Developmental Validation of the EZ1&2™ DNA Investigator® Kit on the EZ2® Connect Fx

Mario Scherer[1], Daniele Terramagra[1], Amy Liberty[2], Anke Prochnow[1]

[1] QIAGEN Strasse 1, 40724 Hilden, Germany

[2] QIAGEN LLC, Germantown, MD, USA

Abstract

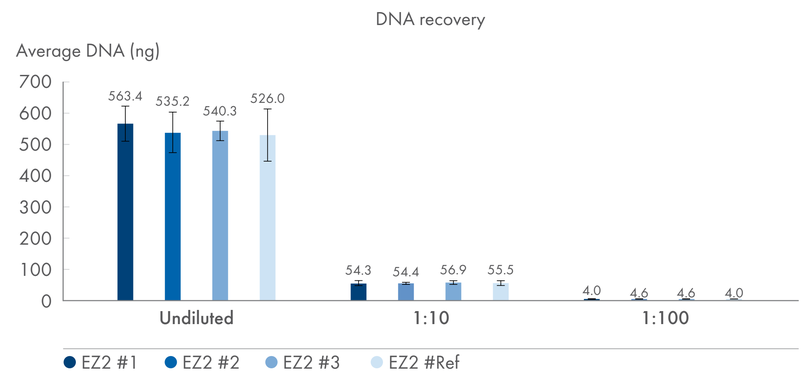
The EZ1&2 DNA Investigator Kit allows automated purification of total DNA from forensic and human identity samples on the EZ2 Connect Fx instrument. Its proven magnetic-bead technology provides high-quality DNA, suitable for direct use in sensitive downstream applications such as quantitative PCR, STR analysis and NGS. The purified DNA obtained is free of proteins, nucleases and inhibitors.

Investigators can choose from a wide range of pretreatment protocols, available for various casework or reference sample types, and instrument protocols, available for different sample lysate volumes, normalized extraction and lysates containing solid sample substrates. Up to 2 ml of sample lysate can be processed using a dedicated Large Volume Rack available for the instrument. Specific protocols for bones enable the input of increasing amounts of starting material to improve sensitivity. Furthermore, the protocols allow the processing of casework items that demand large lysis volumes, such as cartridge casings, tapes or combinations of multiple swabs. Purified DNA can be eluted in as little as 20 µl, providing maximum sensitivity for challenging samples.

The performance of the EZ1&2 DNA Investigator Kit on the EZ2 Connect Fx was evaluated using typical casework sample types and conditions commonly encountered in forensic and parentage laboratories. Sensitivity, reproducibility, accuracy, stability and absence of cross-contamination were tested. Wherever applicable, this validation study followed the recommendations of the European Network of Forensic Science Institutes (ENFSI) and the validation guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM).

Equivalence and Reproducibility

The EZ2 Connect Fx instrument has been designed to replicate protocols available on previous EZ1® instruments. DNA from serial dilutions of blood lysates was extracted using the EZ1&2 DNA Investigator Kit on the EZ2 Connect Fx and the EZ1 Advanced XL instruments. Side-by-side comparisons using the Tip Dance Protocol showed equivalent performances. Consistent results were obtained across all runs on both instruments.

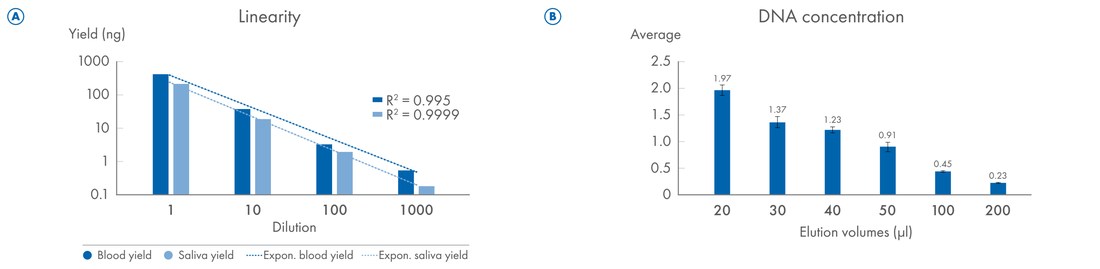


Equivalence of performance on the EZ2 Connect Fx and the EZ1 Advanced XL. DNA was extracted using the Tip Dance Protocol and eluted in 50 µl water. Three runs were performed on the EZ2 Connect Fx and one run on the EZ1 Advanced XL. Each blood dilution was run in four replicates.

Sensitivity and Linearity

Sensitivity across dilutions was tested to determine the suitable range of sample input. DNA from dilutions of blood and saliva (corresponding to 10, 1, 0.1 and 0.01 µl) was extracted in triplicates using the Trace Protocol and eluted in 40 µl TE buffer. Quantification shows a linear correlation between input sample amount and purified DNA yield (Figure A). This linearity was observed for both sample types over the range of input material tested. Full profiles were obtained for all samples.

Lower elution volumes increase the sensitivity by providing higher concentrations of extracted DNA. In the EZ2 Connect Fx, DNA can be eluted in 20–200 µl of water or TE buffer. To analyze the impact of elution volumes on the total yield and the concentration of samples, DNA was extracted from 1:10 dilutions of blood in six replicates per elution volume. Recovered eluate volumes were measured. As expected, the DNA concentration increased from the highest to the lowest elution volume (Figure B).



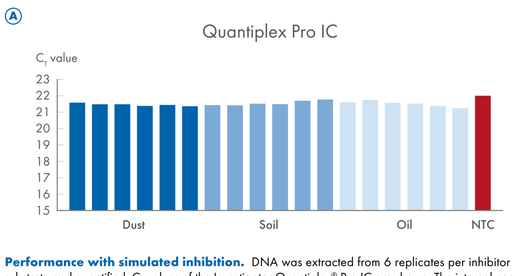
Linearity across dilutions. DNA from blood and saliva dilutions was extracted in triplicates using the Trace Protocol and eluted in TE buffer. The dilution series corresponds to 10, 1, 0.1 and 0.01 µl blood or saliva samples.

Correlation of DNA concentration and elution volumes. DNA extraction was performed using the using the Trace Protocol and eluted in TE buffer. Six replicates were run for each elution volume.

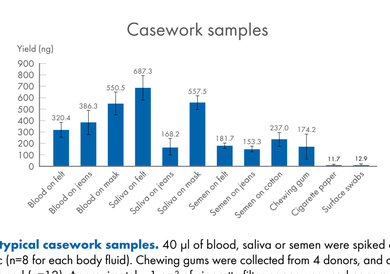
Robustness

Forensic casework samples are frequently associated with potential PCR inhibitors. Therefore, we tested the ability of the system to fully remove inhibitors from samples. Swabs were taken from three potential inhibitor-rich environments: (1) dust close to a road, (2) soil and (3) an old engine covered with used oil. Subsequently, 50 µl of blood was spiked onto the area of the swab carrying potential inhibitors, and the swabs were dried for two days before extraction. DNA was extracted using the Large-Volume Protocol and eluted in 50 µl. The downstream qPCR quantification showed no shift of the internal control (Figure A), indicating the absence of any inhibition. Full STR profiles were obtained for all samples using an analytical threshold of 50 RFU.

In another experiment, DNA was extracted from a selection of typical casework samples using the Large-Volume Protocol or the Tip Dance Protocol (cigarette filter paper) and eluted in 50 µl. All samples gave sufficient yield to obtain full STR profiles using an analytical threshold of 50 RFU (Figure B). No inhibition was observed in any of the samples, as indicated by the internal quantification control and the STR Quality Sensors.



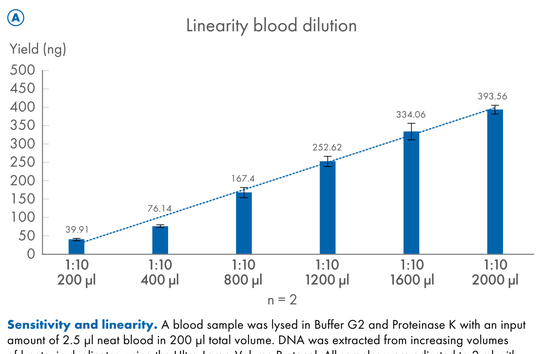
Performance with simulated inhibition. DNA was extracted from 6 replicates per inhibitor substrate and quantified. C_T values of the Investigator Quantiplex® Pro IC are shown. The internal control (IC) of a negative control indicates the expected value without any inhibition.



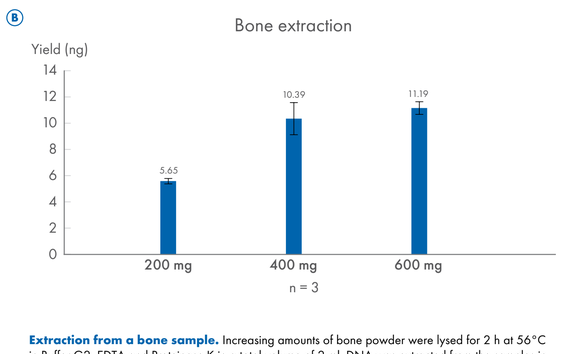
Yield from typical casework samples. 40 µl of blood, saliva or semen were spiked on different types of fabric (n=8 for each body fluid). Chewing gums were collected from 4 donors, and approximately 50 mg were lysed (n=12). Approximately, 1 cm² of cigarette filter paper was used as sample (n=12). Surface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

DNA Extraction from Bones Using the Ultra-Large-Volume Protocol

The Ultra-Large-Volume Protocol makes use of the EZ2 Large Volume Rack, allowing the use of 7 ml sample tubes for DNA binding. The protocol can accommodate up to 2 ml sample lysate and the run takes approximately 50 minutes. It is suitable for larger casework items, such as cartridge casings, or to increase yields from bone samples. Here we tested the use of the protocol to extract DNA from large amounts of bone powder. For dilutions of blood or other non-bone sample types, yields increase linearly with sample input. However, for bone samples, saturation may be observed when the input amount exceeds 500 mg of bone powder. This saturation is likely caused by the competitive binding of DNA to hydroxyapatite released from the bone.



Sensitivity and linearity. A blood sample was lysed in Buffer G2 and Proteinase K with an input amount of 2.5 µl neat blood in 200 µl total volume. DNA was extracted from increasing volumes of lysate, in duplicates, using the Ultra-Large-Volume Protocol. All samples were adjusted to 2 ml with Buffer G2. Samples were quantified with the Investigator Quantiplex Pro Kit.



Extraction from a bone sample. Increasing amounts of bone powder were lysed for 2 h at 56°C in Buffer G2, EDTA and Proteinase K in a total volume of 2 ml. DNA was extracted from the samples in triplicates using the Ultra-Large-Volume Protocol and quantified using the Investigator Quantiplex Pro Kit.

ct Fx provides a reliable solution for high-quality DNA

.

erformance compared to EZ1 instruments, allowing

vailable exclusively on the EZ2 Connect Fx, provides higher

lentity, and paternity testing. These products are not intended for the diagnosis, prevention or treatment

GEN kit instructions for use or user operator manual. QIAGEN instructions for use and user manuals are
ur local distributor).