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New Y-Screen Assay using the Quantifiler™ Trio DNA Quantification Kit

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ABSTRACT -

Sexual Assault Kit (SAK) samples are among the most difficult sample types encountered by many forensic laboratories. Differential extraction procedures used as part of a sexual assault workflow are both time consuming and labor intensive. For many laboratories, SAKs represent a high percentage of current and backlogged cases and often yield no presence of male DNA, when analyzed downstream by autosomal and Y STR amplification. To assist in the decision making process of whether to take a sample forward to differential extraction, we have developed a novel DNA screening workflow allowing customers to quickly assess whether swab evidence from an SAK contains a male contributor prior to the standard labor-intensive differential extraction procedures used in forensic laboratories. This Y-Screen assay starts with a small cutting of an SAK swab placed into a buffer which lyses cells (including sperm) in only 10 minutes. This is immediately followed by a quick neutralization step and dilution before addition to the Quantifiler® Trio assay. We demonstrate that the sensitivity of the assay correlates to the results obtained from differential extraction procedures with our studies and STR analysis, as well as the results from forensic laboratories using a range of sample types, differential extraction procedures and STR chemistries. We show that this technique can provide a complementary DNA confirmative assay to complement the current presumptive screening techniques commonly used by labs.
This Y-Screen assay solves important sample screening and processing problems, allowing forensic laboratories to more rapidly process SAK samples and therefore help to assist in decreasing overall SAK turnaround times.

INTRODUCTION -

Forensic laboratories are looking for new tools to enable rapid sample assessment for more informed casework lab processing decisions. Samples collected in SAKs can vary greatly based on the sexual assault case situation, but cotton swabs are commonly found in these kits as key sample collectors for orifice and body samples swabbed from the victim. The SAK sample is evaluated for the presence of sperm and if detected, labor intensive and time consuming differential extraction procedures are used to process these samples to separate male sperm fraction from the suspect from the epithelial cells from the victim for improved DNA-based identification downstream. SAKs often yield no male DNA, when analyzed downstream by autosomal and Y short-tandem repeat (STR) amplification.
We describe a novel DNA screening workflow for the rapid assessment of swab evidence from SAKs to quickly assess the presence of a male contributor prior to the standard labor-intensive differential extraction procedures used commonly in forensic laboratories. By using this new DNA-based Y-Screen assay in conjunction with other presumptive serological screening methods, forensic laboratories are able to confirm both conclusive and inconclusive serology results as well as having a useful aid in detecting male/sperm DNA when slide search results are questionable.

MATERIALS AND METHODS -

The steps in the Y-Screen assay are as follows- First, cut a small piece of each cotton swab sample type, saving the rest of swab for differential extraction. Place swab cutting in 100 uL 1N NaOH (in LySep™ column) for 10 min at 80C with shaking at approximately 750 rpm. Centrifuge column for 2 minutes at 12k RPM. Add 4 uL glatial acetic acid and dilute the sample 1/5 in low TE. For example, add 10 uL sample lysate to 40 uL low TE. Add 2uL of diluted lysate into Quantifiler® Trio real-time PCR reaction and follow the standard protocol for the Quantifiler® Trio assay. A positive male quant result with the Quantifiler Trio assay is indicative of the presence of male DNA on the swab and a recommendation to move forward with DNA extraction (differential DNA extraction or standard DNA extraction).
The Y-Screen assay has been validated in-house using mock casework samples and differential extraction using standard Proteinase K/DTT lysis procedures followed by cleanup of both the sperm and epithelial cell fractions on the Automate Express™ Forensic DNA Extraction System. This was followed by analysis with Identifiler ® Plus, GlobalFiler® and YFiler™ Plus. The lysis procedures employed by our test sites are shown in Table 2. A cutting of a swab sample was analyzed with the Y-Screen assay.
Following this, the remainder of the swab (excluding any part of the swab which may have been removed for serology) was used for DNA extraction, quantification and STR.

Figure 1. Quantifiler® Trio Kit

RESULTS -

Table 1. Extraction Procedure for Outside Test Sites

Sample Class/Test Site	Extraction procedures
Post-Coital Swabs (Site 1)	Organic extraction for both differential extractions and non-differential extractions. Elution volume is 75uL.
Non-probative casework samples (Site 2)	Differential extraction for all of the samples, but all of the F1 (epithelial) samples were extracted by the Qiagen EZ1® Advanced XL robot using the Investigator Kit. The F2 (sperm) samples were extracted by organic extraction with phenol chloroform and purified with Millipore microcons.
Mock casework samples (Site 3)	Organic extraction for both differential extractions and non-differential extractions followed by Maxwell® 16 (Promega) to purify
Mock casework samples (Site 4)	SEB, Proteinase K
Mock casework samples (Site 5)	Differential extraction with Chelex®, Microcon® cleanup for some samples

Table 3. Y-Screen Assay Results for Test Site 1

Sample Description	Extraction method	Y-Screen Y Quant	YFiler™ Plus results for Sperm Fraction or Standard DNA extraction
C5- 1 day	differential	+	Full profile
C5- 2 day	differential	+	Full profile
C5- 3 day	differential	+	Full profile
C5- 1 day-2	differential	+	Full profile
C5- 2 day-2	differential	+	Full profile
C5- 3 day-2	differential	+	Full profile
C5- 1 day-3	differential	+	Full profile
C5- 2 day-3	differential	+	Full profile
C5- 3 day-3	differential	+	Partial profile
C2- 1 day	differential	+	Full profile
C2- 2 day	differential	+	Partial profile
C2- 2 day-2	differential	+	Full profile
C2- 6 day	Non-differential	-	No profile
C2- 7 day	Non-differential	-	No profile
C3- 5 day	Non-differential	-	No profile
C3- 7 day	Non-differential	-	No profile
C4- 6 day	Non-differential	+	Full profile
C4- 7 day	Non-differential	+	Partial profile
C4- 8 day	Non-differential	+	Partial profile

Table 3. 100% Concordance between Y-Screen & STR Results for 19 Post-Coital Samples.

Table 2. Results for Test Site 2

	Sample	P30	micro	TMB	Y-Screen SA Quant	Y-Screen Y Quant	Identifiler® Plus results for Male Fraction
R1	Swab	+	+		+	+	Mixture
R2	Swab	+	+		+	+	Full male
R3	Swab of semen stain	+	-		+	- See Fig.1	Full male
R9	Vag Swab	-	-		+	+ See Fig.1	Full female profile
R10	Anal Swab	-	-		+	+	Full female profile
R11	Vag Swab	-	-		-	- See Fig.1	Full female profile
R12	Anal Swab	-	-		-	-	No profile for F1, F2
R13	Oral Swab	-	-		+	-	Full female profile
R14	Vag Swab	-	+		+	+	Full male profile
R15	Anal Swab	-	-		+	+	Partial male profile
R16	Vag Swab	-	+		+	+	Full female, w. few alleles from single male
R17	Oral Swab	-	-		+	2/3 reps +	Full female profile
R18	Swab	-	-	+	+	-	Full female profile
R19	Swab	-	-	+	+	+	Full male profile
R20	Swab	-	-	+	+	-	Full female profile
R21					+	+	No profile for F1, F2

Table 2. Concordance between Y-screen assay and Identifiler® Plus. The amplification curves for the 3 samples which do not show correlation between Y-Screen and STR results are shown in Figure 2A.

Figure 2- Non-probative Casework Sample Failures (Site 2)

A. 3 Sample Failures **B. 3 Successful Samples**

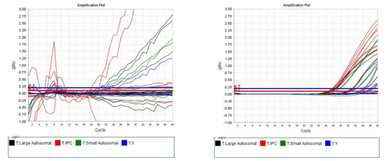

Figure 2. Amplification plots demonstrating the visual difference between non-probative casework samples from Site 2 which failed to correlate quantification results with the Y-Screen assay to the resulting Identifiler® Plus profile results and accurate correlation of three samples ("Successful Samples").

Table 4. Correlation Between Y-Screen Assay and Corresponding STR Results

Sample Class/Test Site	N	Correlation between Y-Screen Assay and					Samples with Zero Male in Y Screen & no STR run post DNA extraction bc of insufficient quantity*
		Identifiler® profile	YFiler® profile	Identifiler® Plus profile	GlobalFiler™ profile	YFiler® Plus profile	
Post-Coital Swabs (Site 1)	19					100% (N=19)	
Non-probative casework samples (Site 2)	16			92% (N=16)			
Mock casework samples (Site 3)	40		100% (N=29)	100% (N=22)			1
Mock casework samples (Site 4)	19			78% (N=19)	78% (N=19)	74% (N=19)	
Mock casework samples (Site 5)	12	100% (N=8)	100% (N=1)				3

Table 4. The Y-Screen correlation between Y-Screen assay and post DNA extraction DNA quantity/STR results is shown. Correlation between Y-Screen Assay and resulting STR profile was achieved if one of the 2 following conditions was met. First, if male DNA was detected with the Y-Screen assay, a positive male quant result with the Trio assay post DNA extraction was also obtained. Second, if no male DNA was detected with the Y-Screen assay, male DNA was not be obtained with DNA extraction protocols or was obtained at such a low level that useful information (>10 loci with our STR technologies) was not obtained (or the lab chose not to run the sample through STR because the DNA quantification level did not meet their thresholds). In addition, for test site 4, two samples had a negative result for the small autosomal (human) quantification target in the Y-Screen assay and still provided useful information from the female donor when the Identifiler® and GlobalFiler™ assays were used. These samples were still treated as not concordant, although the Y-Screen assay is primarily for the detection of male DNA. In most cases, if the DNA quantity obtained post-DNA extraction was insufficient for STR analysis, the laboratory still chose to amplify the sample and little to no information was obtained. These samples are grouped with the corresponding STR kit and are considered positive correlations. *In some cases, the DNA quantity obtained after DNA extraction was too low to amplify and the laboratory chose not to amplify the sample.

Table 5 –Correlation between Serology Tests/Y-Screen Assay and STR Results

Test	Correlation between Y-Screen Assay and STR Genotyping Results
P30/PSA	77%
Acid Phosphotase	87%
Slide Screen (Sperm Search)	66%
Y-Screen Assay	91%

Table 5. Two customer sites ran serology tests in addition to the Y-Screen assay. Success rates for these serology tests as well as the Y-Screen assay are shown here with the same criteria for correlation described in Table 4 legend.

CONCLUSIONS -

The Y-Screen assay is a useful DNA confirmatory screening tool when used complementary to other presumptive screening methods. We demonstrate that the sensitivity of the assay correlates well to the results obtained from differential extraction procedures from forensic laboratories using a range of differential extraction procedures and STR chemistries. The Y-Screen assay provided better correlation to resulting STR profiles when compared with commonly used serology methods. In addition, the assay provides useful indicators that the result may not be valid. By confirming conclusive and inconclusive serology results, the assay adds valuable insurance about sample quantity. This Y-screen assay solves important sample screening and processing problems, allowing forensic laboratories to more rapidly process SAK samples and therefore helps to assist in decreasing overall SAK turnaround times and backlogs.

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TRADEMARKS/LICENSING -

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