Developmental Validation of the New EZ2® DNA Investigator® Sep&Prep Kit for Automated Processing of Sexual Assault Samples

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Reliable and robust processing of sexual assault samples

Analysis of sexual assault samples remains challenging due to incomplete separation of cellular material from different contributors, often resulting in mixed DNA profiles. The EZ2 DNA Investigator Sep&Prep Kit (Sep&Prep) enables efficient, automated, differential extraction with improved recovery of sperm-derived DNA, resulting in effective isolation of male-derived DNA and reduced female DNA carryover.

Here, we present data from the developmental validation of the EZ2 DNA Investigator Sep&Prep kit including:

- Linearity and sensitivity
- Differential separation efficiency in post-coital samples
- Accuracy of differential separation
- Repeatability with multiple biological samples

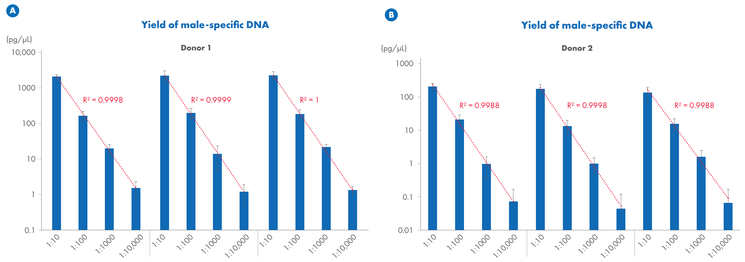
Unless otherwise noted, quantification was performed with the Investigator Quantiplex® Pro Kit or RGQ Kit, while STR profiling was performed with the DNA Investigator 24plex QS Kit. The Sep&Prep method is compatible with established forensic systems, including qPCR and STR kits, and sperm fractions can be directly used for amplification without further purification.

The Sep&Prep workflow is suitable for a broad range of forensic samples, including challenging low template samples with high female background.

High linearity, sensitivity, and reproducibility

Linearity, sensitivity, and reproducibility were evaluated using serial dilutions (1:10, 1:100, 1:1000, 1:10,000) of semen from two donors, spiked on swabs, and processed in multiple replicates across three instruments. DNA yield showed highly linear recovery of male DNA from both high (Donor 1) and low (Donor 2) DNA semen samples, demonstrating consistent performance across a broad dynamic range.

High reproducibility was observed between biological and technical replicates. Sensitivity analysis indicated successful DNA recovery and STR profile generation (data not shown) across the dilution series.



Linearity and reproducibility. Six replicates were extracted for each sample (50 µL elution volume). Y-specific quantification (n=2). **A** Donor 1. **B** Donor 2.

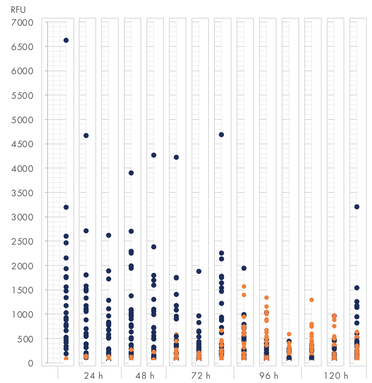
Precise separation even with challenging samples

Post-coital samples (24–120 h) showed that the Sep&Prep workflow enables reliable differential separation despite decreasing sperm content.

Early samples produced full male profiles with minimal female contribution, whereas later samples showed reduced male allele recovery and increased female-specific alleles.

Nevertheless, male alleles remained consistently detectable, demonstrating effective sperm isolation and robust performance in low-template, high-background samples.

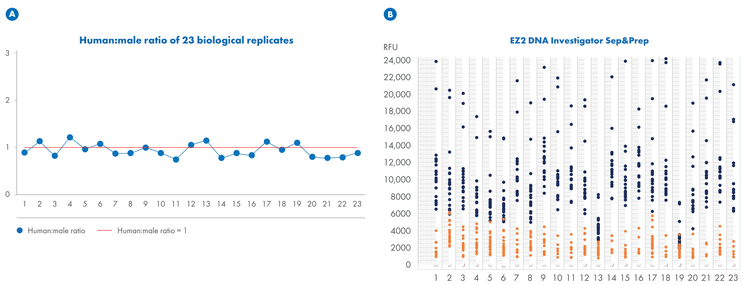
	24 h	48 h	72 h	96 h	120 h
Male DNA conc. (ng/µl)	2.867	0.279	0.028	0.007	0.029



Individual RFU heights of 14 sperm fraction profiles. Analytical threshold: 50 RFU; locus-specific minimum peak height threshold: RFU ≥9% of highest allele per STR locus. Female donor (orange dot), male donor alleles (blue dot). Shared alleles not included. 1 vertical strip = 1 STR profile.

Separation accuracy

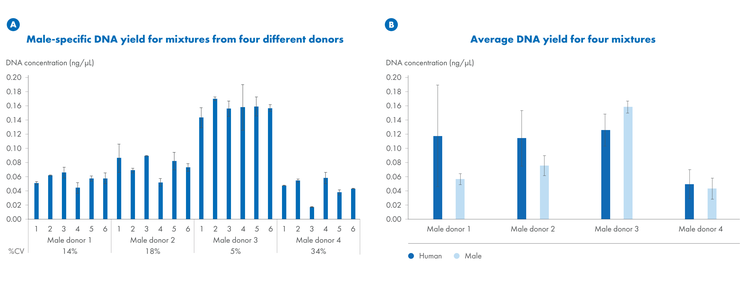
Effectiveness of differential separation was demonstrated using the human-to-male DNA ratio. Across 23 replicates of 1:200 diluted semen spiked on vaginal swabs, ratios were consistently close to 1 (mean ~0.94), indicating efficient sperm DNA isolation with minimal female carryover. All ratios were within the expected range for predominantly male profiles with only low female background, which was confirmed by STR analysis. Overall, the results demonstrate reliable separation performance with minute reduced female carryover.



Separation accuracy (A) and allele comparison (B). STR analysis (right), threshold set to 200 RFU. Locus-specific minimum peak height threshold: RFU ≥9% of the highest allele within the STR locus. Female donor (orange dot), male donor (blue dot). Shared alleles not included. 1 vertical strip = 1 sample.

Repeatability

Results of 24 vaginal swabs from one female donor, spiked with 1:200 diluted semen from 1 of 4 male donors showed consistent male DNA recovery within each donor group, with low variability across biological and technical replicates. For three donors, %CV ranged from 5–18%, indicating stable intra-run performance. Increased variability (34%) was observed under low DNA input conditions, reflecting expected stochastic effects at low-template levels. Overall, the workflow demonstrated robust and consistent performance across replicates.



Repeatability of six biological replicates. Elution in 100 µL. **A** Y-specific quantification n=2. The coefficient of variation (%CV) describes the variability among the six biological replicates. **B** Mean DNA concentrations (ng/µL) of autosomal ('human') and Y-specific ('male') targets shown per donor.

Conclusions

Validation studies of the EZ2 DNA Investigator Sep&Prep Kit confirmed:

- Precise separation between sperm-derived DNA and DNA from non-sperm cells
- Strong performance for challenging samples with low sperm content and high female DNA background, such as post-coital samples
- Consistent generation of interpretable STR profiles even under low-template conditions
- High reproducibility across instruments, biological replicates, experimental conditions

Additional validation studies included in the upcoming validation report confirmed:

- Reliable processing of a wide range of sexual assault sample types
- Robustness of the kit components and protocol
- Freedom from cross-contamination

Key advantages of the automated EZ2 Connect Fx workflow include:

- Standardized and automated processing ensuring reproducible results
- Low variability across replicates and strong inter-instrument agreement
- Efficient integration into existing forensic workflows through compatibility with commercial qPCR and STR kits
- Direct usability of sperm fractions without additional purification steps

Overall, the automated Sep&Prep workflow demonstrated high robustness and reproducibility with low variability across replicates and instruments, while improving male DNA isolation and STR profile clarity. These features highlight its particular suitability for reliable analysis of challenging forensic samples.

The EZ2 DNA Investigator Sep&Prep Kit is intended for Research Use Only, not for use in diagnostic procedures. The product is suitable for Forensic and Paternity Use. For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user operator manual. QIAGEN kit instructions for use and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services (or your local distributor).

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Sample to Insight