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The Y-Screen Assay: Validation and Evaluation of Subsequent STR Success

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Abstract

There are hundreds of untested sexual assault kits in police custody. As of 2017, New York State passed a law stating that all untested kits must be turned over to forensic laboratories to be tested, causing backlogs at laboratories to increase. The Y-Screen assay is a DNA extraction protocol which utilizes NaOH to rapidly lyse epithelial and sperm cells, and the Quantifiler Trio DNA quantification kit to detect the presence or absence of male DNA prior to standard sample processing. The Y-Screen assay is a commercial product used in this research to test a defined set of validation samples with or without a male contributor. Here we found that there is a correlation between the results of the Y-Screen assay and the results of the standard extraction and DNA typing. For most samples that tested positive with the Y-Screen assay, male DNA profile results were detected after amplification and electrophoresis of the same sample. If the Y-Screen results were extremely low (below 0.0007ng/µl) then, it is likely that the downstream DNA profile will not yield male DNA either. But there were exceptions. After implementation of the Y-Screen assay a total of 669 casework swabs were tested and from that number 352 (approximately 53%) swabs tested positive for the presence of male DNA without undergoing serology testing. The types of swabs testing positive were vaginal, vulvar, anal, perianal, and dried secretion swabs. The swabs that usually tested negative were the oral swabs. In conclusion the Y-Screen assay effectively streamlines the processing of sexual assault kits (SAK) samples.

Introduction

Sexual Assault Kits

Sexual violence is a pervasive social problem; national epidemiological data indicate that 18%-25% of women are sexually assaulted in their adult lifetimes (Black et al., 2011). The victims are advised to have a medical forensic exam, which includes caring for injuries sustained in the assault, emergency contraception if applicable, administering prophylaxis for sexually transmitted infections that could have been contracted, and the collection of a sexual assault kit (SAK)(Campbell & Fehler, 2015). A SAK is a standard kit that is comprised of multiple envelopes containing swabs, slides, and other trace evidence collected from the victim. There can be a total of 10 different sets of swabs collected from different areas of the victim, as well as five slides and then underwear and trace evidence. Examples of some items collected in a kit are head hair, pubic hair, swabbing of the vagina, anus, mouth, and/or breast for the collection of semen (Campbell & Fehler, 2015). The SAK has instructional forms on how each item should be collected and packaged in order to prevent contamination and degradation of the sample. The items that can be found in one of these standardized kits are as follows: oral swab and smear, exemplar, trace evidence, underwear, debris, dried secretion swabs, fingernail scrapings, pulled head hair, pubic hair combing, pulled pubic hair, perianal and anal swabs and smear, vaginal swabs and smear, and cervical swabs and smear (“SWGDAM”, 2016). The collection process for SAKs is time-consuming and highly invasive for the victim. After the SAK has been collected by a SANE (Sexual Assault Nurse Examiner) nurse, it is taken into custody by law enforcement personnel, who then become responsible for submitting it to a forensic laboratory for testing (Campbell & Fehler, 2015). At the laboratory the kits are screened for biological evidence, and analyzed for DNA. The resulting DNA profiles are uploaded to the Combined DNA Index

System (CODIS), the national forensic DNA database consisting of DNA profiles from arrestees/convicted offenders and crime samples. The samples are compared to those references and, if there is a match (hit), it provides investigators with a lead (Campbell & Fehler, 2015).

Sexual assault evidence is collected focusing on alleged actions during the assault. Studies have shown that evidence collection for each body fluid has a time frame for post-coital DNA persistence time. Semen has the longest period of time for being present on the surface of the body, and within the body after a sexual assault (National Institute of Justice, n.d.). The goal of processing SAK's is to obtain a CODIS eligible profile. The factor that determines CODIS eligibility is: if it is a sample collected from a crime, and if it is does not match the victim's exemplar, and if it is believed to be from the perpetrator ("SWGDAM", 2016). High throughput is a standard approach done with consistent sample types and a defined workflow using automated technology and robotic instrumentation to produce uniform reported results ("SWGDAM", 2016).

SAKs account for a significant portion of the cases received in most labs. Processing SAK evidence is time consuming, labor intensive, and expensive because each item is tested separately. The first test done with the swabs from SAK's is a serological test for the presence of semen.

Acid Phosphatase and PSA Testing

It can take 4-6 hours to screen SAK evidence (Holt A.,Marfori M., Olson,S., &Yong Ning Oh, D., 2015). The screening of a sexual assault kits begins with an inventory and itemization of all the items collected in the kit. All the slides get submitted for staining and microscopic examination for sperm cells. The time frame of 4-6 hours does not include the

reading or running of any samples. This can include serological testing such as Acid Phosphatase (AP) and Protein Specific Antigen (PSA) detection. If sperm is detected, the sample is processed using differential extraction. After the serology testing is complete, the slides associated with those samples are stained and read.

A limitation of using AP and PSA serology testing to triage samples that will get pushed downstream for DNA extraction, is the fact not all SAK scenarios are the same. The serological AP and PSA tests screen the samples for the presence of a semen protein. For example, if the victim reports that the perpetrator ejaculated on her, there is a good chance that the protein screening will detect the semen on the samples. But say the victim stated that the perpetrator forcibly penetrated her but there was no ejaculation. With only a semen test being used to triage which samples get extracted, those samples that have the perpetrators saliva or epithelial cells will be missed. Another limitation with AP and PSA serology testing is that it can be time consuming and expensive. It is expensive to run each swab on its own P30 card. A single P30 kit can process only 25 samples. A SAK can have up to 10 swabs in a single kit, which means one P30 kit can only test at most 2 and a half SAKs. This current method of triaging samples is also considered to be time consuming, since most forensic laboratories batch samples. Once the samples have been tested with AP and PSA, if the results aren't concordant, the original slide and an extract slide would need to be examined. It can take up to four weeks to have a sample be put in for DNA extraction.

Backlog

The problem is that there are an increasing number of untested kits in police custody that will be turned over to forensic laboratories for testing. With the increased numbers of SAK's

being submitted to labs, the lack of resources to test the kits is causing a backlog. The term "backlog" is often used by jurisdictions that have identified "un-submitted" (not unreported) SAKs during their audit or inventory in a forensic laboratory. The definition of backlog is as follows: a case(s) received by the laboratory that exceeds the laboratory's capacity and is (are) awaiting testing ("SWGDAM", 2016).

In a National Institute of Justice (NIJ) funded survey of 1,692 law enforcement agencies, it was estimated that there were 169,000 rape cases dating back to 1982 that contained untested biological evidence (Campbell & Fehler, 2015). A more recent study conducted by Strom and Hickman in 2010, surveyed 2,250 law enforcement agencies and estimated that 10% (27,595) of all unsolved sexual assault cases since 2003 contained un-submitted forensic evidence (Strom &Hickman, 2010). In a study done by Campbell and Fehler it was found that 41% of SAKs were not submitted for analysis, and a similar percentage of kits collected from minors were also not submitted (Campbell & Fehler, 2015). This leads to the question why are some kits submitted and others not; and, are there certain criteria that must be met?

Strom and Hickman, conducted a study in 2010 that looks into why some SAKs are submitted and others not. In their study, they determined that 44% of the respondents did not submit evidence if a suspect had not been identified; 24% if the suspect had already been adjudicated; 19% if the case had been dismissed; 17% if they did not think it was useful; 15% if testing was not requested; and, 12% if the suspect had been identified but not charged (Strom & Hickman, 2010). Another reason as to why police agencies would not submit the SAKs for analysis was based on the victim reporting bathing post-assault, and the belief that the evidence would be compromised (Campbell & Fehler, 2015). A study by Campbell also determined that victims between the ages of 13 to 15 were more likely to have their kit submitted than victims

aged 16 to 17 years old, because the agencies find children more credible than adolescents (Campbell & Fehler, 2015). For adult victims it was found that cases in which the victim was injured, the perpetrator used force, and the assault involved penetration the SAKs were submitted. This suggested that if the police did not submit the evidence it was because they doubted the evidentiary value of the kits and/or doubted the credibility of the victim (Campbell & Fehler, 2015).

In recent years the problem of high SAKs backlog has come to light. 2016 has been a year of addressing the issue with new laws being put in place and depicting criteria on how to handle sexual assault kits delineated. One problem affecting the backlog is the fact that police agencies, and hospitals, have many untested SAKs in their possession. They then transferred all those untested kits to the custody of forensic laboratories.

This issue of untested SAKs is not only relevant in New York, but all over the United States. Another place were the SAK backlog is extremely high is Detroit, Michigan. Detroit has one of the largest backlogs of untested SAKs in the United States. In order to combat this problem the Detroit Sexual Assault Kit (SAK) Action Research Project (ARP) was created (Campbell & Fehler, 2015). The Detroit lab proved to have a backlog issue of SAKs. In 2008; after a census was conducted the lab had a total of 11,219 SAKs spanning from 1980 to 2009 that were untested (Campbell & Fehler, 2015). According to an article in Forensic Magazine, Alaska is also dealing with this backlog issue. Alaska State Troopers have more than 1,000 untested kits containing DNA evidence from sexual assaults. In 2016, the Juneau Police Department had about 350 untested kits and the Anchorage Police Department had another 1,400. Unlike most states Alaska received $1.1 million last Fall to test the kits, but that hasn't happened yet. A statewide group, known as the Sexual Assault Kit Initiative working group is

developing a testing priority to make sure the money is used as efficiently as possible (Associated Press, 2017). According to another article in Forensic Magazine, Virginia has had 44 "Hits" on the old testing of backlogged rape evidence. The article states that "DNA testing in 431 cases of collected, but previously untested biological evidence recovered in Virginia rape cases, has resulted in 44 DNA database "hits" (Green, 2017, pg.1), which shows the value of processing backlog kits. In 2015, a statewide inventory of law enforcement agencies by the Virginia Department of Forensic Science found that there were nearly 3,000 untested "PERKs" (physical evidence recovery kits)for crimes that occurred from 1985 to June 30, 2014 (Green, 2017).

The NIJ (National Institute of Justice) has convened a multidisciplinary team called the Sexual Assault Forensic Evidence Reporting (SAFER) Act working group SART (Sexual Assault Response Team) (National Institute of Justice, n.d.). The SAFER group has suggested to eliminate the smear slides from the collection kit. The slides have been shown to take up time and have been deemed as unnecessary because they contain epithelial cells, bacteria and other debris that do not help in subject identification (National Institute of Justice, n.d.). YSTR testing analyzes the STRs (Short Tandem Repeats) on the Y-chromosome. YSTR can be combined with autosomal STRs for additional information that can be used to determine the perpetrator. The SAFER group has a few recommendations they feel if followed, will help streamline the analysis process of SAKs. One of the recommendations is that examiners should concentrate the collection of evidentiary samples to no more than two swabs per collection area as to not dilute the sample (National Institute of Justice, n.d.).

Rape Kit Law

To prevent backlogs in the future a new law was passed in 2017. The law states that every untested kit must be submitted to a laboratory for testing, and has placed some criteria for new kits that are collected (New York State, 2016). Some of the criteria are as follows; each forensic laboratory receiving sexual offense evidence kits after the effective date shall develop Combined DNA Index System (CODIS) eligible profiles from potential perpetrators from the evidence tested within 90 days of receipt, each laboratory within one hundred and twenty days after receiving kit shall develop a CODIS eligible profile from old kits (New York State, 2016).

The rape kit backlog law was created by subdivision 838-a of the executive law, as proposed by A.10067-A and S.8117 ("SWGDAM", 2016). The bill is identified as S8117 bill. The law was signed into Congress in November of 2016, but was not put into effect until February 26 2017. The law has several different components. The first component is that each police agency and prosecutorial agency must submit any sexual assault kits to a forensic laboratory within 10 days of receipt of kit into their custody (New York State, 2016). The next component is that each forensic laboratory then assesses the case specific information for CODIS eligibility and develop profiles. The labs have 90 days from receipt of kit to accomplish this. Another component of the law deals with the backlog and the kits currently in police custody. Police agencies have 90 days since this law was put into effect to turn over all kits to forensic laboratories. The labs have 120 days upon receipt to generate CODIS eligible profiles and inventory the kits. The law even details the reporting requirements for the labs and investigating agencies (New York State, 2016). This law caused a push for streamlining SAK processing procedures of SAKs.

Extraction

There are two different types of extractions used in this validation. Both of the extraction methods are performed using robotic instrumentation such as the QiaSymphony from Qiagen and the Automate from Applied Biosystems (ABI). The first type of extraction is the differential extraction, which is used on samples that are positive for male semen. It lyses the DNA from the substrate, in this case swabs, and separates the DNA into two fractions, the epithelial fraction (EF) and the sperm fraction (SF) (Butler, 2005). For this validation the SF is the relevant fraction because it is where the male DNA is expected to be. The SF fraction can be tested for autosomal or Y-chromosome specific Short Tandem Repeats (STRs). The second type of extraction is the standard robotic extraction for non-semen evidence, meaning this would be done on samples that had saliva or touch male DNA. This extraction lyses the DNA from the swab and purifies it (Westchester County Forensic Laboratory, 2017). The extracts are then quantified using the Quantifiler Trio quantification kit. Once the concentration of the sample is determined they are amplified and STR typed.

YSTRs

The Y-chromosome is approximately 60Mb in length. The breakdown of the 60Mb lengths is a transcribed region 35Mb in length, while the remaining 25Mb length corresponds to the non-transcribed region. What makes the Y-chromosome unique is the fact that it is rich in many different kinds of repetitive sequences. The Y-chromosome is characterized by 200 SNPS and a 10^{-3} hotspot on the chromosome that is known for mutations to occur. The YSTR approach targets male DNA and is advantageous in cases with female victims and a male perpetrator. Since the vast majority of sexual assaults involve male perpetrators, Y-chromosome specific

DNA testing is designed to only look at the male portion of mixtures, in which autosomal testing revealed high levels of female DNA (Butler, 2005). The use of Y chromosome specific PCR primers improves the chances of detecting low levels of the perpetrator's DNA in high background of the victim's DNA (Butler, 2005).

YSTRs are mainly used when evidence is positive for semen but no DNA foreign to the victim can be detected, when the evidence in question is amylase positive and a male/female mixture is expected, when a large number of semen stains need to be screened and when the number of semen donors needs to be determined (Butler, 2005). The ability to identify male-specific DNA and highly variable Y-chromosomal polymorphisms, is an invaluable addition to the standard panel of autosomal loci used in forensic genetics (Roewer, 2009). The use of Y-STR markers also extends the post-coital interval for successful male DNA detection (Ballantyne, 2012).

Y-Screen

The Y-Screen test is a quick assay to be run on swabs collected from sexual assault kits to assess whether male DNA is present on the swab to then proceed with either differential extraction, or standard robotic extraction. A previous study conducted by Applied Biosystems, created the Y-Screen assay to help labs deal with untested SAKs in a more efficient way (Thermo Fisher Scientific, 2015). It utilizes sodium hydroxide (NaOH) to lyse the cells and Quantifiler trio (ABI) to detect the absence or presence of male DNA prior to processing the kit. The Quantifiler Trio Kit is a Real Time Polymerase Chain Reaction (RT-PCR) kit that determines how much DNA is in the sample. There are four targets: two autosomal human specific (80 bp and 214 bp) DNA sequences, one male target with 75bp, and the IPC (internal

positive control) with 120bp (Holt & Marfori et.al. 2015). In a DNA screening assay study conducted by Holt, the results showed that male DNA profiles could be developed using this approach. The study used one autosomal STR kit (ID+, Identifier Plus), and one Y-chromosome specific STR kit (YF, Y-Filer) kits to obtain their results (Holt & Marfori et.al., 2015). This study concluded that there is a correlation between pre-extraction quantification results and downstream STR results.

Screening for male DNA using quantification and YSTRs is significantly more sensitive than the regular serology screening ("SWGDAM", 2016).The technique used is a quick non-differential extraction of ⅛ of the swab to determine, if male DNA is present. If no male DNA is detected, then no further processing of the sample is needed ("SWGDAM", 2016). The Y-Screen assay is a useful DNA confirmatory screening tool when used with other presumptive screening methods. The manufacturer's validation claims that the sensitivity of the assay correlates well to the results obtained from differential extraction procedures, using a range of differential extraction procedures. The assay also provides a better correlation to resulting STR profiles when compared with commonly used serology methods. In addition the assay has shown to be more sensitive than the current serology tests. By confirming conclusive and inconclusive serology results, the assay is able to add assurance about the type of sample quantity. The Y-Screen assay promises to solve important sample screening and processing problems, allowing forensic laboratories to more rapidly process SAK samples (Holt & Marfori, et.al, 2015).

Eliminating the rape kit backlog requires a new processing procedure to keep up with the high throughput of some labs. The Y-Screen assay can be used as a tool to triage sexual assault kits by confirming whether or not swabs contain a male contributor. Filtering negative kits from downstream DNA analysis can save time, money and effort. Here, we perform a validation study

using a set of mock casework samples and control samples. A portion of each swab will be cut for Y-Screen; a second cutting will be used for a standard DNA extraction, and quantitation and short tandem repeat (STR) analysis according to standard operating procedures (SOP). The Y-Screen assay is only to be used for swab samples. Fabric samples may contain Polymerase chain reaction (PCR) inhibitors and since this is a crude lysis procedure, PCR inhibitors will not be removed by this assay. The FBI Quality Assurance Standard (QAS) were used to determine which studies to conduct at the Westchester County Forensic Laboratory for the internal validation of the Y-Screen assay (FBI, 2011). Based on the FBI and the ANAB standards for accreditation, the studies that must be conducted in an internal validation are as follows: a known and non-probative samples study, reproducibility and precision, sensitivity study, mixture study, and a contamination study.

Methods and Materials

The Y-Screen assay from Applied Biosystems was only validated for sexual assault kit swabs. This assay is not suitable for fabric samples that may contain PCR inhibitors. (ThermoFisher Scientific,2015). This assay is a two- step procedure which uses a crude extraction procedure followed by running the extract with the Quantifiler Trio kit to assess whether male DNA is present.

Known/Mock Evidence Samples

Known and mock casework samples were created to mimic positive and negative rape kit swabs (male DNA present or not present). Table 4 lists the known/ mock evidence samples that contained semen; this includes several post coital samples from volunteer donors. Table 5 lists the known/ mock evidence samples that did not contain semen. These samples were designed to

mimic dried secretion swabs which are found in rape kits. They consisted of mixtures of female body swabs with either male saliva or other male DNA.

Sensitivity/Mixture samples

Two sets of mixture swabs were prepared for this study. The first set consisted of female buccal swabs with male saliva dilutions. The male saliva dilutions were made as follows: 1:2, 1:10, 1:100, 1:1k, 1:5k, 1:10k, 1:15k, 1:20k, 1:50k. The second set of swabs consisted of female buccal swabs and semen dilutions. The dilutions of semen were made as follows: 1:2, 1:10, 1:100, 1:1k, 1:5k, 1:10k, 1:15k, 1:20k, 1:50k, 1:100k. Fifty microliters of each dilution was then added to the female buccal swabs, keeping the female component constant. The dilutions were made with the same female and male contributors.

Reproducibility/Repeatability samples

These were four mixture swabs of 1:1 mixtures of semen: saliva that were cut in triplicate and pooled to demonstrate the reproducibility of the assay. Two analysts were assigned the same four swabs. The goal was to extract the DNA from the pooled swabs and correctly identify, if there was male DNA present or not.

Contamination samples

For the contamination study blank swabs were cut into the tubes and extracted alongside the samples to ensure there was no contamination in the reagents being used or caused by the extraction method for the assay (see table 11).

Y-Screen Assay

The first step in the Y-Screen assay is to cut 1/8 piece of the cotton swab, saving a portion for differential/standard extraction. The cutting is placed in a LySep column/tube (ABI)

with 100 microliters of 1N NaOH and incubated for 10 minutes at 80°C at 750rpm. The LySep column is then centrifuged for 2 minutes at 12,000rpm and discarded. 4 microliters of glacial acetic acid is then added and the sample is diluted 1:5 in low TE (10mM Tris-HCL,0.1mM EDTA. 400 microliters of low TE are added.

DNA Extraction, Quantitation and STR Typing

All of these procedures were conducted using the Westchester County Forensic Laboratory's current SOP: Extraction for non-semen samples was performed on the Automate Extraction Robot with the Prepfiler Kit (Westchester County Forensic Laboratory "Extraction Manual", 2017). Differential extraction for semen samples initially followed several manual extraction steps to separate the two fractions. The two fractions are then placed on the Automate Express using the Prepfiler kit to purify the DNA. Quantitation was performed with the Quantifiler Trio kit and 7500 Real-Time PCR system with HID Real-Time Analysis Software v1.2 (all from Thermo Fisher Scientific Lifetechnologies). For human STR amplification and electrophoresis the study used GlobalFiler™ and Yfiler® for 29 cycles on GeneAmp 9700 Thermal Cyclers. 3500 &3130 Genetic Analyzers were used for electrophoresis with data collection software v2.0 and GeneMapper ID-X Software v1.4 for data analysis (all from Thermo Fisher Scientific Lifetechnologies). Analysis thresholds and stutter filters were kept based on the Westchester County Forensic Laboratory's current SOP. For autosomal STR testing the following results are possible: mixture of male and female DNA (the male may be partial); single source (SS) female or male DNA; no amplification. For Y-STR testing the following results are possible: full Y-STR profile with all expected alleles detected, partial Y-STR profile, and no profile.

Results

We found that there is a correlation between the results of the Y-Screen assay and the results of the secondary standard extraction and STR DNA typing. For most samples that tested positive with the Y-Screen assay, male DNA profile results were detected after the standard extraction, amplification and capillary electrophoresis (CE) of the same sample. If the Y-Screen results were extremely low (around or below 0.0006ng/µl) then, it is likely that the downstream DNA profile will not yield male DNA. For those samples testing negative for the Y-Screen assay, no male interpretable STR DNA profiles were generated.

This Y-Screen assay was able to detect male DNA in samples that were negative with our conventional AP and PSA screening tests. Male DNA detection proved to be reproducible among different cuttings of the same sample and different analysts sampling the same sample. It proved to be sensitive with dilutions of male semen and saliva, and detected male DNA on post coital swabs. See below for a detailed description of the results.

Sensitivity

Table 1 lists the results for the sperm fractions of the mixed buccal: semen swabs. Male DNA was detected down to the 1:100K dilutions in both the Y-Screen assay and the standard extraction/quant method. A Y-STR profile was obtained down to a dilution of 1:1K. Samples with dilutions greater than 1:1K generated no male DNA profiles with GlobalFiler™ or Yfiler®. Based on the quantitation values for those samples, we would not expect a male profile to be detected since the values are below our amplification threshold set by the laboratory for both kits. Samples with a Y-Screen male target value below 0.0007ng/ µL did not result in STR types after extraction. These samples can be considered false positives, since male DNA is being

detected but no STR profile is being generated. However, the samples are from different and larger cuttings, female DNA interferes with GlobalFiler typing, and the amount of male DNA was very low. This data set shows that Y-Screen results below 10pg/µL are predictive of a lack of success for STR typing.

Table 1. Sensitivity for semen

Sample Type	Yscreen Human Target Quantity	Y-Screen Male Target Quant	Y-screen M:F Ratio	Fraction	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
buccal:semen neat	4.6020	4.9388	1:1.07	not tested					
buccal:semen(1:2)	2.3160	2.9659	1:1.28	not tested					
buccal:semen(1:10)	0.6792	0.2217	1:2.1	SF	0.2021	0.3295	1:1.6	Mixture	Full Y-STR
buccal:semen (1:100)	0.4138	0.0469	1:7.8	SF	0.0160	0.0215	1:1.3	Mixture	Full Y-STR
buccal:semen(1:1k)	0.2867	0.0038	1:75	SF	0.0050	0.0020	1:1.4	Mixture	Full Y-STR
buccal:semen(1:5k)	0.2239	0.0006	1:357	SF	0.0073	0.0001	1:83	Mixture - 2 low level male alleles	No Profile
buccal:semen(1:10k)	0.5303	0.0003	1:1842	SF	0.0024	0.0003	1:7.5	Partial female - 1 male peak called	No Profile
buccal:semen(1:20k)	0.0814	0.0000	n/a	SF	0.0004	0.0002	1:2	Partial female - 3 low level peaks (no male called)	No Profile
buccal:semen1:50k)	0.1564	0.0000	n/a	SF	0.0049	0.0003	1:16	Partial female - 1 male peak	No Profile
buccal:semen(1:100k)	0.0498	0.0001	1:357	SF	0.0010	0.0002	1:3	Partial female - 3 low level peaks (no male called)	No Profile

y-screen extract
Secondary extraction

DNA quantitation values are in ng/µL the term "regular" refers to the quantitation value obtained for the sample after a differential extraction (secondary extraction) occurred.

There were a total of twelve samples that were tested using AP/PSA and the differential extraction method (see table 2). For the eight positive Y-Screen samples male DNA profiles were generated for GlobalFiler™ and YFiler®. The AP/PSA positive samples all yielded male DNA. Of the eight samples that tested AP and PSA negative there were mixed results. In three of the samples that were AP/PSA positive, male DNA profiles were detected using YSTR's. Two samples detected low level male alleles and the other three samples did not detect male DNA profiles. The five samples in the chart that did not generate male DNA profiles all had very low

Y-Screen male target quant values (lower than 0.0007 ng/µL) or were negative with the male quant. The results of these samples demonstrate that the Y-Screen method is more sensitive than the conventional AP/PSA screening test. Even if the AP and PSA results are negative, the Y-Screen assay is sensitive enough to detect male DNA, because the DNA target is amplified with the polymerase chain reaction. It will be an effective way of screening sexual assault kit swabs that may or may not have semen present on them.

Table 2. AP/PSA results

(This table contains only SF results)

Sample Type	AP	PSA	Yscreen Human Target Quantity	Y-Screen Male Target Quant	GlobalFiler Results SF	Y-Filer SF
Blood:semen	3	Positive	1.1726	1.4794	Mixture	Full Y-STR
peri anal:semen (fecal material)	3	Positive	0.3722	0.4598	SS Male	Full Y-STR
48 Hr PC*	0	Negative	6.0153	0.0019	Mixture	3 Loci
72 Hr PC*	0	Negative	3.8376	0.0127	Mixture	Full Y-STR
buccal:semen(1:10)	1	Positive	0.6792	0.2217	Mixture	Full Y-STR
buccal:semen (1:100)	0	Weak Pos	0.4138	0.0469	Mixture	Full Y-STR
buccal:semen(1:1k)	0	Negative	0.2867	0.0038	Mixture	Full Y-STR
buccal:semen(1:5k)	0	Negative	0.2239	0.0006	Mixture (low level male)	No Profile
buccal:semen(1:10k)	0	Negative	0.5303	0.0003	SS Female	No Profile
buccal:semen(1:20k)	0	Negative	0.0814	0.0000	SS Female	No Profile
buccal:semen(1:50k)	0	Negative	0.1564	0.0000	Female profile w/ 1 male peak	No Profile
buccal:semen(1:100k)	0	Negative	0.0498	0.0001	SS Female	No Profile

DNA quantitation values are in ng/µL. * The 48 hour Post Coital and 72 hour swabs were from different sample sets.. These swabs were used from prepared material from the 2012 “Post Coital Time Interval Study”.

Table 3 lists the results for the mixed buccal: saliva swabs. The Y-Screen assay was able to detect male saliva DNA down to 1:100 dilution. The 1:100 sample gave a partial male Y-STR profile. The standard extraction method gave male quantitation values down to 1:50K, but these

swab samples yielded no male STR DNA profiles. From this dilution series of nine samples, five were Y-Screen positive, and four of the five generated Y-STR profiles. The 1:20K dilution gave a weak positive result with the Y-Screen assay and the standard extraction but no male DNA profile was generated with the GlobalFiler TM or Yfiler® kit. Here a low Y-Screen value of 0.0006 ng/µL still gave a partial Y-STR profile. As expected based on autosomal STR mixture ratio detection levels (Butler 2005), a male Globalfiler component was only seen for the neat, 1:2 and 1:10 samples.

Table 3. Sensitivity for Male Saliva

Sample Type	Yscreen Human Target Quantity	Y-Screen Male Target Quant	Y-screen M:F Ratio	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
buccal:saliva neat	0.7155	0.2608	1:1.7	1.1100	0.2299	1:3.8	Mixture	Full Y-STR
buccal:saliva (1:2)	0.3514	0.1156	1:2	2.1199	0.1034	1:20	Mixture	Full Y-STR
buccal:saliva (1:10)	0.5815	0.0123	1:46	3.9049	0.0739	1:52	Mixture	Full Y-STR
buccal:saliva (1:100)	0.5061	0.0006	1:903	2.3208	0.0046	1:508	SS Female	10 loci
buccal:saliva(1:1k)	0.1419	0.0000	n/a	0.7976	0.0010	1:831	SS Female	No profile
buccal:saliva(1:5k)	0.6368	0.0000	n/a	3.6653	0.0001	1:35065	SS Female	No profile
buccal:saliva(1:10k)	0.3842	0.0000	n/a	6.8773	0.0006	1:10644	SS Female	No profile
buccal:saliva(1:20k)	0.2712	0.0002	1:1482	1.1957	0.0001	1:13411	SS Female	No profile
buccal:saliva(1:50k)	0.2429	0.0000	n/a	1.9378	0.0005	1:3574	SS Female	No profile

y-screen extract
Secondary extractior

DNA quantitation values are in ng/µL. The dilution swabs are all made the same way, using the same contributors.

Known/Mock Samples

Table 4 lists mock casework samples that contain semen. All samples were extracted with the Y-Screen assay procedure and quantified using the Quantifiler Trio kit. A second cutting was taken and the samples were extracted with a differential extraction, quantified and carried through to CE using both the GlobalFiler™ kit and the Yfiler® kit.

Table 4. Known/Non-Probative sample with semen

Sample Type	Y-Screen Human Target Quantity	Y-Screen Male Target Quant	Y-Screen M:F Ratio	Fraction	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
48 Hr PC	6.0153	0.0019	1:3198	SF	0.0623	0.0010	1:63	Mixture	3 loci
72 Hr PC	3.8376	0.0127	1:301	SF	0.0196	0.0078	1:1.5	Mixture	Full YSTR
Blood:semen (50ul each)	1.1726	1.4794	1.3:1	SF	0.9967	1.2109	1.2:1	SS male	Full YSTR
peri anal:semen (fecal material)	0.3722	0.4598	1.2:1	SF	9.792	4.784	1:2.0	SS male	Full YSTR
4 Days PC	12.3990	0.0004	1:28629	SF	0.0555	0.0022	1:24	SS female	No profile
5 Days PC	7.8373	0.0000	n/a	SF	0.0353	0.0001	1:240	SS female	No profile

y-screen extract
Secondary extraction

DNA quantitation values are in ng/µL Note: 48 Hr PC & 72 Hr PC swabs were from different sample sets. PC= post coital.

All of the samples that gave a positive result with the Y-Screen assay and the differential extraction procedure yielded profiles that contained male DNA ranging from full single source profiles, to mixtures, and full or partial Y-STR profiles. The DNA profiles detected with both the GlobalFiler™ and Yfiler® kits were concordant with the known contributors to the samples. This was true for both the SF and EF fractions of these mixtures (only the results of the semen fractions are listed in table). Please note that the peri anal sample did not have a female profile even though the swab was taken from a female donor. It should be noted that the purpose of this swab was to show that the Y-Screen assay would not be inhibited by the presence of fecal matter.

Table 5 lists mock casework samples that contained male DNA from sources other than semen. Again, each swab was subjected to the Y-Screen assay and then quantified. A second cutting was taken and the swab was extracted on the Automate, quantified and carried through to CE. All of the swabs that tested positive with the Y-Screen assay generated some type of male DNA profile after the standard extraction was performed and analyzed with the GlobalFiler™

and Yfiler® kits. Some samples generated male DNA profiles in GlobalFiler™ and Yfiler®, while others generated a male DNA profile only in Yfiler®. The profiles generated in both the GlobalFiler™ and Yfiler® results were concordant with the known contributors to the samples.

Table 5. Known/Non-Probative non-semen

Sample Type	Y-Screen Human Target Quantity	Y-Screen Male Target Quant	Y-Screen M:F Ratio	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
Vulvar (oral contact)	3.2074	0.0673	1:48	8.3812	0.224	1:36	SS Female	Full YSTR
Vulvar(Digital penetration)	5.7122	0.0065	1:873	39.9436	0.0681	1:585	SS Female	15 loci
Vulvar swab (penetration - no semen)	3.2642	0.0010	1:3256.6	16.125	0.01	1:1615	SS Female	12 loci
Dried Secretion (f:m)	0.0002	0.0001	1:2	0.07	0.0527	1:1.3	Mixture	Full YSTR
(Bite mark) (f:m)	0.0060	0.0068	1.3:1	0.2138	0.2239	1:1	SS Male	Full YSTR
Dried Secretion(Neck) (f:m)	0.0297	0.0195	1:1.5	0.5071	0.4965	1:1	Mixture male is major	Full YSTR
Arm swabbing (grabbed by male)	0.0044	0.0036	1:1.2	0.0773	0.0973	1.3:1	Mixture - male is major	Full YSTR
Oral (no male)	0.0269	0.0000	n/a	1.7605	0	n/a	SS Female	No profile
Dried secretions (no male)	0.0243	0.0000	n/a	0.0886	0.0001	1:947	SS Female	No profile
Peri anal (no male)	0.0974	0.0000	n/a	0.0061	0	n/a	SS Female	No profile
Vulvar (no male)	0.0162	0.0000	n/a	0.0792	0	n/a	SS Female	No profile
vaginal (no male)	0.0232	0.0000	n/a	0.0347	0.0003	1:134	SS Female	No profile

y-screen extract
Secondary extractior

DNA quantitation values are in ng/µL

The samples of table 5 which are known to contain male DNA but not from semen are typically not flagged for DNA testing by our traditional serological screening methods, since the traditional screening is testing for components of semen (AP, PSA and microscopic screening for sperm cells). We occasionally screen for saliva but only when requested, or if the case scenario suggests that it may be present. So these STR profiles may have been missed. In addition, five mock samples from a female were collected that did not have male DNA present. All of these samples tested negative with the Y-Screen assay, and after the standard DNA analysis procedure

was performed, no male DNA profiles were detected with either the GlobalFiler™ or the Yfiler® kits. Two of the samples (dried secretions and vaginal swabs) had been negative with the Y-Screen assay, but then gave low male Quantifiler Trio target values after standard extraction. The values for these two samples were below our STR amplification threshold (Westchester County Forensic Laboratory "Y-Screen", 2017).

Mixtures

Table 6 combines previously listed samples, this time sorted by the male to female mixture ratio after standard or differential extraction. It is expected that with an increasing amount of female DNA male DNA cannot be detected using autosomal markers anymore. Due to the lack of PCR competition it is still possible to find Y-STR signals (Roewer, 2009). This was the case for most of the samples.

Looking at table 6, it can be seen that two samples, the vulvar (oral contact- no semen) and the buccal: saliva (1:10) dilution had a sufficient amount of male DNA present but produced only female STR results. Since the samples contained enough male DNA to be amplified, it was the mixture ratio of greater than 1:20 that prevented the autosomal kit to detect some presence of male even if at a low level. When the same samples were tested with Y-Filer, full Y-STR profiles were generated. More extreme mixtures >1:100 still yielded partial Y-STR profiles. Basically, for samples with a M:F ratio from 1:1 to 1:20, a male DNA profile can be generated with the Globalfiler kit, M:F ratios between 1:20 and 1:100 generated low level male DNA profiles using the Globalfiler kit, and no male DNA profiles were detected with M:F ratios from 1:500 to 1:1600. Y-STRs were detected and generated for all M:F ratios ranging from 1:1 to 1:1600. It is important to note, that these samples were Y-Screen positive. The combination of Y-Screen and Y-STR will increase success rates in cases with male female mixtures.

Table 6. Selected Y-Screen positive samples sorted by M:F ratios

Sample Type	Y-Screen Human Target Quant	Y-Screen Male Target Quant	Y-Screen M:F Ratio	Fraction	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
peri anal:semen (fecal material)	0.37219	0.45982	1:1.2	SF	9.7921	4.784	1:2	SS male	Full YSTR
buccal:semen(1:1k)	0.28667	0.00381	1:75	SF	0.00500	0.00200	1:2.5	Mixture	Full Y-STR
72 Hr PC	3.83756	0.01273	1:301	SF	0.0196	0.0078	1:2.5	Mixture	Full YSTR
buccal:saliva neat	0.71552	0.26079	1:2.7	std	1.11000	0.22990	1:4.8	Mixture	Full Y-STR
buccal:saliva (1:2)	0.35140	0.11556	1:3	std	2.11990	0.10340	1:20	Mixture	Full Y-STR
buccal:semen(1:10)	0.67922	0.22166	1:3	EF	7.13650	0.31620	1:23	Mixture - low level male (2 alleles)	Full Y-STR
4 Days PC	12.39898	0.00043	1:28630	SF	0.0555	0.0022	1:25	SS female	No profile
Vulvar (oral contact - no semen)	3.20742	0.06734	1:48	std	8.3812	0.224	1:37	SS Female	Full YSTR
buccal:saliva (1:10)	0.58146	0.01232	1:47	std	3.90490	0.07390	1:53	Mixture - low level male	Full Y-STR
48 Hr PC	6.01527	0.00188	1:3197.7	SF	0.06230	0.00100	1:63	Mixture	3 loci
buccal:semen (1:100)	0.41380	0.04686	1:7.8	EF	1.76900	0.01650	1:106	Poss. mixture - 1 male peak in stutter position	9 loci
buccal:saliva (1:100)	0.50606	0.00056	1:903		2.32080	0.00460	1:508	SS Female	10 loci
l penetration (vulvar	5.71221	0.00654	1:872.6		39.94360	0.06810	1:585	SS Female	15 loci
72 Hr PC	3.83756	0.01273	1:301	EF	30.4826	0.0222	1:1373	SS female	9 loci
buccal:semen(1:1k)	0.28667	0.00381	1:75	EF	1.99300	0.00140	1:1423	SS female	1 locus
Vulvar swab (penetration - no semen)	3.26419	0.00100	1:3257	std	16.1251	0.01	1:1612	SS Female	12 loci

y-screen extract
Secondary extractior

DNA quantitation values are in ng/μL. This table is not a list of new samples but rather a re-assortment.

In Table 7 we can see that, one condition for detecting Y- STR results is that the male DNA component has sufficiently high quantitation results. Table 7 shows previously listed samples where the Y-Screen had been positive, but no Y-STR results were obtained. All samples had low amounts of DNA and were below the STR amplification cutoff value of 10pg/μL based on the male Quantifiler Trio target.

Table 7. Selected Y-Screen positive samples negative for Y-STRs

Sample Type	Y-Screen Human Target Quantity	Y-Screen Male Target Quant	Y-Screen M:F Ratio	Fraction	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
buccal:semen (1:100k)	0.04982	0.00014	1:357	SF	0.001	0.0002	1:3	Partial female - 3 low level peaks	No profile
buccal:semen (1:10k)	0.53033	0.00029	1:1842	SF	0.00240	0.00030	1:7.5	Poss Mixture - 1 male peak called (not stutter position)	No profile
4 Days PC	12.39898	0.00043	1:28629	SF	0.0555	0.0022	1:25	SS female	No profile
buccal:semen (1:5k)	0.22386	0.00062	1:357	SF	0.00730	0.00010	1:83	Mixture - 2 low level male alleles	No profile
buccal:saliva (1:20k)	0.27120	0.00018	1:1482		1.19570	0.00010	1:13411	SS Female	No profile

y-screen extract
Secondary extraction

DNA quantitation values are in ng/µL. This table is not a list of new samples but rather a re-assortment.

Table 8. Y-Screen negative results and STR typing outcome

Sample Type	Y-Screen Human Target Quantity	Y-Screen Male Target Quant	Fraction	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	GlobalFiler Results	Y-Filer Results
5 Days pc	7.8373	0.0000	EF	40.267	0.0001	1:323801	SS female	No profile
			SF	0.0353	0.0001	1:240	SS female	No profile
Oral (no male)	0.0269	0.0000	std	1.7605	0.0000	n/a	SS Female	No profile
Dried secretions (no male)	0.0243	0.0000	std	0.0886	0.0001	1:947	SS Female	No profile
Peri anal (no male)	0.0974	0.0000	std	0.0061	0.0000	n/a	SS Female	No profile
Vulvar (no male)	0.0162	0.0000	std	0.0792	0.0000	n/a	SS Female	No profile
vaginal (no male)	0.0232	0.0000	std	0.0347	0.0003	1:134	SS Female	No profile
buccal:saliva(1:1k)	0.1419	0.0000	std	0.7976	0.0010	1:831	SS Female	No profile
buccal:saliva(1:5k)	0.6368	0.0000	std	3.6653	0.0001	1:35065	SS Female	No profile
buccal:saliva(1:10k)	0.3842	0.0000	std	6.8773	0.0006	1:10644	SS Female	No profile
buccal:saliva(1:50k)	0.2429	0.0000	std	1.9378	0.0005	1:3574	SS Female	No profile
buccal:semen(1:20k)	0.0814	0.0000	EF	0.3723	0.0000	n/a	Poss Mixture - 1 male peak called (in stutter position)	No profile
			SF	0.0004	0.0002	1:1.3	Partial female - 3 low level peaks	No profile
buccal:semen1:50k)	0.1564	0.0000	EF	4.8830	0.0002	1:24415	SS female	No profile
			SF	0.0049	0.0003	1:16	Poss Mixture - 1 male peak	No profile

y-screen extract
Secondary extraction

DNA quantitation values are in ng/µL. This table is not a list of new samples but rather a re-assortment.

Table 8 again combines previously listed samples that were Y-Screen negative but still extracted using either standard or differential extraction. Of the 12 samples that tested negative for the presence of male DNA with the Y-Screen assay, none produced Y-STR profiles. These results were concordant with the expectation, that if there is no male DNA present there should be no Y-STRs detected. Autosomal typing detected the female contributor and generated mostly single source female profiles. There were two exceptions: as can be seen in table 8, for the buccal:semen (1:20K) sample the epithelial fraction (EF) which had a zero male value with the Y-Screen and after differential extraction, had a possible male profile. It was determined that there was one peak, which was not from the female called in a stutter position making the results a possible mixture. This was unexpected due to the fact that, if there is no male DNA detected with the Y-Screen assay or with the differential extraction, then no male DNA profile should have been detected. For the (1:50K) sample, there was a single peak called in the sperm fraction (SF), was considered a possible mixture, since the major component was the female profile. The one peak that did not belong to the female profile was not located in a stutter position, but could be allelic drop in. In both cases no Y-STR profile was generated and the single peak called in Globalfiler STR typing may be deemed an artifact and not from the male contributor.

Reproducibility/ Repeatability

The repeatability/reproducibility study conducted for this validation had four mixture swabs of 1:1 mixtures of semen: saliva that were cut in triplicate and pooled to demonstrate the reproducibility of the assay. The two analysts attempted to take similar sized cuttings (approximately 1/8 of the swab) and performed the Y-Screen assay on different dates using

different 7500 Real Time instruments. The results of the two sets of samples were compared and appear in Table 9.

Table 9. Reproducibility/ Repeatability

Sample Name	Analyst	Date	Instrument	Yscreen Human Target Quantity	Y-Screen Male Target Quant	Mean Y-Screen Male Target Quant	Std Dev. Y-Screen Male Target Quant	T- Test Y-Screen Male Target Quant
Semen:Saliva	LS	1/12/2017	7500A	0.37461	0.31213	0.35168	0.03206	0.003
Semen:Saliva				0.31156	0.35224			
Semen:Saliva				0.33841	0.39066			
Semen:Saliva	AD	1/20/2017	7500B	0.82756	0.74659	0.902	0.12177	
Semen:Saliva				1.11761	1.04396			
Semen:Saliva				1.03629	0.91545			

DNA quantitation values are in ng/µL. The t-test was conducted using pooled standard deviation values.

The results show that the assay is reproducible in detecting the presence of male DNA in the sample. The assay also proves to be repeatable in that the analysts cutting from the same sample obtained similar DNA concentrations. The reproducibility/repeatability study was done to satisfy the FBI QAS criteria, if when the test is repeated the results are the same. While the detection of male DNA was consistent, both analysts had different DNA concentration results. To determine if there was a significant difference between the analysts carrying out the assay, a T test was done based on the male real time values. The T test results stated that the samples had a p-value of 0.003. Since the p-value is less than 0.05, it is concluded that there is a significant difference between the two analysts. Since the study was used to verify if male DNA was detected the difference between analysts sampling is not detrimental to the validation and the

assay is still reproducible. The significant difference in concentration between the two sets is attributed to sampling variation, or how much of the swab the analysts cut.

Contamination

Table 10. Extraction Negative controls

Sample Name	Sample Type	Yscreen Human Target Quantity	Y-Screen Male Target Quant
EN 1 011217	Extract negative	0.0000	0.0000
EN 2 011217	Extract negative	0.0000	0.0000
EN 3 012017	Extract negative	0.0000	0.0000
EN 4 012017	Extract negative	0.0000	0.0000

DNA quantitation values are in ng/μL.

Table 10, shows several extraction negatives that were run with each set of samples for the Y-Screen assay, and demonstrates that no contamination occurred during the extraction. There were no instances of contamination noted with the Y-Screen assay.

Additional Results

The samples shown in table 11 are different from those previously listed samples and represent examples of Y-screens performed by the lab. This table focuses on the STR profiles obtained from samples organized from the smallest Y-Screen value and up after either standard or differential extraction. As can be seen values >0.1ng/μL in the Y-Screen consistently generated CODIS eligible male autosomal profiles. Lower values need to be examined to determine, if a cutoff using the Y-Screen male real time value can be used to decide what gets chosen for further testing. Previously (see table 1) a value of below 0.0007 ng/μL was considered predictive of negative STR results. Table 11 now shows one sample that had a Y-Screen male value of 0.0003ng/μL generating a CODIS profile. The secondary extraction uses a

slightly bigger cutting and the result was a mixture in autosomal STR typing. This profile would have been missed with a 6-10pg/µL cutoff. Therefore the cutoff value should be lower, only if the real time Y-Screen value is zero, the sample should not be taken through to a secondary extraction. This is also supported by two samples in table 5, where Y-Screen male target values of 0.0010 and 0.0001 ng/µL produced Y-STR results.

Table 11. Samples sorted based on Male Y-Screen value

Y-Screen Male Target Quant	Y-Screen M:F Ratio		Type of Extraction	Fraction	Regular Ext. Human Quant	Regular Ext. Male Quant	Regular Ext.M:F Ratio	DNA Profile Type	CODIS Eligible
0.0001	1:87	+	Standard		0.1401	0.003	1:46	mixture	N
0.0003	1:23	+	Standard		0.042	0.0023	1:18	mixture	Y
0.0017	1:61	+	Standard		0.8379	0.0153	0.079167	poss mixture	N
0.0042	1:2	+	Standard	N/A	0.2373	0.0673	1:3	mixture	Y
0.0046	1:5	+	Standard	N/A	0.2119	0.0326	1:6	mixture	N
0.0105	N/A	+	Standard		0.5332	0.3582	1:2	mixture	Y
0.0132	1:171	+	Differential	SF	0.0103	0.0056	N/A	mixture	Y
0.0176	1:7.07	+	Differential	EF	0.0037	0.0021	N/A	mixture	N
0.0301	1:214	+	Differential	SF	0.0159	0.0034	1:3.67	low level mix	N
0.1793	1:8	+	Differential	SF	0.0805	0.0917	N/A	male (MJ)	Y
0.4393	N/A	-	Standard		0.2528	0.2093	N/A	mixture	Y
0.6423	N/A	+	Differential	SF	0.0375	0.0356	N/A	ss full profile	Y
1.1997	1:5	+	Differential	SF	0.171	0.1871	0.042361	full profile	Y
2.5358	1:1	+	Differential	SF	0.5126	0.5095	N/A	partial female	Y
3.0726	1:6	+	Differential	SF	0.1466	0.1067	N/A	male (MJ)	Y

y-screen extract
Secondary extractior

DNA quantitation values are in ng/µL.

Casework Results

The purpose of including an overview real casework results is to show that the Y-Screen assay is a faster processing method for SAKs. After a year of having the Y-Screen assay

implemented in the laboratory for use with casework, a total of 110 kits have been tested, a total of 669 samples were run through the assay, and their subsequent STR results were obtained. The average number of swabs tested per kit was six. Of these swabs 53% were positive for Y-Screen and 47% were negative. After secondary extraction and autosomal STR testing, the number of CODIS eligible profiles was higher for positive orifice swabs (58%) than body swabs (42%). This may be due to the fact that the sample is more preserved in an orifice than, if it had been deposited on the surface. A total of 130 swabs were taken all the way through to CE. From those samples the majority of STR profiles that were developed were from the dried secretion swabs and the vaginal swabs. Next were the perianal, anal and vulvar swabs. No eligible profiles were developed from the oral or cervical swabs collected from the kits (see figure 1). Along with the STR success there was some database success generated from the Y-Screen assay. 26 samples of the 130 swabs taken through STR typing were eligible for CODIS and therefore entered into the database. This is approximately 17% of all samples that were tested. From the 26 samples entered, eight samples had state offender hits, meaning the profile entered matched a profile from a convicted offender already in the database. This is a 30% hit rate.

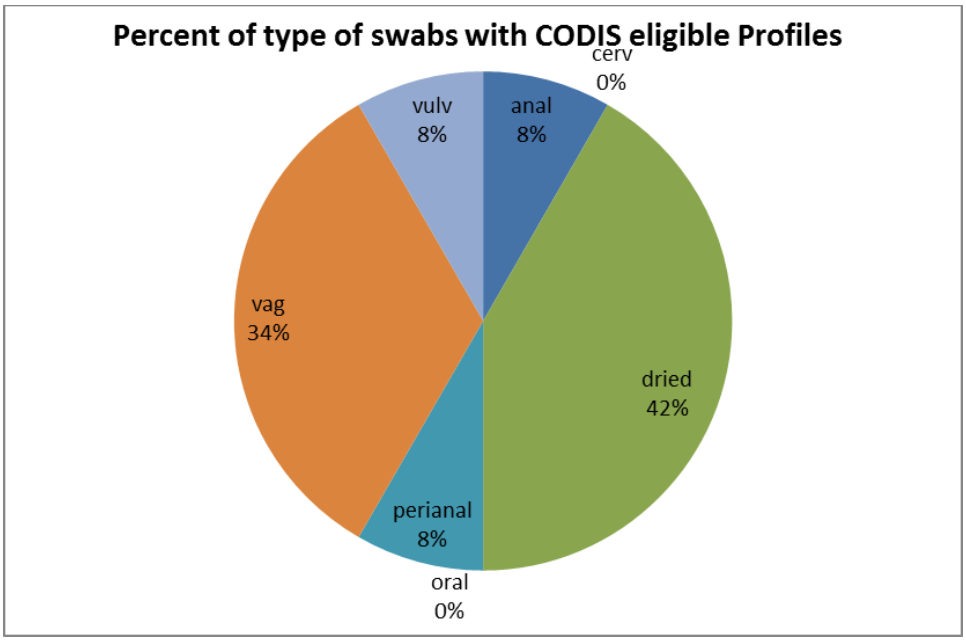

Figure 1. Percent of Swabs eligible for CODIS. The figure shows a pie chart of the types of swabs with CODIS eligible male DNA profiles.

Discussion

All internal validations conducted by an accredited lab, must follow the FBI guidelines. The guidelines provide criteria that must be met in each of the studies conducted. For an internal validation, it is required to have a known and non-probative study, a reproducibility and precision study, a sensitivity study, a mixture study and a contamination study. The internal validation of the Y-Screen conducted at the Westchester County Forensic Laboratory, met all of these requirements in order to implement the assay in casework. For the sensitivity study, it was required that low quality and quantity samples be evaluated. For the known/mock study, it is required that known samples with known contributors be used to test the ability of the assay. For the reproducibility/repeatability study, it was required that the lab obtain the same results when the test is repeated. For the mixture study, it was required that mixture samples be used to establish guidelines for mixture interpretations. For the contamination study, it is required that

the lab test samples to ensure no exogenous DNA is introduced into the samples during processing.

Sensitivity

The Y-Screen assay will be a very useful screening tool to be used on swabs from sexual assault kits for the detection of male DNA. It is more sensitive than conventional screening tests for semen and will detect non-semen male DNA as well. The sensitivity study was conducted to determine the limits or at how low male DNA, the Y-Screen assay could obtain a STR profile. There were two sets of dilution mixtures generated to test these limits, the first being semen dilutions and the second being saliva. The two sets were used in order to test the limits of the Y-Screen not only with semen DNA but other types of male DNA that could be present in real swabs. The Y-Screen assay was able to detect male DNA in samples that were negative with conventional AP and PSA screening tests; it can detect male DNA in the form of saliva or touch, which would be missed with the presumptive AP test, since the AP tests for a semen specific enzyme. The Y-Screen can detect male DNA in semen dilutions down to 10,000x. Samples that gave negative results for AP and PSA, still provided Y-STR profiles. For the male saliva dilution set, the Y-Screen was able to detect saliva down to a 1:100 dilution. The Y-Screen assay is able to detect the presence of male DNA down to a lower concentration since it utilizes PCR amplification and targets DNA rather than a protein as in the case of AP and PSA. It proved to be sensitive with dilutions of male semen and saliva, as well as detect male DNA on post coital swabs.

Known/Mock

The QAS requires that an internal validation have a known and mock sample study (FBI, 2011). The validation provided known samples with known contributors to test the ability of the

assay. The known mock samples were a combination of semen male DNA and saliva/touch DNA, including negative samples. The results determined that all positive Y-Screen samples yielded STR profiles concordant with the known contributors. All negative samples were negative for the Y-Screen.

Reproducibility/ Repeatability

The Y-Screen also proved to be reproducible among different cuttings of the same sample and different analysts sampling the same sample. The results show that the assay is reproducible in detecting the presence of male DNA in the sample when the analyst takes multiple cuttings from the same sample. The assay has also been shown to be reproducible across different instruments on different days, as well as across different analysts.

Mixture

The criteria that is implemented by the FBI QAS is that mixed DNA samples should be used to represent those typically encounter by the testing laboratories, to establish guidelines for mixture interpretations (FBI, 2011). The samples that were generated for this study were a mixture of female and male DNA. These samples were used to mimic swabs typically encountered in sexual assault kits. The Quantifiler Trio software produces a male to female ratio for samples that contain mixtures of male to female DNA. The results from these samples support that as the ratio of female DNA increases relative to male DNA, the ability to detect the minor male component may be limited with autosomal STR analysis and compared to the Y-STR analysis.

Contamination

The contamination study criteria set by the QAS, is that the samples are run through the assay to test for the unintentional introduction of exogenous DNA into a DNA sample or PCR

reaction (FBI, 2011). To ensure no exogenous DNA was coming into contact with the samples during the extraction with the Y-Screen assay, an extraction negative with a blank swab was run alongside for each batch of extractions. The expected results were to see no presence of DNA. The four extraction negatives evaluated had no human or male DNA detected using the Quantifiler Trio kit.

Casework Impact

The Y-Screen result was shown to have a correlation with the autosomal and Y-STR results. The swabs that are positive with the Y-Screen assay for the presence of male DNA, are pushed forward meaning they get extracted a second time and go through the whole DNA process of amplification to STR typing. The Y-Screen assay determines which sample has male DNA, but the quality of the STR profile is based on the secondary extraction of that sample. The correlation to the Y-Screen assay is more of an indication of what should be expected. If there is a high quantitative value of male DNA present, it can be expected that the sample will provide a full Y-STR profile. If the sample had a low quantitative value of male DNA then it is expected to see mixtures in STR typing. In the case were a low quantitative value of male DNA is detected, there may be a single source female profile detected, if the female DNA overpowers the male component in the mixture.

This assay will affect current practices on the processing of sexual assault kits by cutting down on the cost, number of samples extracted for DNA, and improving downstream sample processing. Only positive Y-Screen assay samples need to be put through for the second extraction and it is possible to select the most promising (highest Y screen value) for the kit. This will improve turnover time and reduce backlog. When compared to casework processing using the old workflow, 82 SAK kits were received and of those kits received only 67 were processed

in a year. Of those 82 kits received a total of 472 swabs were tested for AP/PSA; approximately 354 swabs had to be taken through to CE. Using the Y-Screen assay the laboratory was able to process 110 kits in one year with a total of 130 samples take through to CE. This reduction was due to only one swab getting extracted. Figure 2 shows the previous SAK workflow.

Prior to the use of the Y-Screen assay, the processing of swabs from a SAK was very time consuming demonstrated by the flowchart. The way the samples were processed is as follows; a cutting of the swab was take and tested with the AP reagents, this takes approximately five minutes. Then another sample of that same swab is cut and placed in a tube to be tested for PSA at a later date. In most labs, samples are batched in groups. The samples that were cut for PSA testing are batched and soaked once a week, meaning if you did not have them cut for the testing date that week you would have to wait an additional week before the samples get tested. In the testing phase the samples take approximately 3 hours to obtain PSA results. Therefore it can take a whole week from when the analyst itemizes the SAK, to when the samples are incubated and tested with PSA. From there if the samples are PSA positive, they get sent onward for differential DNA extraction which is also batched once a week, meaning that if the sample is not cut in time for the following week, the sample would not get incubated for an additional week. If the sample is negative for PSA, an extract slide is made and the original slide collected with the kit would be stained and read. It takes approximately an hour and a half to stain the extract slide and an hour to stain the original slide. The stained slide must then be read; this could be the same day or any day. Depending on the results, the slide would determine if the sample was sent to DNA extraction. Therefore it can take anywhere from two to four weeks to process samples from one SAK. The Y-Screen assay takes 30-45 minutes to extract the sample. Then it would get placed on the 7500 Real time either that day or the next and the analyst can

have the results of which samples to take through to extraction within one week of itemization of the kit.

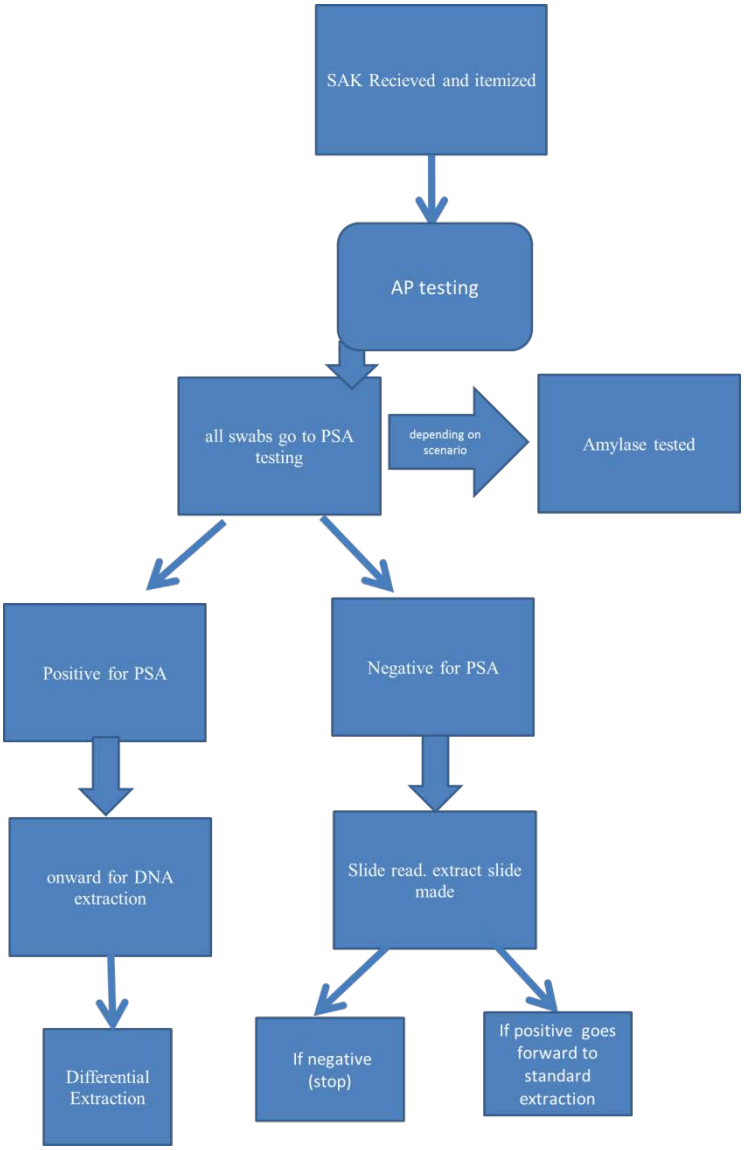

Figure 2. SAK Workflow prior to Y-Screen Assay. This workflow was followed in the processing of SAK's prior to the Y-Screen assay. It shows how the processing was more serological dependent and how the samples were sent downstream for extraction.

Figure 3 is the flowchart of how the Y-Screen assay is used to processes SAK's.

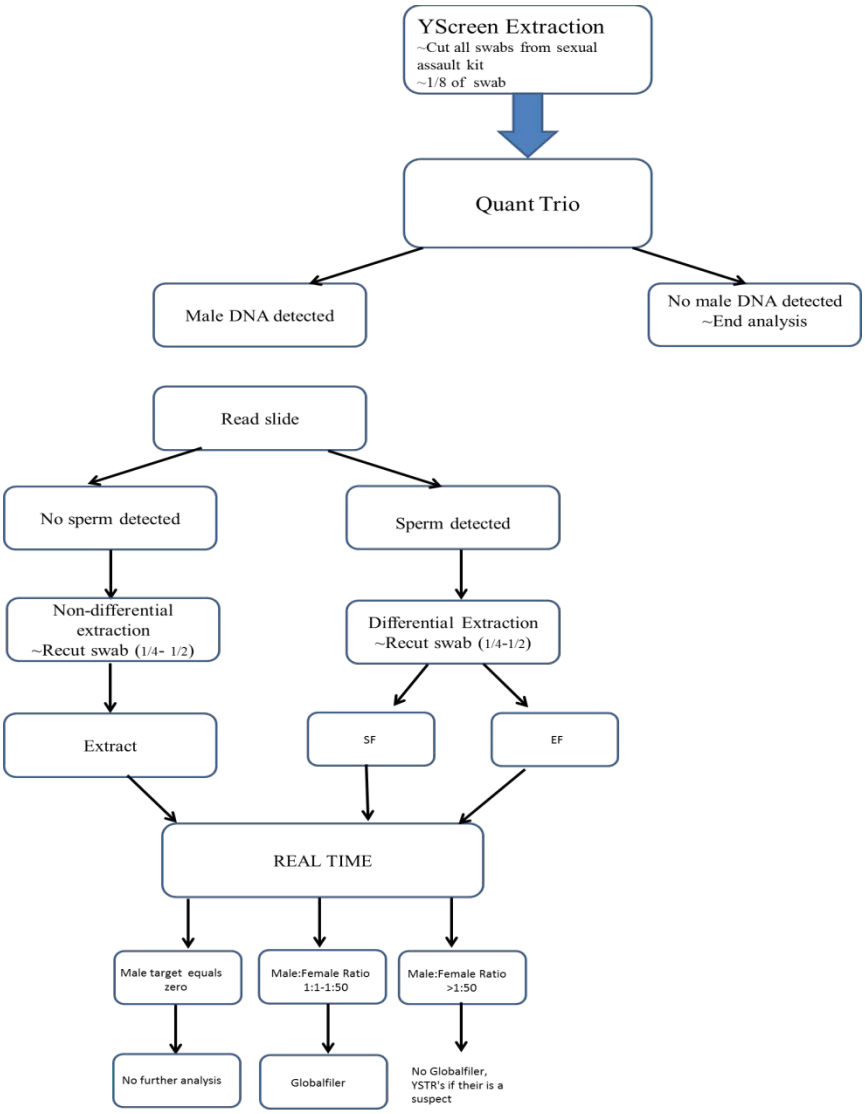

Figure 3 SAK flowchart after implementation of the Y-Screen assay. It shows the downstream decision making based on the presences or absence of male DNA. Unlike the old work flow if no male DNA is detected the analysis ends there and no slides have to be stained or read.

It is clear to see from the flow chart that the Y-Screen assay provides for a more streamlined process. To extract a sample takes approximately half an hour. This will reduce the amount of time and cost it takes to process the kit with the elimination of AP/PSA testing. The

slide staining and reading is performed only for swabs that are candidates for differential/standard extraction. The time taken for reading the slide has been shortened by having a scan of the slide submitted from the Y-Screen assay and then reviewed. A full scan is conducted in ten minutes. If no spermatozoa are detected in that time, the slide is recorded as being negative. The slide results determine, if a differential extraction or non-differential (standard) robotic extraction is to be conducted on the swab. Negative slide results indicate a non-differential extraction and a positive spermatozoa slide means the sample needs differential extraction. The Y-Screen implementation data shows its success in generating CODIS profiles; this addresses the high throughput needs of forensic laboratories. The casework implementation shows the success of the Y-Screen assay in improving the turnaround time. Of the 110 cases run through this assay in a year, every single deadline was met, be it an old or new case for the 3-month due date.

Limitations

The Y-Screen assay was predictive of the downstream results. A positive male Y-Screen quantification result correlated with a positive male quantification result post extraction. The assay was proven useful with mixtures as well. A low quantification result with the Y-Screen generally correlated to low quantification results post extraction. The manufacturer has emphasized limitations to this correlation due to the fact that there have been some cases seen, where a sample had a Y-Screen value below the amplification threshold that when taken through to CE actually generated a Y-STR profile (ThermoFisher Scientific, 2015). This was also observed in this validation.

The assay can only be used on swabs due to it being a crude lysis extraction and inhibitors might be present that may impact the IPC (internal positive control) and the degradation marker results. In the Y-Screen assay this is a limitation. In this study the Real Time PCR, showed elevated out of range values for the internal PCR control (IPC) for several samples. The samples that had this elevated IPC were negative for male/human DNA and normally run the same day they were extracted. When the samples were requantified later their IPC values were in range with the parameters for the lab. It was unclear as to why the samples worked the second time. The theory was tested with fresh sodium hydroxide made in smaller batches and more frequently. When the issue continued to occur, it was believed that the inhibition may be caused by the glacial acetic acid that is added during the lysate.

To study this issue further, a test was conducted alongside a Y-Screen assay extraction, by adding a sample of neat glacial acetic acid to the Quantifiler Trio quantitated batch. All samples for this run worked with no problems with the IPC's except for the straight glacial acetic acid, which had caused inhibition. It was concluded that a way to remove this problem was to vortex the samples after the addition of glacial acetic acid. It was found that there was no inhibition for the samples when the samples were vortexed.

Advantages/Disadvantages

With any new method or technique, it has its advantages and disadvantages. The Y-Screen assay has several advantages. The first advantage of the implementation of the assay is that it improves the overall turnaround time in the processing of SAK's. The assay allows analyst to extract 1-2 samples instead of consuming swabs and time to test for AP and PSA and reading all the slides in the kit, which means the Y-Screen is quicker in pinpointing which swabs have

male DNA present. This is also more cost efficient. Another advantage of the assay is its ability to detect the presence of male DNA, even in minute amounts not only for semen but saliva and touch sources. SAK's previous testing was based on serological tests (AP and PSA). These tests were shown to not be as sensitive or reliable as using Y-Screen to detect male DNA in samples. The assay only uses a small cutting, then a slightly larger cutting for the secondary extraction, which prevents the consumption of the whole sample.

There are some disadvantages with the assay. One disadvantage is that the assay is so sensitive it detects low male values; these low values sometimes do not yield a male STR profile. This could be instrument noise or the male DNA is too low to produce a male STR profile. Another disadvantage is that the first cutting which gets extracted with the Y-Screen cannot be used for amplification, since it is a crude lysis extraction and with no purification the inhibitors are still present. Samples always have to be extracted twice to be taken through to STR typing.

Conclusion

The Y-Screen assay has proven to be a useful tool in the processing of SAK's. The assay has shown to be more sensitive than the conventional AP/PSA testing in generating Y-STR profiles. The Y-Screen assay not only detects male DNA in semen but rather any type of male DNA (saliva and touch). From all the mock samples that have been run through this assay, there were no false negatives observed, meaning if there was no Y-Screen male Real Time value, no STR result was obtained. If male DNA was detected with the assay, the STR success depended on the Y-Screen value. Y-Screen values greater than 10 pg/µL always had some type of male signal detected after the second extraction. Below that 10 pg/µL value, some samples yielded Y-STR profiles, but others were negative. The Y-Screen assay has demonstrated a sufficient

correlation between the first extraction results with downstream STR results, and is an appropriate tool to help solve the rape kit backlog issue.

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