Application Note

Comparison of two DNA extraction platforms for use in forensic casework applications: EZ2® Connect Fx versus Maxwell® FSC Instrument

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Introduction

In 2021, QIAGEN launched the EZ2 Connect Fx, a new instrument for end-to-end automation of nucleic acid extraction and purification in human identification applications. The EZ2 Connect Fx platform is based on the well-known and trusted EZ1® Advanced XL magnetic-bead purification technology.

Performance of the EZ2 Connect Fx was compared with performance of an instrument regularly encountered in forensic laboratories, the Maxwell FSC Instrument (Promega Corporation). Testing on the Maxwell FSC was performed at the Institute of Legal Medicine, Ulm and followed their validated workflow. Testing on the EZ2 Connect Fx instrument was undertaken by Lisa Dierig (ILM Ulm) in QIAGEN Hilden Research & Development Laboratory. Duplicate mock forensic casework samples were prepared in the Hilden laboratory and randomly distributed for extraction by the EZ2 Connect Fx or Maxwell FSC Instrument. All purified DNA samples were quantified with the Investigator® Quantiplex® Pro Kit and amplified with the Investigator 24plex QS Kit for STR analysis.

This kit includes the innovative Quality Sensor, enabling additional data to be generated for quality control and evaluation of extraction performance.

Materials and Methods

DNA samples

Samples were chosen to reflect real casework submissions: blood, saliva, cigarette butts and touch DNA samples. Liquid samples such as saliva and blood were pipetted onto a sterile glass slide and collected with a sterile moist swab, or a DNA-free swab previously lightly coated with soil or oil then allowed to dry. Some liquid samples were pipetted directly on to an inhibiting substrate.

Saliva samples

Saliva is a non-homogenous liquid. This can be problematic when saliva is pipetted and can lead to uneven cell distribution. Sample homogenization was achieved by adding nuclease-free water (1:1) to the neat saliva followed by thorough vortexing. This first dilution is referred to as "Saliva-Dil1". Further dilutions were made as described below in Table 1. For each sample, 10 µl of diluted saliva was pipetted onto a glass slide then sampled by swabbing with a clean swab pre-wet with 10 µl of nuclease-free water. Four replicate swabs were made for each dilution; two were ▶

randomly assigned to be processed by the EZ2 Connect Fx and two to be processed by the Maxwell FSC.

Table 1. Preparation of saliva samples

Sample name	Saliva dilution	Method
Saliva-Dil1	Saliva: nuclease-free water 1:1	Pipette 10 µl of Saliva-Dil1 on to a slide then swab
Saliva-Dil2	Saliva-Dil1: nuclease-free water 1:10	Add 50 µl of Saliva-Dil1 to 450 µl of nuclease-free water Pipette 10 µl on to a slide then swab
Saliva-Dil3	Saliva-Dil2: nuclease-free water 1:10	Add 50 µl of Saliva-Dil2 to 450 µl of nuclease-free water Pipette 10 µl on to a slide then swab

Blood samples

Blood is a non-homogenous viscous liquid and may exhibit uneven cell distribution when it is pipetted. Sample homogenization was achieved by adding nuclease-free water (1:1) to the blood followed by thorough vortexing. This first dilution is referred to as "Blood-Dil1". Further dilutions were made as described below in Table 2. For each dilution, 10 µl of blood sample was pipetted on to a glass slide then sampled by swabbing with a sterile swab pre-wet with 10 µl of nuclease-free water. Four replicate swabs were made for each dilution; two were randomly assigned to the EZ2 Connect Fx and two to the Maxwell FSC.

Table 2. Preparation of blood samples

Sample name	Blood dilution	Method
Blood-Dil1	Blood: nuclease-free water 1:1	Pipette 10 µl of Blood-Dil1 on to a slide then swab
Blood-Dil2	Blood-Dil1: nuclease-free water 1:10	Add 50 µl of Blood-Dil1 to 450 µl of nuclease-free water Pipette 10 µl on to a slide then swab
Blood-Dil3	Blood-Dil2: nuclease-free water 1:10	Add 50 µl of Blood-Dil2 to 450 µl of nuclease-free water Pipette 10 µl on to a slide then swab

Inhibited samples

Soil- or oil-inhibited samples were produced by pipetting 20 µl of "Blood-Dil-Inh" (Table 3) on to a sterile slide. The samples were then collected with a swab previously prepared with soil or oil as described in Table 4. For the other samples, the blood "Blood-Dil-Inh" (Table 3) was directly deposited into the substrate. Blood samples were prepared as described in Table 4 and were allowed to dry. Substances such as soil, oil or cloth dyes are known or expected to have an inhibitory effect on amplification of an inadequately purified DNA sample. Four replicates of each sample were prepared for each inhibitor; two swabs per sample type were randomly assigned to the EZ2 Connect Fx and two to the Maxwell FSC.

Table 3. Preparation of blood for inhibited samples

Sample name	Blood dilution	Method
Blood-Dil-Inh	Blood: nuclease-free water 1:3	Add 300 µl blood to 600 µl of nuclease-free water

Table 4. Preparation of inhibited blood samples

Sample name	Method
Soil	Pipette 20 µl of Blood-Dil-Inh on to a slide and swab; the swab was previously used to swab soil from a compost area
Oil	Pipette 20 µl of Blood-Dil-Inh on to a slide and swab; the swab was previously used to swab motor oil
Black leather	Pipette 20ul of Blood-Dil-Inh directly on the piece of black leather
Brown leather	Pipette 20ul of Blood-Dil-Inh directly on the piece of brown leather
Black cotton T-shirt fabric	Pipette 20ul of Blood-Dil-Inh directly on the piece of black cotton fabric

Touch samples

Touch samples were created with the consent of all subjects. Eight microscope slides were handled for one minute by eight different persons. Handled microscope slides were arbitrarily divided into two zones: obverse side, and reverse side. The obverse and reverse surfaces of each slide were swabbed separately giving 2 x 8 samples (labelled "Fingerprint").

Four different keyboards and four different mobile phones were swabbed. Phones and keyboards were divided into two zones at the middle of the object; one swab was used to swab the left part and another swab was used for the right part. Duplicate swab samples from each object were then randomly assigned to either the EZ2 Connect Fx or Maxwell FSC.

Cigarette butts

Eight cigarette butts were collected from consenting individuals. The filter material was cut into two equal parts and duplicates were randomly assigned to either the EZ2 Connect Fx or Maxwell FSC for extraction of genomic DNA.

Lysis and purification

For lysis and substrate removal, all samples for the EZ2 Connect Fx were processed with the Investigator Lyse&Spin Basket Kit. For the Maxwell FSC, all samples were processed with the Casework Extraction kit and the Maxwell FSC DNA IQ™ Casework Kit. Sample lysis and DNA purification was set up following manufacturer's recommendations as summarized in Table 5.

DNA quantification

Data was quantified with the Investigator Quantiplex Pro Kit, a ready-to-use system using quantitative real-time PCR for the detection of human and male DNA and parallel assessment of DNA degradation. This assay possesses four specific targets to provide information about the quantity of human DNA (short fragment), DNA degradation (longer fragment of human DNA), the presence of male DNA (male-specific DNA fragment) and an internal PCR control to assess inhibition. The assay characteristics are listed in the Table 6.

Table 6. Characteristics of the Investigator Quantiplex Pro Kit

Human target, large autosomal	Human target, small autosomal	Human male target, small Y	Internal PCR control (IPC)	Limit of detection
353 bp	91 bp	81 bp	434 bp	0.5* pg/µl – 200 ng/µl

*Stochastic effects might appear for the lower quantities.

The Investigator Quantiplex Pro Kit was prepared following handbook recommendations. An input volume of 2 µl of purified DNA was analyzed for each template. All samples were quantified in duplicate, and the average of the results are shown.

DNA quantification was carried out on an Applied Biosystems® 7500 Real-Time PCR System and the results were analyzed using HID Real-Time PCR Analysis Software v.1.2 from Thermo Fisher Scientific.

All the quantification results were exported from 7500 HID 1.2 software and imported under QIAGEN Quantification Assay Data Handling and STR Setup Tool. This tool is freely available on the QIAGEN website and can be found on the Investigator Quantiplex Kits page, in the Additional Resources folder. The tool gives the user an overview of the data and presents a clear indication of potential mixture, degradation and/or inhibition. ▶

Table 5. Manufacturer's recommendations for lysis and purification of genomic DNA from one casework sample

EZ2 Connect Fx Instrument EZ1&2 DNA Investigator Kit	Maxwell FSC Instrument Casework Extraction Kit and Maxwell FSC DNA IQ Casework Kit
For casework samples on swab and solid substrate	For all samples: Extraction of samples on a solid support
475 µl buffer G2 25 µl proteinase K	386 µl Casework Extraction Buffer 10 µl proteinase K 4 µl 1-thioglycerol
Lysate volume: 500 µl for each sample	Lysate volume: 400 µl for each sample
Incubation at 56 °C; 900 rpm, 60 minutes Centrifuge 10000 rpm for 1 minutes	Incubation at 56°C; 750rpm, 60 minutes Centrifuge 10000 rpm for 2 minutes 200 µl of lysis buffer added 600 µl transferred to the cartridge
Instrument protocol: Large-Volume	Instrument protocol: DNA IQ Casework method
Run time: 18 minutes for 24 samples	Run time: 24 minutes for 16 samples
Elution volume: 50 µl	Elution volume: 50 µl

Calculation of these events is based on the ratio of the quantity of the different DNA fragments in a sample; ratios are compared to the thresholds set up on the tool.

The following thresholds were used to indicate the presence of mixtures, degradation, and inhibition:

- Mixture index (Human quantity/Male quantity): 2 (will flag when the human quantification results is two times or more than the male quantification result).
- Degradation index (Human quantity/Human degradation quantity): 10 (will flag when the small human target quantification is 10 times or more than the large target quantification).
- Inhibition Index (IC Shift): 1 (the average of the IPC standard – IPC sample).

Results and discussion

Yield of extracted DNA

Saliva samples

The quantification results from extractions by the EZ2 Connect Fx and Maxwell FSC for the range of saliva dilutions tested are presented in Figure 1. Two samples for each dilution were analyzed and the average of the results are shown.

The concentration of DNA recovered from swabs with the EZ2 Connect Fx procedure is higher than with the Maxwell FSC for all the saliva dilutions tested.

Blood samples

The quantification results from the blood swabs extracted with the EZ2 Connect Fx and the Maxwell FSC are presented in Figure 2. Two samples for each dilution were analyzed and the average of the results are shown.

The concentration of DNA recovered from swabs with the EZ2 Connect Fx procedure is higher than with the Maxwell FSC for all the blood dilutions tested.

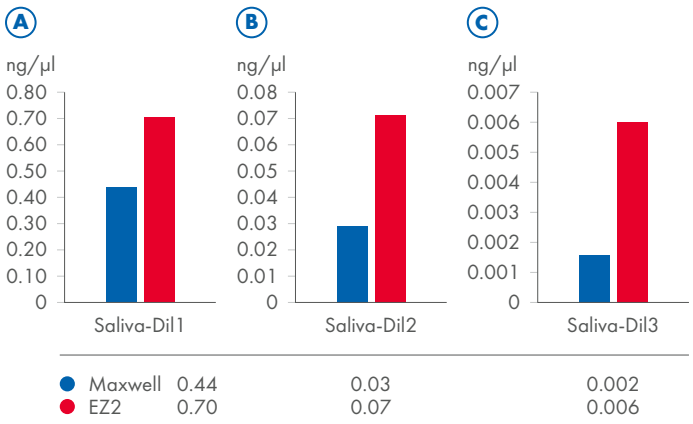


Figure 1. Saliva: Concentration of DNA (ng/µl) recovered from saliva swabs by Maxwell FSC and EZ2 Connect Fx.
Saliva swabs were prepared in duplicate from three different dilutions. The graphs A, B and C are presented at different scales to better show the data. (Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).

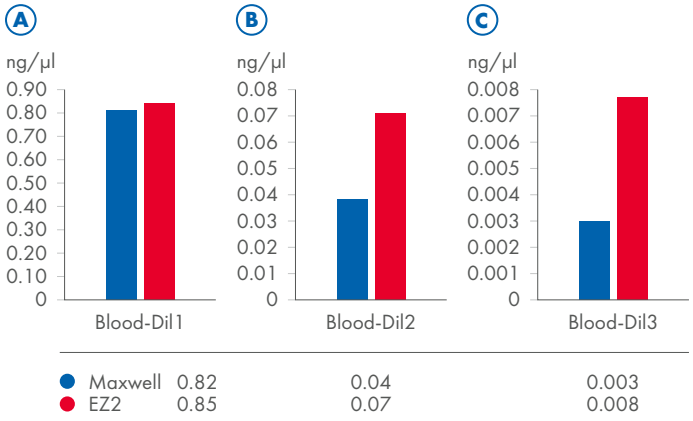


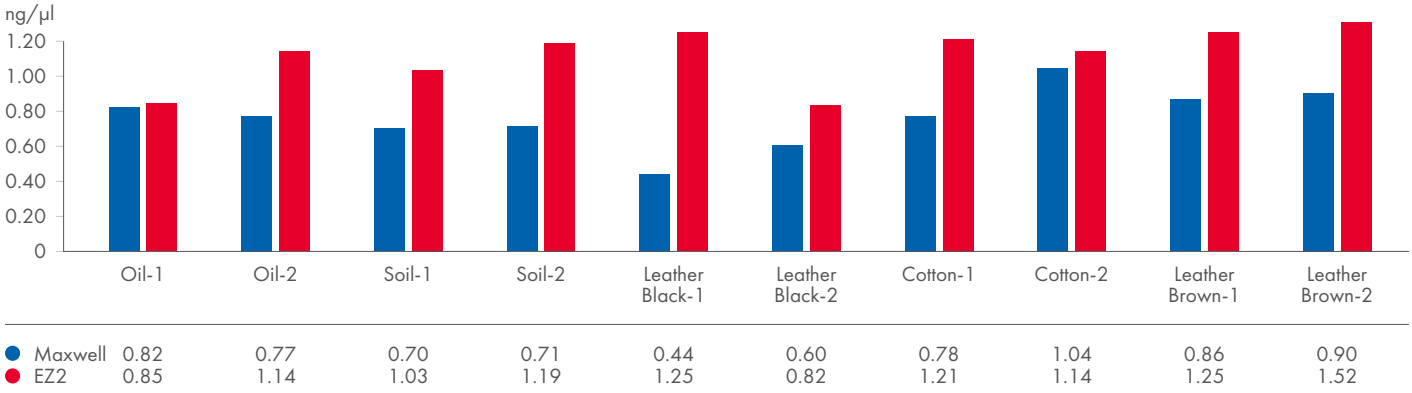
Figure 2. Blood: Concentration of DNA (ng/ul) recovered from blood swabs by Maxwell FSC and EZ2 Connect Fx.
The blood swabs were prepared in duplicate from three different dilutions. The graphs A, B and C are presented at different scales to better show the data. (Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).

Inhibited samples

The quantification results from swabs of blood exposed to various inhibitors then extracted with the EZ2 Connect Fx and the Maxwell FSC are presented in Figure 3. Purification efficiency and DNA recovery were tested by these challenging samples.

The quantification results for the samples exposed to potential inhibitors show a higher concentration of recovered DNA with the EZ2 Connect Fx than for the Maxwell FSC. The analysis conducted with the QIAGEN

Quantification Assay Data Handling and STR Setup tool did not show any residual inhibition in either the Maxwell FSC extracted samples or EZ2 Connect Fx extracted samples. The EZ2 Connect Fx system produced higher yields than the Maxwell FSC for all ten samples tested in this study.



	Oil-1	Oil-2	Soil-1	Soil-2	Leather Black-1	Leather Black-2	Cotton-1	Cotton-2	Leather Brown-1	Leather Brown-2
Maxwell	0.82	0.77	0.70	0.71	0.44	0.60	0.78	1.04	0.86	0.90
EZ2	0.85	1.14	1.03	1.19	1.25	0.82	1.21	1.14	1.25	1.52

Figure 3. Inhibitors: Concentration of DNA (ng/µl) recovered from inhibited blood swabs by Maxwell FSC and EZ2 Connect Fx.
The samples contain blood with a possible inhibitor (oil and soil) or were applied to different substrates such as dyed leather and cotton. (Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).

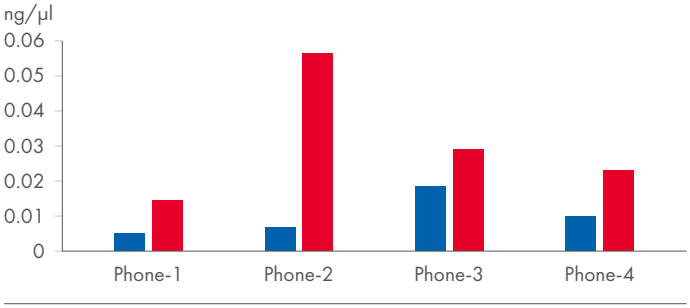
Touch samples

Phones and keyboards

Figure 4 and Figure 5 present the DNA concentration results from four different phones and four different keyboards, respectively. Substrate surfaces were divided into two equal regions, and each was swabbed.

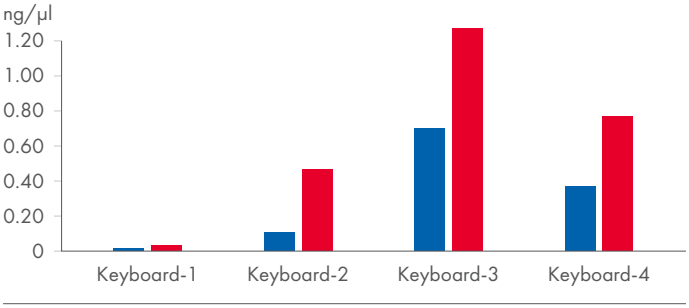
Processing of a duplicate swab was assigned randomly to the EZ2 Connect Fx or Maxwell FSC. Differences in the DNA concentration were expected between swabs processed on the EZ2 Connect Fx and duplicate swabs processed on the Maxwell FSC as touch samples cannot be normalized.

The results in Figure 4 and Figure 5 show that for all samples, the DNA concentration recovered by the EZ2 Connect Fx was higher than the DNA concentration recovered by the Maxwell FSC.



	Phone-1	Phone-2	Phone-3	Phone-4
Maxwell	0.005	0.007	0.018	0.010
EZ2	0.015	0.057	0.029	0.023

Figure 4. Phone: Concentration of DNA (ng/ul) recovered from surface swabs by processing with Maxwell FSC and EZ2 Connect Fx.
(Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).



	Keyboard-1	Keyboard-2	Keyboard-3	Keyboard-4
Maxwell	0.01	0.10	0.71	0.37
EZ2	0.04	0.47	1.27	0.77

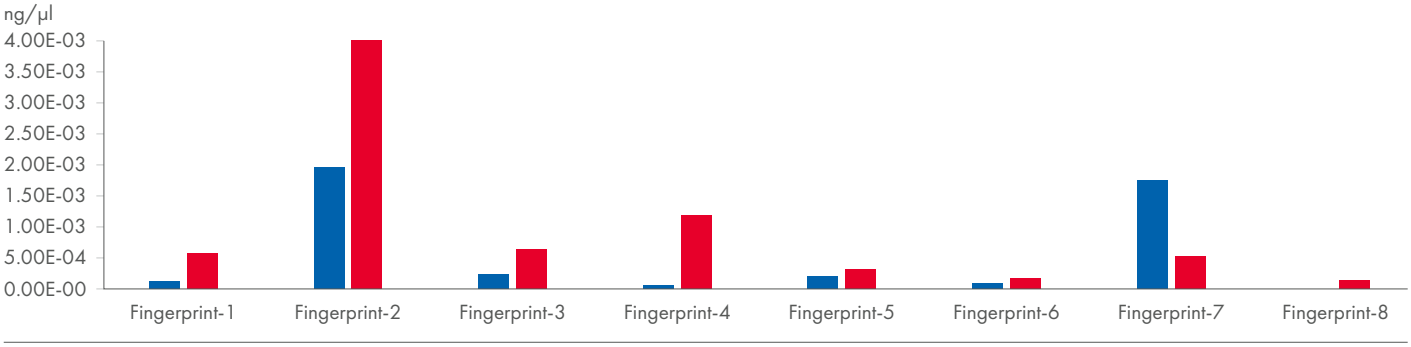
Figure 5. Keyboard: Concentration of DNA (ng/ul) recovered from surface swabs by processing with Maxwell FSC and EZ2 Connect Fx.
(Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).

Fingerprints on glass slides

Figure 6 presents the DNA concentration results from the swabs taken from the fingerprint slides. Each slide produced one swab for processing on the EZ2 Connect Fx and one for processing on the Maxwell FSC.

The DNA trace samples showed a high level of variability among individuals who touched the slides.

The results show that for seven out of the eight samples, the DNA concentration recovered by the EZ2 Connect Fx was higher than the DNA concentration recovered from the Maxwell FSC. The Maxwell FSC returned a higher yield from the Fingerprint-7 sample.



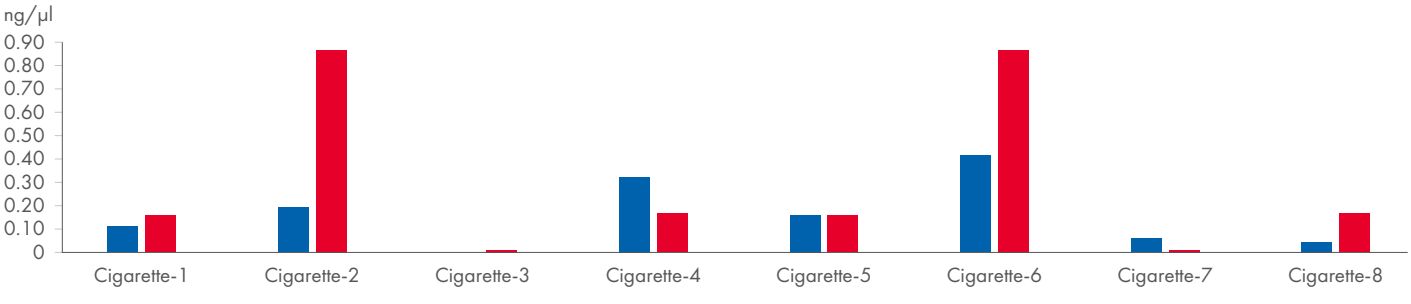
	Fingerprint-1	Fingerprint-2	Fingerprint-3	Fingerprint-4	Fingerprint-5	Fingerprint-6	Fingerprint-7	Fingerprint-8
Maxwell	1.19E-04	1.97E-03	2.18E-04	4.87E-05	1.76E-04	7.72E-05	1.75E-03	0.00E+00
EZ2	5.68E-04	5.96E-03	6.51E-04	1.17E-03	3.06E-04	1.71E-04	5.11E-04	1.09E-04

Figure 6. Fingerprint slides: Concentration of DNA (ng/µl) recovered from glass slides swabbed and then processed with Maxwell FSC and EZ2 Connect Fx.
(Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).

Cigarette butts

Figure 7 presents the DNA concentration results for the different cigarette butts analyzed. The quantification was run in duplicate for each sample and the average of the results is shown.

For this set of samples, the EZ2 Connect Fx outperformed the Maxwell FSC, returning a higher concentration of DNA for five of the eight duplicate samples analyzed. The quantifications showed identical recovery results for the Cigarette-5 sample. Results were higher on the Maxwell FSC for Cigarette-4 and Cigarette-7.



	Cigarette-1	Cigarette-2	Cigarette-3	Cigarette-4	Cigarette-5	Cigarette-6	Cigarette-7	Cigarette-8
Maxwell	0.112	0.195	0	0.326	0.161	0.416	0.057	0.044
EZ2	0.157	0.871	0.013	0.169	0.157	0.871	0.013	0.169

Figure 7. Cigarette butt: Concentration of DNA (ng/ul) recovered from cigarette filter material by processing with Maxwell FSC and EZ2 Connect Fx.
(Maxwell: Maxwell FSC; EZ2: EZ2 Connect Fx).

STR analysis and success rate

The STR profiles were generated with the Investigator 24plex QS Kit and were analyzed in GeneMapper™ ID-X v.1.2.

Samples were analyzed using the following rules:

1. Heterozygous peak imbalance set for 50%
2. Analytical Threshold set for 50 rfu
3. Uninterpretable rules based on major profile needing to be twice the height of a minor profile. If this is not possible at a locus, the locus did not pass QC check.

Samples were classified as "Full profile/Full profile mix", "Partial Databaseable/Partial Databaseable mix", "Partial profile/Partial mix" or "No result".

- A Full profile indicates no drop-out or heterozygous peak imbalance greater than 50%, all peaks above 50 rfu and, in the event of a mixture, the major profile being a minimum of two times the height of the minor.
- A Partial Databaseable/Partial Databaseable Mix profile indicates where there are 12 or less incidences of heterozygote imbalance, peaks below 50 rfu and where the major can be called against a minor. This profile is deemed of suitable quality for a good comparison or for loading to a database.
- Partial profile/Partial Mix indicates more than 12 occurrences of the difference rules and the creation of a profile unsuitable for loading or containing enough information for searching.
- A profile is considered as "No result" if no allele is above the analytical threshold (50 rfu).

The number of profiles observed for each category and each instrument, based on the review and classification of the different profiles, is summarized in Table 7.

Table 7. Summary of general STR profiles for Maxwell FSC and EZ2 Connect Fx

Classification	Maxwell FSC	EZ2 Connect Fx
Full profile (including mixes)	24	31
Partial Databaseable Profile (including mixes)	14	8
Partial profile (including mixes)	8	7
No result	0	0

A comparison of these results for the Maxwell FSC and EZ2 Connect Fx, expressed as percentage success rates, is shown in Figure 8.

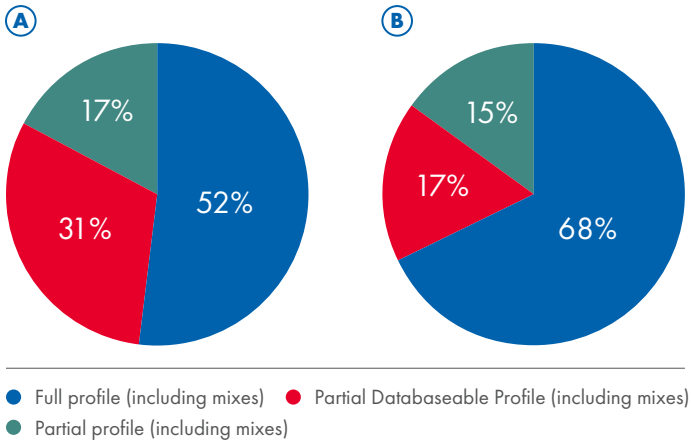


Figure 8. Percentage success rates for STR analysis. (A) Maxwell FSC; (B) EZ2 Connect Fx. Numbers have been rounded to the nearest whole number.

Results for the success rate of STR analysis show that DNA extraction from the mock casework samples with the EZ2 Connect Fx produced a higher proportion of robust samples suitable for downstream STR analysis than DNA extraction of duplicate samples on the Maxwell FSC. More full profiles (68%) were elicited from samples purified on the EZ2 Connect Fx than on the Maxwell FSC (52%). None of the EZ2 samples or Maxwell FSC were classified as "No result", but a slightly higher partial rate was observed with the Maxwell FSC at 17% vs a partial rate of 15% for the EZ2 Connect Fx.

Conclusion

- Quantification results from this study demonstrate that the EZ2 Connect Fx consistently outperformed the Maxwell FSC across the range of mock casework samples tested. This level of performance is critical to the successful processing of low-quantity and low-quality samples from crime scene investigations.
- The IPC analysis with Investigator Quantiplex Pro indicated no inhibition in any of the samples tested. In addition, no inhibitory effects were seen in STR results.
- The STR results were consistent with the quantification results, showing a higher percentage of complete profiles for samples processed on the EZ2 Connect Fx than on the Maxwell FSC. There were more full profiles on EZ2, and less partial profiles when samples were extracted on the EZ2 Connect Fx.

Summary

The EZ2 Connect Fx is a worthy addition to the EZ family of instruments, producing good yields of high-quality clean genomic DNA for excellent results from a wide variety of forensic casework samples.

Ordering Information

Product	Contents	Cat. no.
EZ2 Connect Fx System	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 2x EZ2 Connect racks (EZ2 Connect Fx Tip Rack and the EZ2 Connect Fx Tip Rack – Flip Cap Tubes), EZ2 Connect Fx Cartridge Rack and 1-year warranty on parts and labor	9003220
EZ1&2 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 Kit (200)	For use on Applied Biosystems 7500 Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
Investigator 24plex QS Kit (400)	Primer mix, Fast Reaction Mix including Taq DNA Polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO), and nuclease-free water	382417

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