Application Note

Comparison of two DNA extraction platforms for use in forensic casework applications: EZ2® Connect Fx versus AutoMate *Express*™

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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 AutoMate *Express* (Thermo Fisher Scientific). Duplicates of several different types of mock forensic casework samples were processed by both instruments and results of DNA purification and yield recovery were compared. Testing on the AutoMate *Express* was performed at the Institute of Legal Medicine, Halle/S. University Hospital and followed their validated workflow. Testing on the EZ2 Connect Fx instrument was undertaken by Marina Nastainczyk-Wulf (UKH) in QIAGEN Hilden Research & Development Laboratory. The duplicate mock samples were prepared in the Hilden laboratory and randomly distributed for extraction by the EZ2 Connect Fx or AutoMate *Express*. 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, hair 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 were pipetted directly on to an inhibiting substrate. Inhibitors such as sand, soil, oil and grease were added to air-dried samples on a swab.

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 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 be processed by the EZ2 Connect Fx and two to be processed by the AutoMate *Express*.

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 AutoMate *Express*.

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

Two different types of inhibited samples were prepared. For the first, 20 µl of diluted blood ("Blood-Dil-Inh" as described in Table 3) was pipetted directly onto a leaf, a substrate likely to contain environmental inhibitors such as humic acid. These can interact with the sample to inhibit the DNA amplification step if they are not removed during purification.

The second type of inhibited sample was produced by pipetting 20 µl of "Blood-Dil-Inh" on to a sterile slide and then collecting with a sterile pre-wet swab with 10 ul of nuclease-free water. The blood sample was allowed to dry then the swab was used to collect an inhibiting substance (Table 4). Substances such as soil, sand, oil or grease 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 AutoMate *Express*.

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
Leaf	Pipette 20 µl of Blood-Dil-Inh on to a leaf
Soil	Pipette 20 µl of Blood-Dil-Inh on to a slide and swab; use the swab to add soil
Sand	Pipette 20 µl of Blood-Dil-Inh on to a slide and swab; use the swab to add sand
Oil	Pipette 20 µl of Blood-Dil-Inh on to a slide and swab; use the swab to add oil
Grease	Pipette 20 µl of Blood-Dil-Inh on to a slide and swab; use the swab to add grease

Hair

Two hairs with roots were collected from each of six consenting donors. One duplicate hair root from each donor was processed on the EZ2 Connect Fx and one on the AutoMate *Express*.

Touch samples

Touch samples were created with the owner's consent by swabbing five different keyboards and five different mobile phones. Each object was 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 were then randomly assigned to either the EZ2 Connect Fx or AutoMate *Express*.

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 AutoMate *Express*, all samples were processed with a PrepFiler™ LySep™ Column.
DNA purification of samples was carried out following manufacturer's recommendations with the EZ1&2 DNA Investigator Kit on the EZ2 Connect Fx and with the PrepFiler *Express* Forensic DNA Extraction Kit on the AutoMate *Express* (see Table 5).

DNA quantification

Data was quantified with the Investigator Quantiplex Pro Kit, a ready-to-use system using quantitative realtime 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.

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. ▶

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		**AutoMate *Express* Instrument PrepFiler *Express* Forensic DNA Extraction Kit**
For casework samples on swab and solid substrate	For hairs	For all samples: Large sample spin/filter tube protocol
475 µl buffer G2	460 µl buffer G2	500 µl PrepFiler Lysis Buffer
25 µl proteinase K	20 µl proteinase K	5 µl 1 M DTT
	20 µl 1 M dithiothreitol (DTT)	
Lysate volume: 500 µl for each sample		Lysate volume: 500 µl for each sample
Incubation at 56 C; 900 rpm, 60 minutes		Incubation at 70 C; 750 rpm, 40 minutes
Centrifuge at 10,000 rpm for 1 minute		Centrifuge at 10,000 rpm for 2 minutes
Instrument protocol: Large-Volume		Instrument protocol: Standard protocol
Run time: 18 minutes for 24 samples		Run time: 30 minutes for 13 samples
Elution volume: 50 µl		Elution volume: 50 µl

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.

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. 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 result 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 AutoMate *Express* 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 AutoMate *Express* for all the saliva dilutions tested.

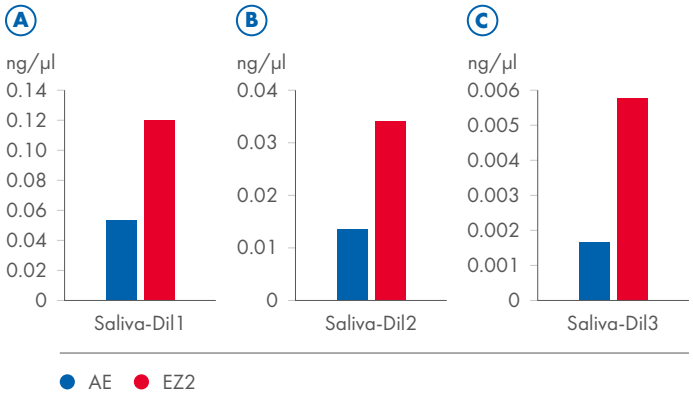


Figure 1. Saliva: Concentration of DNA (ng/µl) recovered from saliva swabs by AutoMate *Express* and EZ2 Connect Fx.
Saliva swabs were prepared in duplicate from three different dilutions. The graphs A,B and C are presented at different scales to better show the data. (AE: AutoMate *Express*; EZ2: EZ2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

Blood samples

The quantification results from the blood swabs extracted with the EZ2 Connect Fx and the AutoMate *Express* 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 AutoMate *Express* for all the blood dilutions tested.

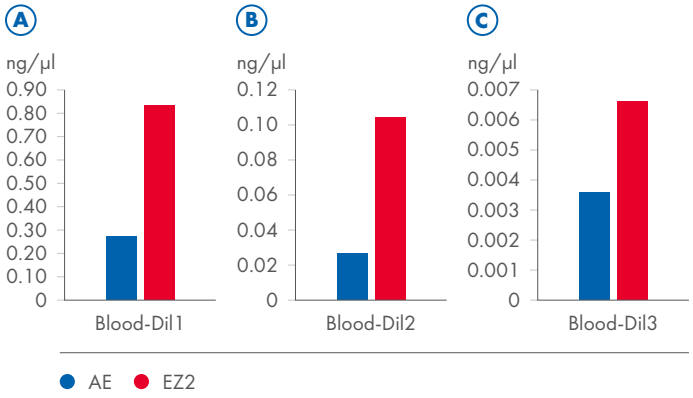


Figure 2. Blood: Concentration of DNA (ng/µl) recovered from blood swabs by AutoMate *Express* and EZ2 Connect Fx.
The blood swabs were prepared in duplicate from three different dilutions. The graphs A, B and C are presented at different scales to better show the data. (AE: AutoMate *Express*; EZ2: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

Inhibited samples

The quantification results from blood swabs exposed to various inhibitors and the leaf substrate extracted with the EZ2 Connect Fx and the AutoMate *Express* 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 AutoMate *Express*. Results on the leaf substrate are comparable for the two systems. The EZ2 Connect Fx system produced higher yields than the AutoMate *Express* for nine out of the ten samples tested in this study. The analysis conducted with the QIAGEN Quantification Assay Data Handling and STR Setup tool did not show any residual inhibition of PCR for samples purified by either workflow. The reduced yield from the Automate *Express* samples is likely a feature of the reduced binding efficiency of the kit when the beads are exposed to challenging conditions, rather than the effects of a residual inhibitor on the downstream PCR reaction.

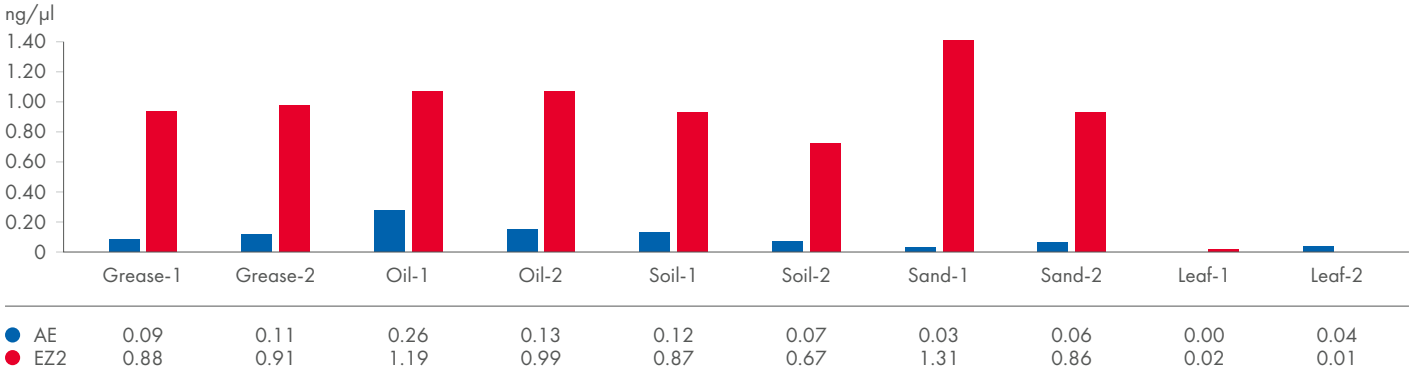


Figure 3. Inhibitors: Concentration of DNA (ng/µl) recovered from inhibited blood swabs or substrate by AutoMate *Express* and EZ2 Connect Fx. The samples contain blood with a possible inhibitor (grease, oil, soil, sand) or were applied to a substrate that could inhibit (leaf). (AE: AutoMate *Express*; EZ2: EZ2 Connect Fx).

Hair roots

The quantification results for DNA extraction from hairs sourced from six different donors are presented in Figure 4. Two hairs from each donor were randomly assigned for DNA extraction to either the EZ2 Connect Fx or the AutoMate *Express*.

Some variation is expected between the results for a given person as hair roots cannot be normalized like other samples, e.g., where a biological fluid can be swabbed. The data in Figure 4 show that, for five of the six people tested, the DNA yields from extraction with the EZ2 Connect Fx are higher than DNA yields from extraction with the AutoMate *Express*.

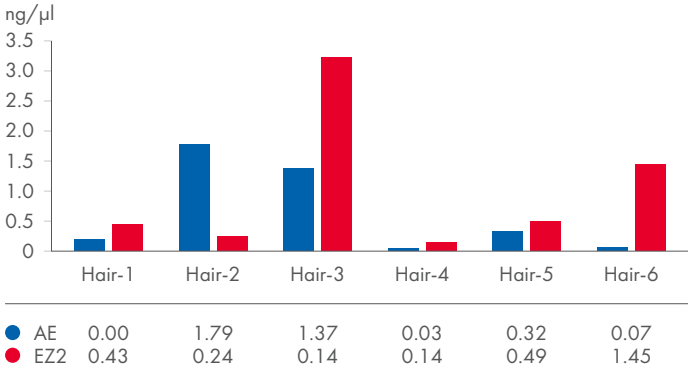
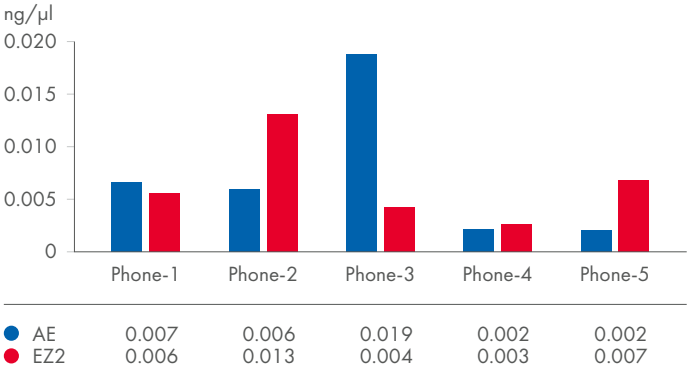


Figure 4. Hair root: Concentration of DNA (ng/µl) recovered from hair samples by AutoMate *Express* and EZ2 Connect Fx. (AE: AutoMate *Express*; EZ2: EZ2 Connect Fx).

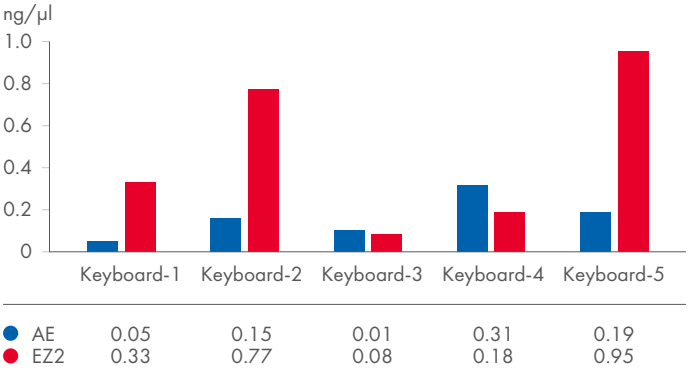
Phones and keyboards swabbed

Figure 5 and Figure 6 present the DNA concentration results from five different phones and five 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 AutoMate *Express*. As with hair roots, variation between duplicates was expected from touch samples as they cannot be normalized. The results showed that for six samples out of ten, the DNA concentration recovered by the EZ2 Connect Fx was higher than the DNA concentration recovered by the AutoMate *Express*. The results were comparable in two cases while in the remaining two cases, the AutoMate *Express* performed better.



AE	0.007	0.006	0.019	0.002	0.002
EZ2	0.006	0.013	0.004	0.003	0.007

Figure 5. Phone: Concentration of DNA (ng/µl) recovered from surface swabs by processing with AutoMate *Express* and EZ2 Connect Fx.
(AE: AutoMate *Express*; EZ2: EZ2 Connect Fx).

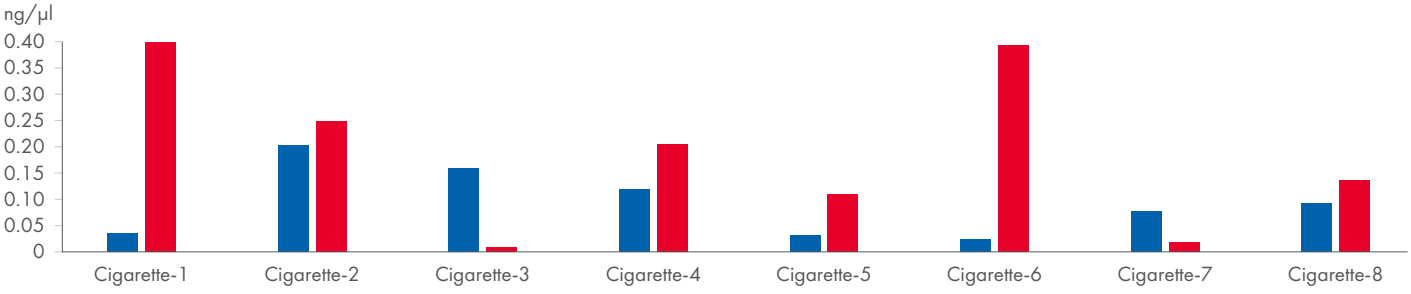


AE	0.05	0.15	0.01	0.31	0.19
EZ2	0.33	0.77	0.08	0.18	0.95

Figure 6. Keyboard: Concentration of DNA (ng/µl) recovered from surface swabs by processing with AutoMate *Express* and EZ2 Connect Fx.
(AE: AutoMate *Express*; EZ2: EZ2 Connect Fx).

Cigarette butts

Figure 7 presents the quantification results for the eight different cigarette butts analyzed. For this set of samples, the EZ2 Connect Fx outperformed the AutoMate *Express*, returning a higher concentration of DNA in six of the eight samples analyzed. Furthermore, the analysis conducted with the QIAGEN Quantification Assay Data Handling and STR Setup Tool showed inhibition of PCR in three of the eight DNA samples recovered from cigarette butts purified with the AutoMate *Express*. There was no inhibition of PCR in the corresponding sample duplicate purified with the EZ2 Connect Fx.



AE	0.04	0.20	0.16	0.12	0.03	0.11	0.08	0.09
EZ2	0.40	0.25	0.01	0.20	0.11	0.11	0.02	0.13

Figure 7. Cigarette butt: Concentration of DNA (ng/µl) recovered from cigarette filter material by processing with AutoMate *Express* and EZ2 Connect Fx.
(AE: AutoMate *Express*; 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.

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

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 AutoMate *Express*. More full profiles (61%) were elicited from samples purified on the EZ2 Connect Fx than on the AutoMate *Express* (50%). None of the EZ2 samples were classified as "No result" whereas 7% of extractions run on the AutoMate *Express* were classified as "No result".

Table 7. Summary of general STR profiles for AutoMate *Express* and EZ2 Connect Fx

Classification	AutoMate *Express*	EZ2 Connect Fx
Full profile (including mixes)	23	28
Partial Databaseable Profile (including mixes)	15	15
Partial profile (including mixes)	5	3
No result	3	0

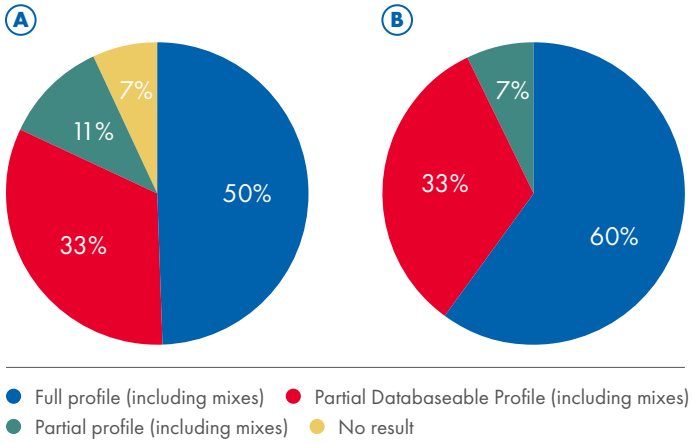


Figure 8. Percentage success rates for STR analysis.
A AutoMate *Express*; **B** EZ2 Connect Fx. Numbers have been rounded to the nearest whole number.

Conclusion

- Quantification results from this study demonstrate that the EZ2 Connect Fx consistently outperformed the AutoMate *Express* 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 inhibition in some of the AutoMate *Express* samples. In particular, the effect of inhibitors in the AutoMate *Express* runs was shown in the lower success rates for cigarette samples.
- Purification with the EZ2 Connect Fx removed all inhibitors; there was no flagging of IPC in the EZ2 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 AutoMate *Express*. There were more full profiles on EZ2, and no sample was classified as "No result".

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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