Validation Report February 2022

Developmental validation of the EZ1&2® DNA Investigator® Kit on the EZ2® Connect Fx instrument

The EZ1&2 DNA Investigator Kit (cat. no. 952034) is designed for automated purification of total DNA from samples encountered in forensic and human identity applications. Proven magnetic bead technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as quantitative PCR amplification reactions, STR, or NGS analyses. Purified DNA is free of proteins, nucleases, and inhibitors. Pretreatment protocols are available for various typical casework or reference sample types.

The kit is compatible with automated extraction on EZ1 instruments and the EZ2 Connect Fx. Various instrument protocols are available to encompass different sample lysate volumes, normalized extraction, and lysates containing solid sample substrates. The EZ2 Connect Fx allows the elution of purified DNA in as little as 20 µl, providing maximum sensitivity for challenging samples.

The performance of the EZ1&2 DNA Investigator Kit was evaluated with regard to various sample types and conditions commonly encountered in forensic and parentage laboratories. Sensitivity, reproducibility, and freedom from cross-contamination were tested. Typical casework sample types were extracted.

The validation study was based on the recommendations, where applicable, of the European Network of Forensic Science Institutes (ENFSI), and on the validation guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM).

Results of developmental validation

The validation study was performed by the QIAGEN R&D department. For DNA extraction, protocols were followed as described in the *EZ1&2 DNA Investigator Kit Handbook* (**www.qiagen.com/HB-2984**), unless stated otherwise. Automated extraction on the EZ2 Connect Fx was performed using standard Investigator protocols available for corresponding sample types. Quantification of human genomic DNA was performed using the Investigator Quantiplex® Pro Kit. STR analysis was performed with the Investigator 24plex QS Kit; capillary electrophoresis was run on 3500 or 3500XL Genetic Analyzers.

Sensitivity

Sensitivity was tested to determine the suitable range of sample input. Dilutions of blood and saliva (corresponding to 10, 1, 0.1, and 0.01 µl) were extracted in triplicates using the Trace protocol. DNA was eluted in 40 µl TE buffer, and quantification shows linear correlation of input sample amount and purified DNA (Figure 1). The experiments showed linearity for both sample types over the range of input material tested. Full profiles were obtained for all samples (Figures 2 and 3 show examples).

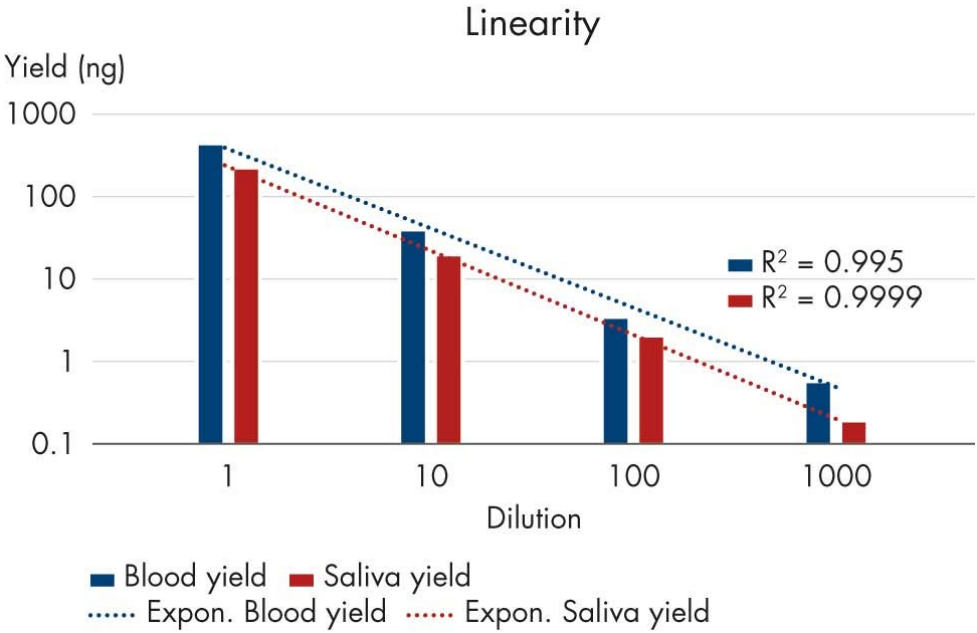

Figure 1. Linearity. Blood and saliva dilutions were extracted in triplicates. The dilution series corresponds to 10, 1, 0.1, and 0.01 µl blood or saliva sample.

Lower elution volumes increase the sensitivity by providing higher concentrations of extracted DNA. The EZ2 Connect Fx provides elution volume options of 20–200 µl water, or TE buffer. To analyze the impact of elution volumes on the total yield and the concentration of samples, 1:10 dilutions of blood were extracted in six replicates per elution volume (Figure 4). Recovered eluate volumes were measured. As expected, the DNA concentration increased from the highest to the lowest elution volume (Figure 5). The total yields observed were in constant range for elution volumes of 40 µl and above. For 20 and 30 µl elution, a slight decrease of total yield was observed (Figure 6). This is expected, as the relative impact of the constant dead volume associated with the beads becomes higher.

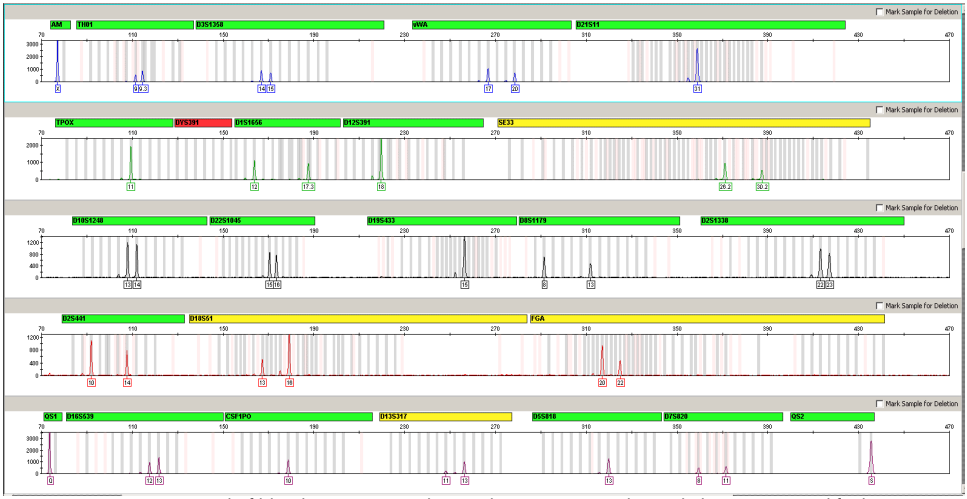

Figure 2. Sensitivity. 0.01 µl of blood were extracted using the Trace protocol. 15 µl eluate were amplified, corresponding to 137 pg DNA template.

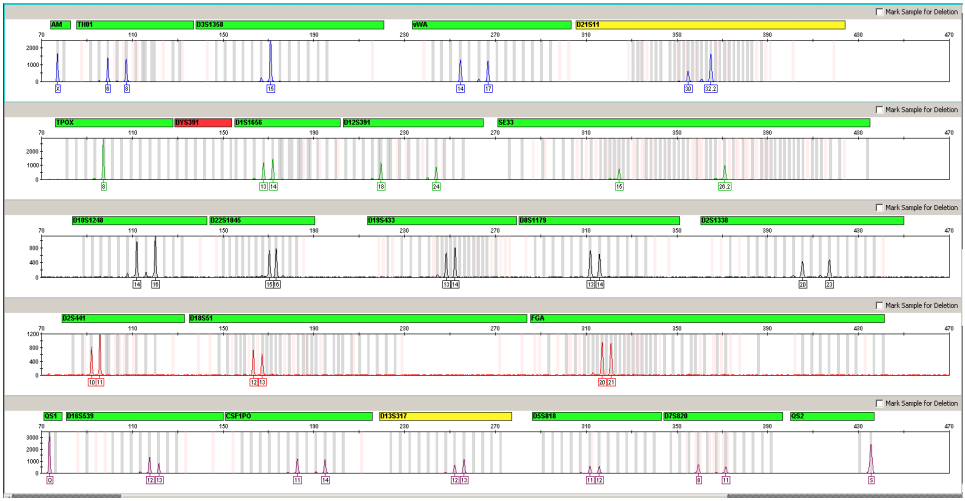

Figure 3. Sensitivity. 0.01 µl of saliva were extracted using the Trace protocol. 15 µl eluate were amplified, corresponding to 93 pg DNA template.

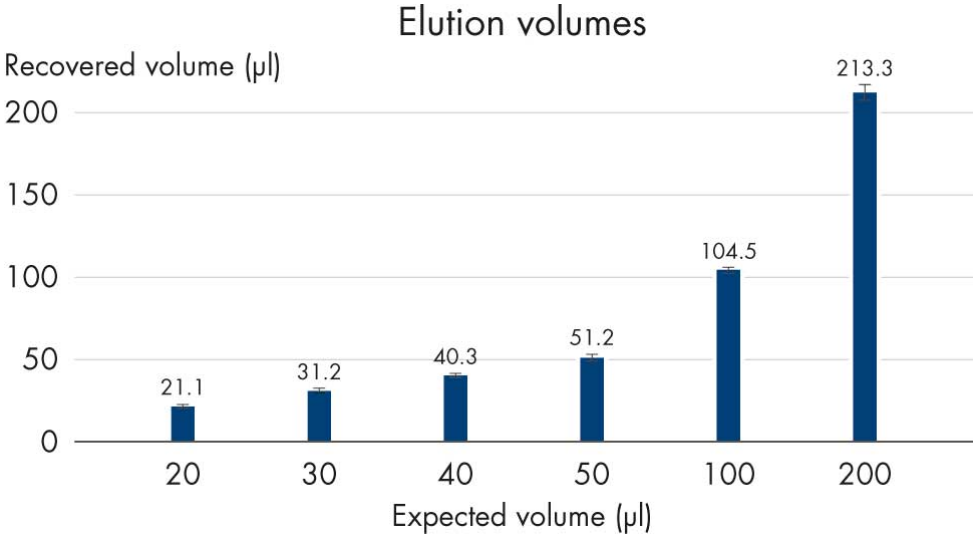

Figure 4. Eluate volumes. Extraction was performed using the Trace protocol with elution in TE buffer. Six replicates were run for each elution volume.

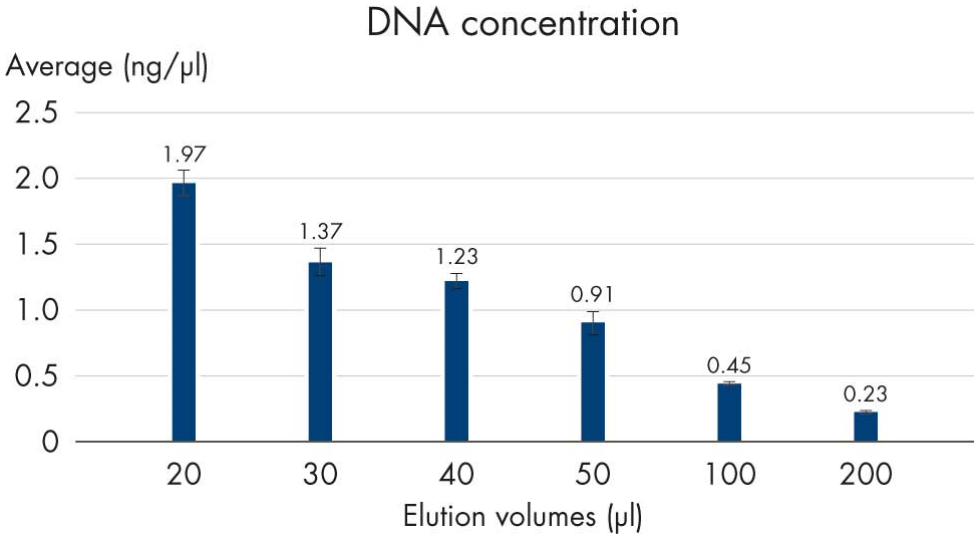

Figure 5. Correlation of DNA concentration and elution volumes. Extraction was performed using the Trace protocol with elution in TE buffer. Six replicates were run for each elution volume.

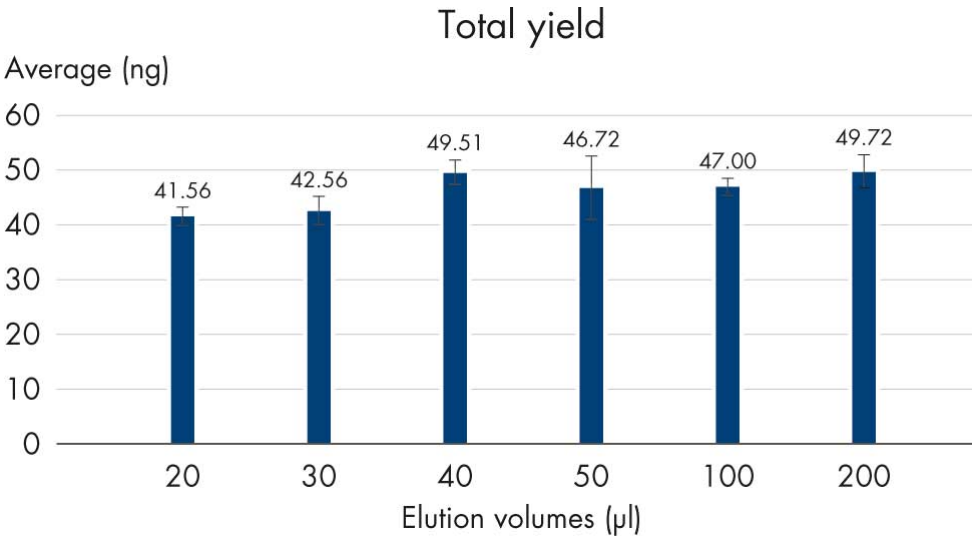

Figure 6. Correlation of total DNA yield and elution volumes. Extraction was performed using the Trace protocol with elution in TE buffer. Six replicates were run for each elution volume. Yields are based on elution volumes measured.

Accuracy

The EZ2 Connect Fx instrument has been designed to replicate protocols available on previous EZ1 instruments. Side-by-side comparisons using the Trace Tip Dance protocol were done to show performance equivalence. Serial dilutions of blood lysates were extracted on the EZ2 Connect Fx, and the EZ1 Advanced XL. Consistent results were obtained across all runs on both instruments (Figure 7).

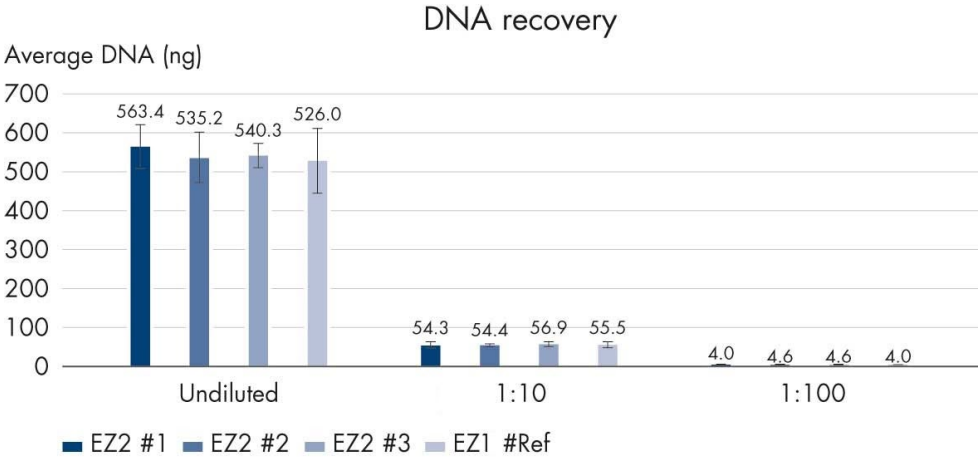

Figure 7. Equivalence of performance on the EZ2 Connect Fx and the EZ1 Advanced XL instrument. The Trace "Tip Dance" protocol was used with elution in 50 µl water. Three runs were performed on one EZ2 Connect Fx instrument, and one run on the EZ1 Advanced XL. Each blood dilution was run in four replicates in each run.

The reproducibility of results was addressed by extracting 2.5 µl of blood and saliva samples on different days. Again, consistent results were obtained (Figure 8).

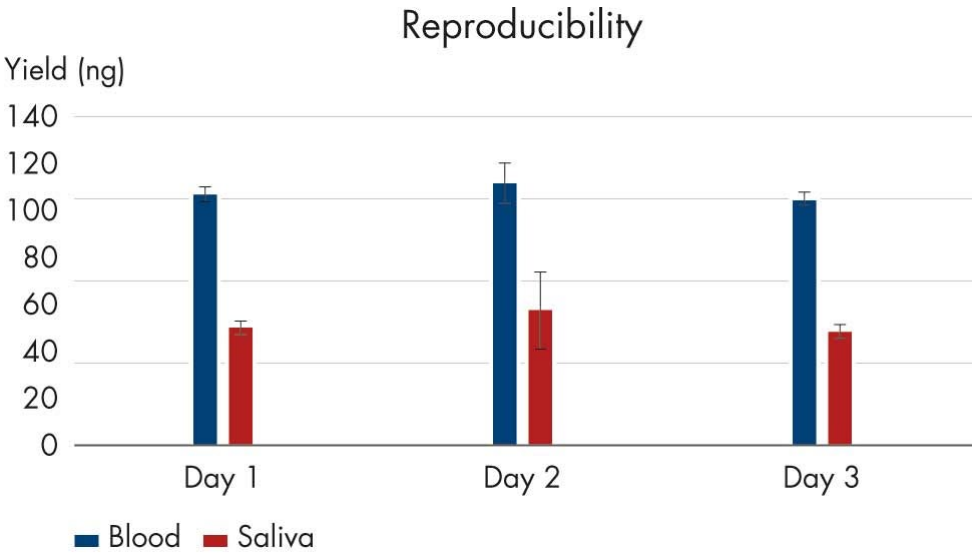

Figure 8. Reproducibility. The Large Volume protocol was used with elution in 50 µl water. Each sample was run in 12 replicates. Three runs were performed on different days.

Freedom from cross-contamination

A cross-contamination study was performed to ensure no contamination between samples takes place within the same run, or in consecutive runs. Twelve buccal swab samples were extracted, alternating with blank extractions. The pattern was reversed in a second run, resulting in a total of 24 blank extractions. The Large Volume protocol was used with an elution volume of 20 µl. For blank extractions, 15 µl eluate were used for STR analysis. No alleles were detected using an analytical threshold of 50 RFU. The presence of the Quality Sensor peaks confirmed successful amplification (Figure 9).

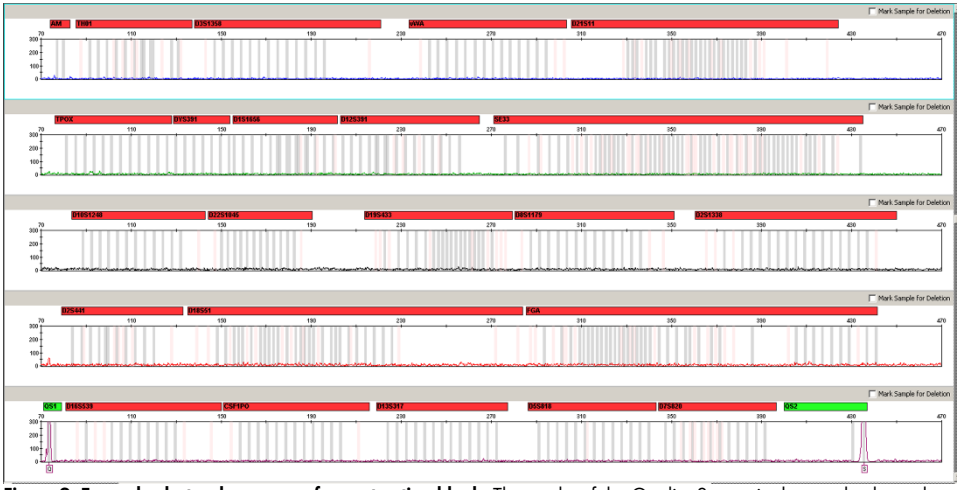

Figure 9. Example electropherogram of an extraction blank. The peaks of the Quality Sensor in the purple channel are truncated due to the scaling of 300 RFU.

Stability

The test was performed to show the ability of the extraction to fully remove inhibitors from samples. Swabs were taken from three environments: (1) dust close to a road, (2) soil, and (3) old engine covered with used oil. Subsequently, 50 µl of blood was spiked onto the area of the swab carrying the potential inhibitors, and the swabs were dried for two days before extraction. The Large Volume protocol was used, and DNA was eluted in 50 µl. During quantification, no shift of the Internal Control was observed (Figure 10), indicating the absence of any inhibition. Full STR profiles were obtained for all samples using an analytical threshold of 50 RFU. All profiles showed the presence of both Quality Sensor peaks, thereby confirming the absence of inhibition (see Figure 11 for an example).

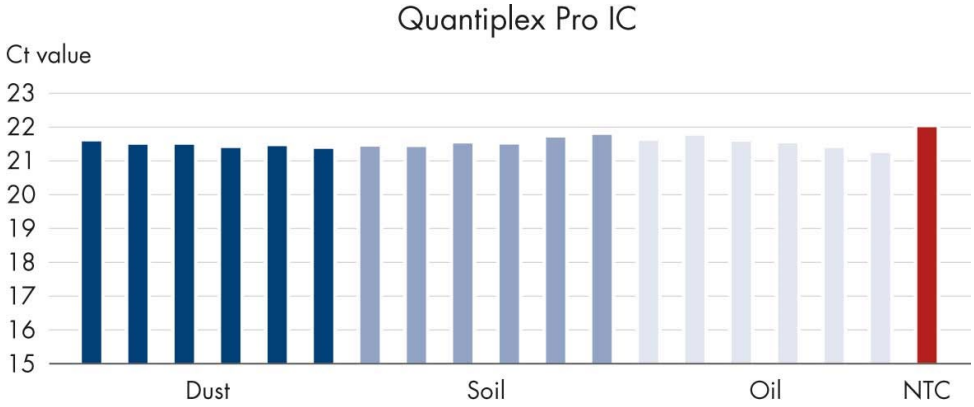

Figure 10. Performance with simulated inhibition. 6 replicates per inhibitor substrate were extracted and quantified. Ct values of the Investigator Quantiplex Pro IC are shown. The IC of a negative control indicates the expected value without any inhibition.

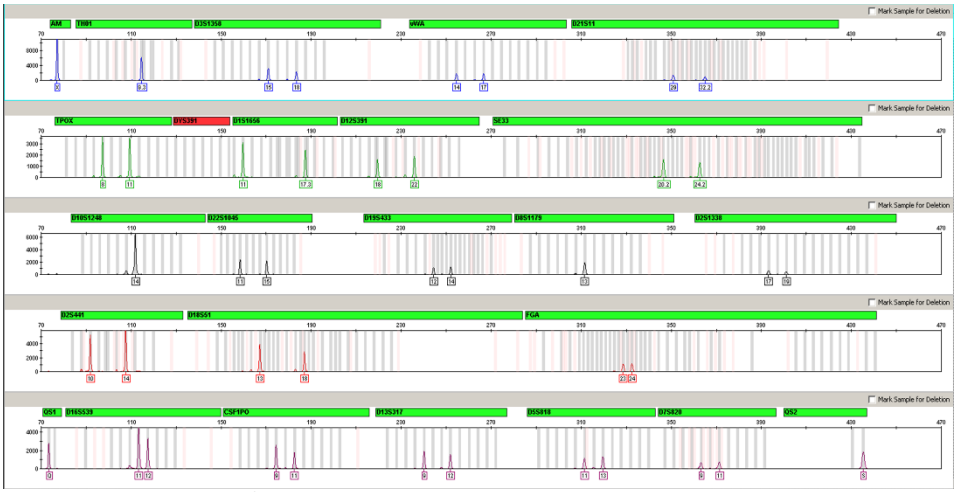

Figure 11. Electropherogram of a blood on soil sample. DNA shows some degradation due to the storage of the mocked swab sample at room temperature. The Quality Sensor confirms the absence of inhibition.

Casework and reference samples

A selection of typical case-type samples were extracted using the Large Volume protocol, or the Trace Tip Dance protocol (cigarette filter paper) with elution in 50 µl. All samples gave

sufficient yield to obtain full STR profiles using an analytical threshold of 50 RFU (Figure 12). No inhibition was observed in any of the samples, as indicated by the internal quantification control, and the STR Quality Sensors. Representative STR profiles for each type of sample are shown (Figures 13–26).

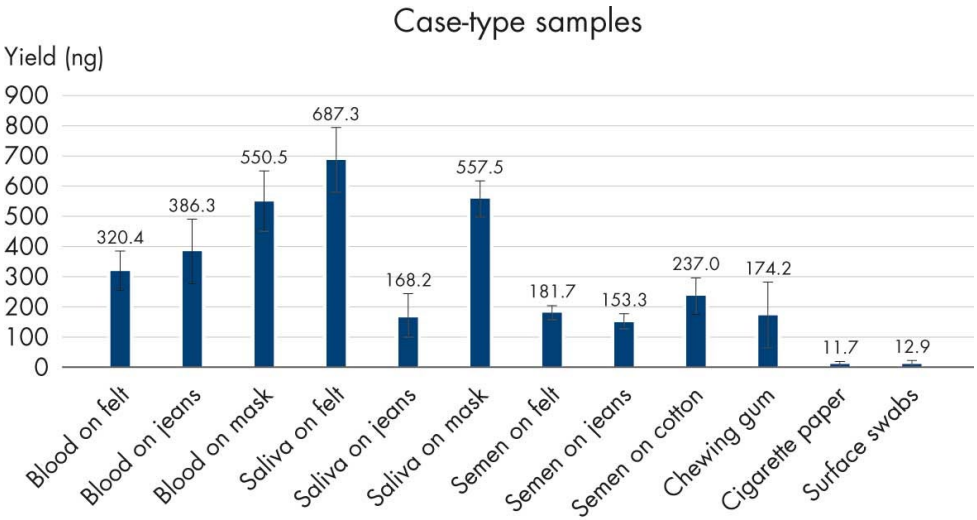

Figure 12. Yield from typical case-type samples. 40 µl of blood, saliva, or semen were spiked on different types of fabric (n=8 for each body fluid). Chewing gum was collected from 4 donors, and approximately 50 mg were lysed (n=12). Approximately 1 cm² of cigarette filter paper was used as sample (n=12). Surface swabs were taken from mobile phones, and computer keyboard and mouse from 3 users (n=12).

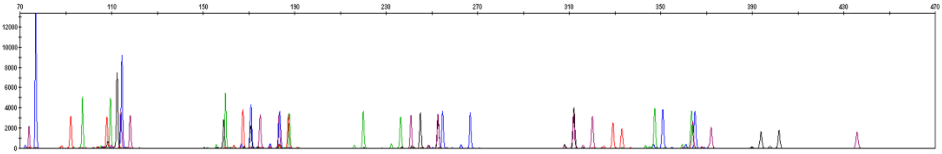

Figure 13. Blood on felt. 500 pg DNA was amplified.

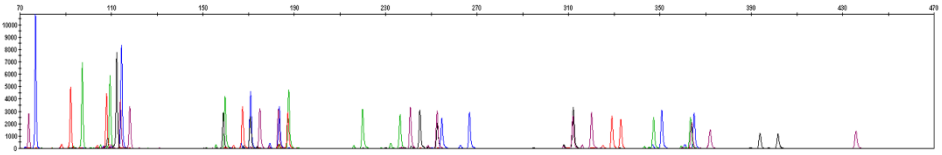

Figure 14. Blood on jeans. 500 pg DNA was amplified.

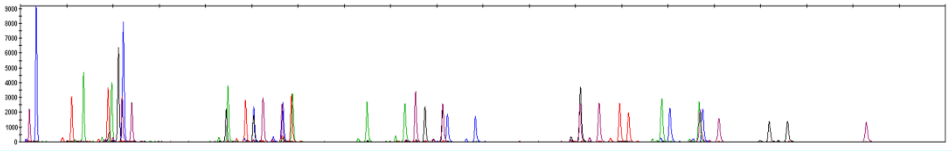

Figure 15. Blood on mask. 500 pg DNA was amplified.

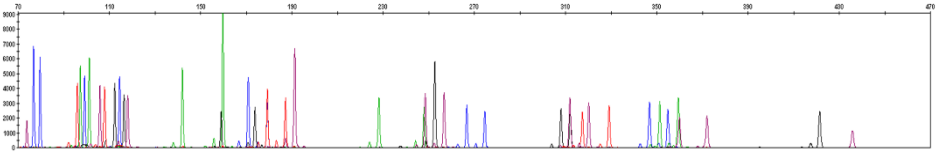

Figure 16. Saliva on felt. 500 pg DNA was amplified.

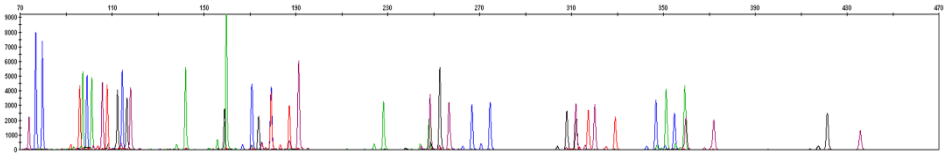

Figure 17. Saliva on jeans. 500 pg DNA was amplified.

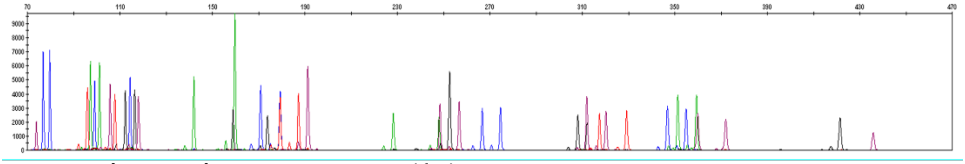

Figure 18. Saliva on mask. 500 pg DNA was amplified.

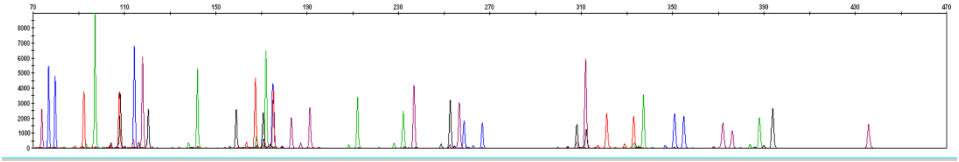

Figure 19. Semen on felt. 500 pg DNA was amplified.

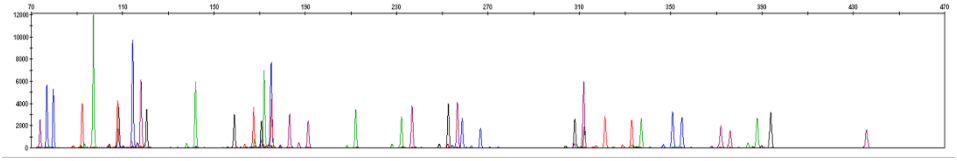

Figure 20. Semen on jeans. 500 pg DNA was amplified.

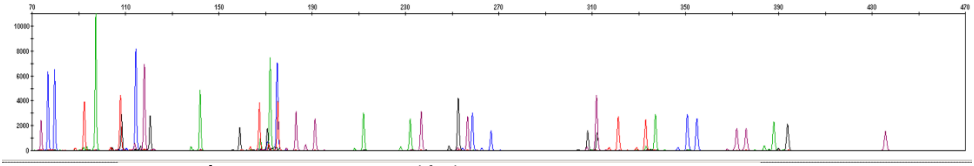

Figure 21. Semen on mask. 500 pg DNA was amplified.

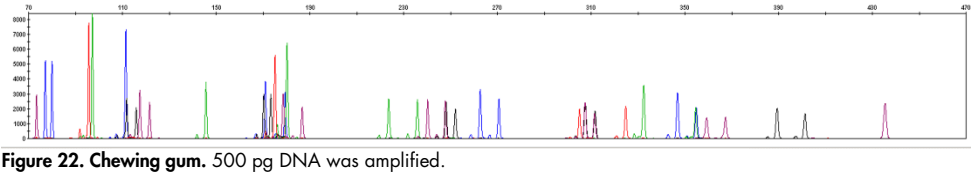

Figure 22. Chewing gum. 500 pg DNA was amplified.

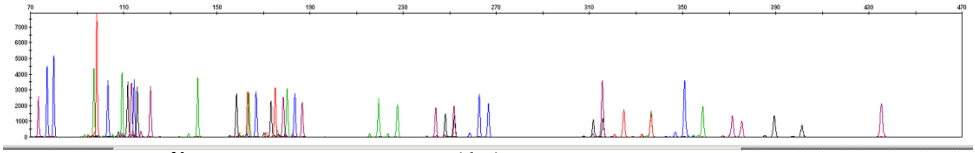

Figure 23. Cigarette filter paper. 500 pg DNA was amplified.

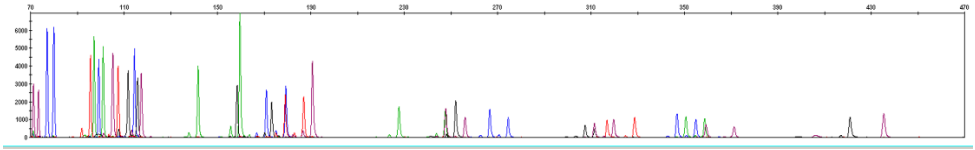

Figure 24. Surface swab from mobile phone. 500 pg DNA was amplified.

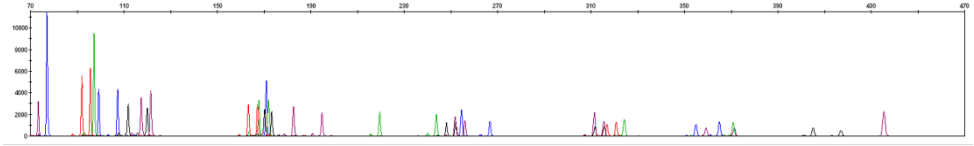

Figure 25. Surface swab from computer keyboard. 500 pg DNA was amplified.

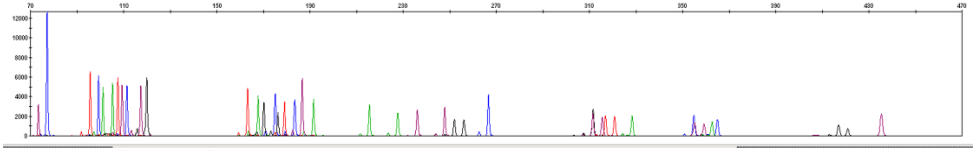

Figure 26. Surface swab from computer mouse. 500 pg DNA was amplified.

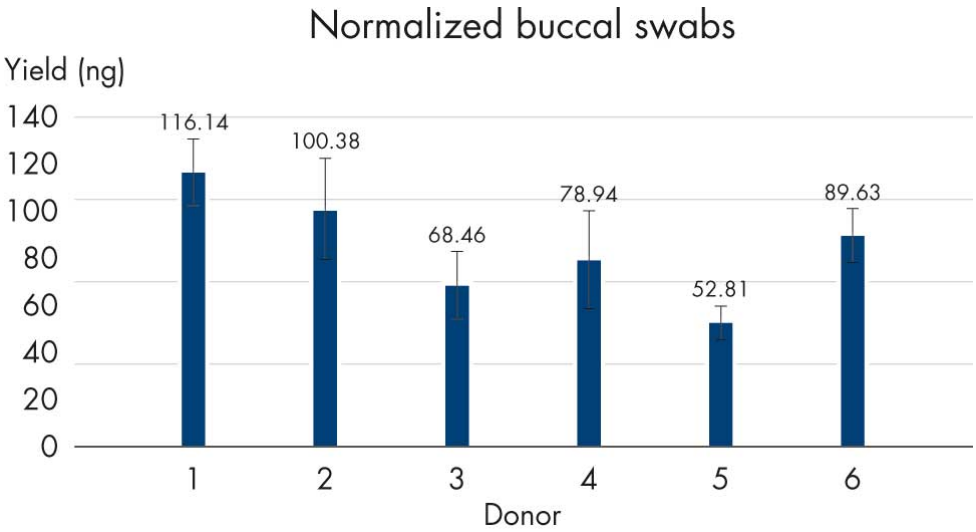

Figure 27. Normalized extraction of buccal swab samples. 4 swabs each were taken from 6 donors and extracted with the Normalization protocol.

The Normalization protocol limits the amount of extracted DNA in order to produce uniform concentrations from samples containing abundant DNA. These normalized samples can be processed at a constant volume for STR, without the need for quantification. Note that total sample DNA is normalized, which leads to some donor dependent variation for buccal swab samples, as the relative amount of microbial background differs. Obtained yields are in a range to allow running STRs at a constant eluate input volume (Figure 27). Four buccal swab samples each were taken from six individuals. The normalization protocol was used to extract samples, with elution in 200 µl. The lowest and highest concentration observed across all 24 eluates was 0.22 ng/µl and 0.67 ng/µl, respectively.

Conclusion

The validation studies confirmed the following:

- The EZ2 Connect Fx instrument is capable of robust and reliable extraction of DNA from samples typically encountered in forensic casework.
- The EZ2 Connect Fx instrument provides reliable results from various sample types and input amounts.
- The EZ2 Connect Fx instrument operates without any cross-contamination of samples.
- The EZ2 Connect Fx instrument shows equivalent performance to well established EZ1 instruments.
- Extracted DNA is free from any inhibitors.

Ordering Information

Product	Contents	Cat. no.
EZ1&2 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, and Reagents; includes Certificate of Analysis	952034
EZ2 Fx Connect	Robotic instrument for automated purification of nucleic acids from up to 24 samples, 1-year warranty on parts and labor	9003220
Investigator Quantiplex Pro Kit (200)	Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387216
Investigator 24plex QS Kit (100)*	Primer mix, fast reaction mix 2.0, control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	382415

* Larger kit sizes available. Please inquire.

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.

Document Revision History

Date	Changes
02/2022	Initial revision

HB-3023-001 02/2022