Development of a new automated EZ2® Connect Fx sexual assault sample processing kit

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Fast and robust processing of sexual assault samples

Analysis of sexual assault samples is often challenging as insufficient separation of cells from the persons involved leads to mixed profiles. A new sexual assault sample processing kit has been developed to address efficient and specific lysis of non-sperm cells (e.g., epithelial cells), as well as improved sperm DNA recovery and precise separation between sperm-derived DNA and DNA coming from non-sperm cells.

The new EZ2 DNA Investigator Sep&Prep Kit offers automated processing on the EZ2 Connect Fx with significantly enhanced throughput and high ease-of-use. With a hands-on time of just 30 minutes for 24 samples, it delivers reproducible results swiftly, addressing the urgent need for timely analysis and backlog reduction in sexual assault evidence kits.

The new method is fully compatible with all common, commercially available kits for downstream applications, such as qPCR and STR profiling on CE and NGS. Sperm fractions are ready-to-use without further purification steps, allowing fast processing and reduced running time.

The EZ2 DNA Investigator Sep&Prep Kit is suitable for processing common sexual assault traces as well as particularly challenging samples that are characterized by very low sperm quantities and a high amount of victim DNA, such as 66-hour-old post-coital (PC) swabs. Here, we present data from our current development of the EZ2 DNA Investigator Sep&Prep Kit and alpha-field test results generated by four laboratories, where various sample types were used.

Samples and workflow used for alpha-field test

A range of sexual assault samples were processed using the EZ2 DNA Investigator Sep&Prep Kit automated on the EZ2 Connect Fx instrument in comparison with the differential wash workflow on the QIAcube® or QIAcube Connect instruments. Vaginal swabs spiked with 1:50 and 1:400 semen dilutions were used in all four laboratories. Additionally, the three external forensic laboratories tested a wide range of specific samples.

	QIAGEN		NCDOJ			LSPCL			ILM Halle		
Sample	**1:50**	**1:400**	**PC 24 h**	**PC 36 h**	**Sheet**	**1:150**	**1:400**	**1:600**	**PC 10 h**	**PC 66 h**	**Oral wash**
Sample type	Vaginal swabs, spiked with semen dilutions (1:50; 1:400)		24 h post-coital (7 years)	36 h post-coital (7 years)	Used sheet + semen stains	Buccal swabs, spiked with semen dilutions: 1:150 (<2 years), 1:400 (10 years), 1:600 (4 years)			10 h post-coital (3 months)	66 h post-coital (1 month)	Mouthwash + semen
STR kit	24plex QS		PowerPlex Fusion 6C; Y23			PowerPlex Fusion 6C			ESX17 Fast		

Up to 24 ready-to-use sperm fraction eluates in 2.5 h

Off-deck sample preparation (1 h 10 min)

- Off-deck lysis
- Centrifugation of sample in spin basket
- Removal of all solid components from tube
- Load sample onto EZ2 Connect Fx

Automated EZ2 Connect Fx process (1 h 5 min)

- Removal of supernatant/epithelial fraction (EPI)
- Separation and purification of sperm fraction (3 steps)
- Efficient sperm cell lysis

Inactivation (15 min)

Purification of EPI fraction (EZ1&2™ DNA Investigator Kit)

Overview of prototype workflow at alpha-test. The final EZ2 DNA Investigator Sep&Prep Kit will be automated on the EZ2 Connect FX with a run time of 1 h 10 min, including the inactivation step.

ILM: Institute of Legal Medicine, Halle; LSPCL: Louisiana State Police Crime Laboratory; NCDOJ: North Carolina Department of Justice.

Comparison of female allele counts of sperm fractions between different methods and laboratories

The STR results of the sperm fractions show effective separation of male alleles from mixed samples processes with the EZ2 DNA Investigator Sep&Prep Kit, leading to single-person STR profiles or mixed profiles with a distinct male major component. The new kit demonstrates good reproducibility across various mixed samples. QIAcube methods perform well with samples containing high male DNA but shows mixed profiles with challenging samples.

	1:50 diluted semen on vaginal swab						1:400 diluted semen on vaginal swab						F*	M†
	QIAcube/QIAcube Connect			Sep&Prep			QIAcube/QIAcube Connect			Sep&Prep				
NCDOJ	31	30	27	5	2	2	31	31	31	4	13	14	43	43
LSPCL	31	31	31	1	1	1	31	31	31	12	10	3	43	43
ILM Halle	23	21	23	0	0	0	22	23	23	0	0	0	31	28
QIAGEN	28	21	29	8	2	1	29	28	28	10	2	14	39	37

LSPCL	QIAcube Connect		Sep&Prep		F*	M†
Semen 1:150	32	30	0	0	42	46
Semen 1:400	29	28	0	7	42	45
Semen 1:600	27	27	5	0	42	48

NCDOJ	QIAcube		Sep&Prep		F*	M†
PC 24 h	0	0	0	1	43	46
PC 36 h	2	2	3	0	43	46

ILM Halle	QIAcube		Sep&Prep		F*	M†
PC 10 h	10	5	0	1	30	31
PC 66 h	20	20	4	9	30	31
Oral wash	15	5	1	1	30	31

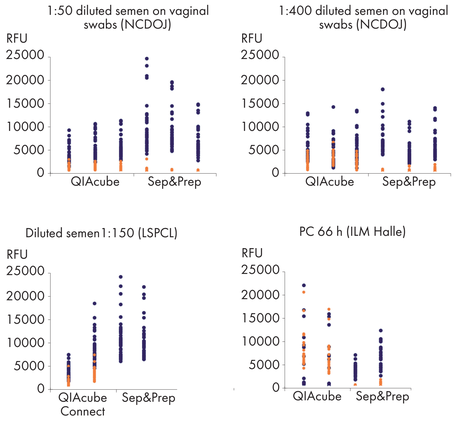
Key: <2 female alleles; 2–8 female alleles; 9–17 female alleles; 18–27 female alleles; >27 female alleles

Reduced number of STR alleles of the female donor in the sperm fractions. Using reference STR profiles, each allele of the STR profiles was assigned to the correct donor. The number inside the cells indicates the number of STR alleles of the female donor. Each cell is the sperm fraction of one biological replicate. The analytical threshold was set to 200 RFU (except NCDOJ samples: 50 RFU) and the locus-specific threshold to RFU ≥9% of the highest allele within the STR locus. For sample 'Sheet' (NCDOJ), no reference profile was present.

*Number of female donor alleles in the STR kit; †Number of male donor alleles in the STR kit.

Precise separation even with challenging samples

The new method precisely separates sperm-derived DNA from mixed samples, producing single-person profiles or mixed profiles with a distinct male major component. It is highly effective in handling challenging samples with high female DNA and low male DNA amounts. These samples, which often result in mixed STR profiles using other methods, show distinct male profiles with the EZ2 DNA Investigator Sep&Prep Kit. The new method yields profiles with fewer alleles and massively decreased RFUs that originated from the female donor as well as alleles of higher RFU that originated from the male donor in comparison to differential wash on QIAcube or QIAcube Connect.

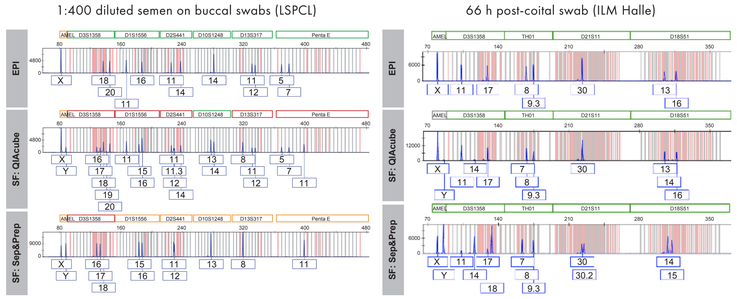


Comparison of representative examples of sperm fractions. Using the respective reference profiles of the DNA donors, each allele of the STR profiles was assigned to the correct donor and highlighted according to origin: Female donor (orange dot), or male donor including shared alleles (blue dot). One strip = one sample, i.e., one specific sperm fraction STR profile (coming from one biological replicate).

Distinct profiles from mixed samples

The sperm fraction of 1:400 diluted semen on buccal swabs separated with the QIAcube Connect differential wash method yields a mixed profile, while the EZ2 DNA Investigator Sep&Prep Kit shows the single-person profile of the male donor from the same sample. No probabilistic genotyping software (PGS) is needed.

Processing of post-coital swabs, collected 66 hours after intercourse, shows mixed profiles with both methods used. The QIAcube profile of the sperm fraction shows a clearly distinguishable female major component, while the Sep&Prep kit achieves a distinct male major component. No PGS is needed.



Representative parts of electropherograms (blue channel). All profiles of the sperm fractions (SF) were tested against the reference profiles of female and male donors. The epithelial fraction (EPI) always shows the profile of the female donor.

Conclusions

The new EZ2 DNA Investigator Sep&Prep Kit for automated processing of sexual samples on the EZ2 Connect Fx instrument demonstrated enhanced precision and reliability, particularly in complex scenarios where traditional methods struggle to provide distinct profiles.

The new EZ2 DNA Investigator Sep&Prep Kit offers:

- Better separation between sperm-derived DNA and DNA coming from non-sperm cells
- Efficient processing of all kinds of sexual assault samples
- Especially advantageous over routine workflows for challenging samples that are characterized by low sperm quantity, high victim DNA amount, e.g., post-coital samples
- Proven performance in benchmarking experiments in four laboratories
- High reproducibility across a wide variety of mixed sexual assault samples

Key advantages of the automated EZ2 Connect Fx workflow:

- Reduced hands-on time and high ease-of-use
- Automated workflow ensures highly reproducible results and increased throughput for faster processing and shorter run times
- Efficient analysis of sexual assault evidence kits to help tackle massive backlogs
- Direct use of sperm fractions without further purification due to full compatibility with commercial kits (qPCR, STR, NGS)

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).

Sample to Insight