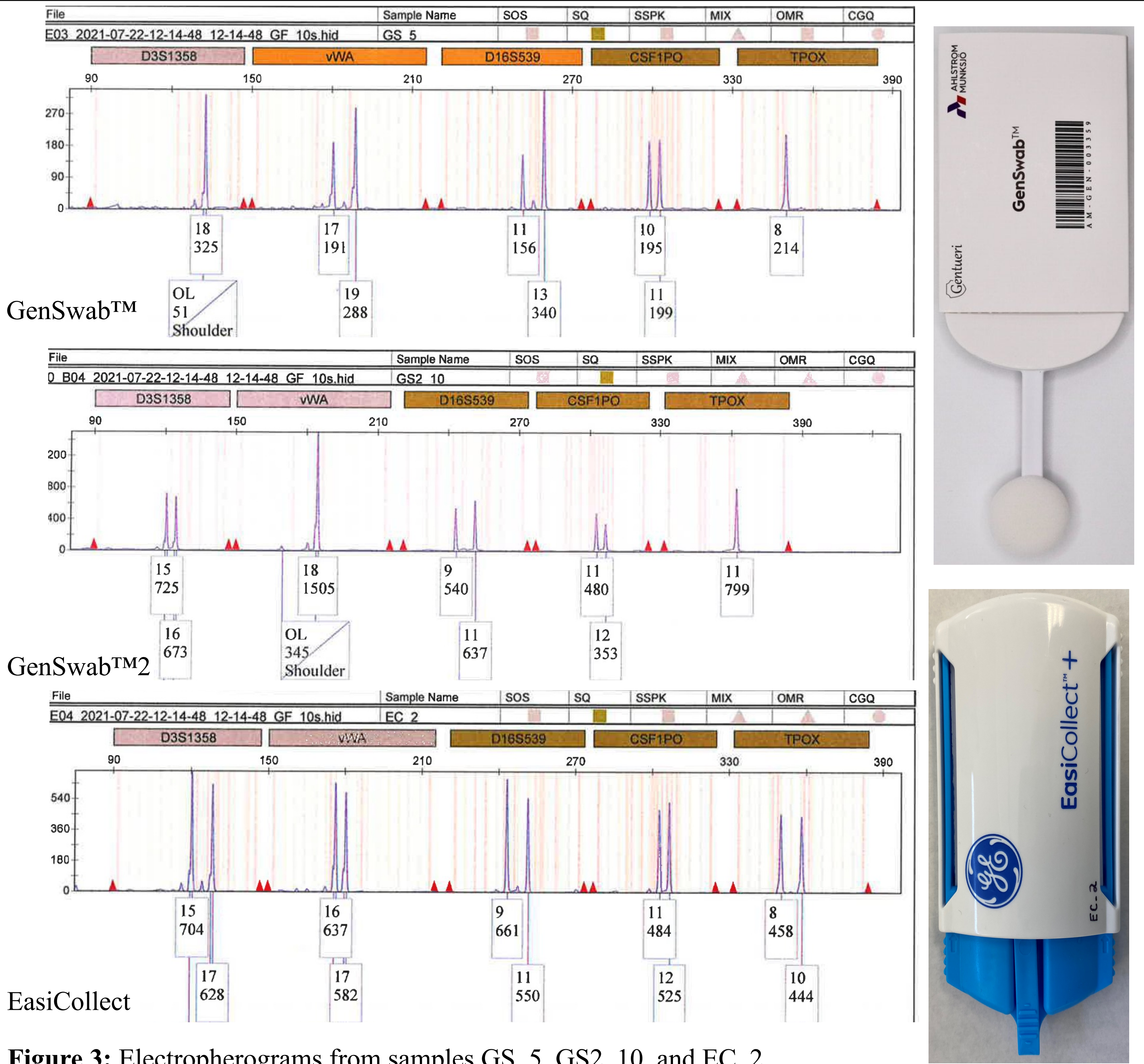
DISCUSSION

- The AutoMate *Express*™ and EZ1® methods both performed successfully using all three device types.
- Sufficient DNA was obtained after extraction in order to produce complete electropherograms for all 12 samples chosen.
- The ANOVA indicated that there was a significant difference in performance between the three devices using the AutoMate *Express*™ method.
- The Tukey Test analyzing the AutoMate *Express*™ method demonstrated that there was a significant difference in performance between the GenSwab™ and EasiCollect devices (p = 0.0081). There was no significant difference in performance between GenSwab™ and GenSwab™2 devices or GenSwab™2 and EasiCollect devices (p > 0.05).
- The ANOVA demonstrated that there was no significant difference in performance between all three devices using the EZ1® method (p > 0.05).
- The data demonstrated that utilizing the AutoMate *Express*™ method optimized the DNA collection for each of the three devices tested in the study.

ACKNOWLEDGEMENTS