Scientific Working Group on DNA Analysis Methods
Next Generation Body Fluid Identification Working Group

Report on Y- Screening of Sexual Assault Evidence Kits (SAEKs)

July 2020

Contents

Introduction

The Next Generation Body Fluid Identification Working Group was convened by the Scientific Working Group on DNA Analysis Methods (SWGDAM) to examine the potential for employing DNA techniques to improve workflow, sensitivity, sample utilization, and efficiency of serological screening. The National Best Practices for Sexual Assault Kits recommended forensic laboratories implement Direct to DNA testing as a best practice: "Laboratories should consider changing the order of processing the evidence by going to Direct to DNA and then, only if needed, proceed to serology" [1]. The Next Generation Body Fluid Identification Working Group's goal is to examine new technologies and techniques, determine which techniques are employed in forensic laboratories, and investigate the potential for best practices and considerations to improve their operations.

Conventional serological testing (i.e., body fluid identification and/or microscopic examination for sperm) is an important aspect of forensic sample processing, especially in cases where sexual assault is suspected. The process, however, can be labor intensive and time consuming, often delaying results and causing backlogs. Sexual assault examination kits (SAEKs) commonly contain swabs to collect biological evidence. In an effort to streamline the examination of sexual assault cases, many laboratories have implemented a Direct to DNA Y-screening case approach for the processing of these samples [2]. This has led to the development and implementation of different Y-screening workflows adapted to regional variations in the SAEKs submitted, and to the resources and organization of the various forensic laboratories.

Y-screening offers a viable alternative to conventional serological methods. It is a Polymerase Chain Reaction (PCR)-based process that replaces conventional serology as the gatekeeper for which samples proceed to Short Tandem Repeat (STR) analysis. The process is more sensitive than conventional serological techniques and can therefore yield higher quality and more accurate information in a shorter timeframe by limiting downstream processing to relevant samples. The improved sensitivity of this method not only relates to the amount of male DNA that can be detected, but more importantly to the number of instances in which male DNA will be detected when compared to conventional analysis. The process offers additional benefits such as enhanced reproducibility and a more consistent workflow, as it eliminates the human interpretation factor inherent to the conventional serological processes. In addition, it lends itself to automation, therefore improving laboratory efficiency.

The Next Generation Body Fluid Identification Working Group's initial strategy was to determine the current methods employed by forensic laboratories and assess the potential for improvement, specifically to include the methods for Y-screening analysis of sexual assault kits. The Working Group determined that a survey tool would be employed to plot the current status of Y-screening implementation strategies, specifically designed to gather information on the analysis of SAEKs from female complainants and alleged male perpetrators. The survey was designed to include the various decision points, along with selections which would capture the variety of methods employed within an array of choices. Decision points included the number of swabs and sampling plans, decisions regarding extraction, quantification, and amplification, when to cease examination, and reporting of results. The final survey was sent to approximately 200 laboratories in the United States and Canada. Insight into the variety of methods employed by forensic laboratories gathered by the survey tool enabled the analysis of trends and assessment of potential best practices. Scrutiny of the Y-screening data provides a framework to identify various factors for laboratories to consider as they evaluate, plan and implement a Y-screening approach.

Glossary

For the purposes of this document, terms are defined as follows:

Conventional Serology

Detection, identification, classification, and study of various bodily fluids such as blood, semen, saliva, urine, breast milk, vomit, fecal matter and perspiration. This generally includes color change tests, enzyme detection tests, antigen/antibody tests, or microscopic observation (e.g., sperm).

Differential Extraction

A process by which the DNA from sperm cells can be separated from remaining cell types (mainly epithelial) and extracted.

Direct to DNA Y-Screening

A workflow that uses qPCR to quantify male DNA, utilizing the male DNA quantity rather than conventional serology to direct downstream DNA STR testing. Some conventional serology may be used in this workflow as determined by laboratory procedure (e.g., for sample selection or determining when to perform a differential extraction). May also be referred to as "Direct to DNA" or "Y-screening."

Item

A set of swabs from a given orifice/body location packaged together (e.g., four vaginal swabs are considered to be one item).

One-Extract Workflow

Extraction and purification of a sample for Y-screening and possible autosomal and/or Y-STR typing with the same DNA extract.

Presumptive Test

Indication that a specific bodily fluid may be present in a sample without confirmation of its presence.

Sample

The portion of a swab tested.

Sampling

Removal of a portion of a swab.

Sexual Assault Evidence Kit (SAEK)

A standardized kit used by medical personnel to collect forensic evidence from sexual assault victims.

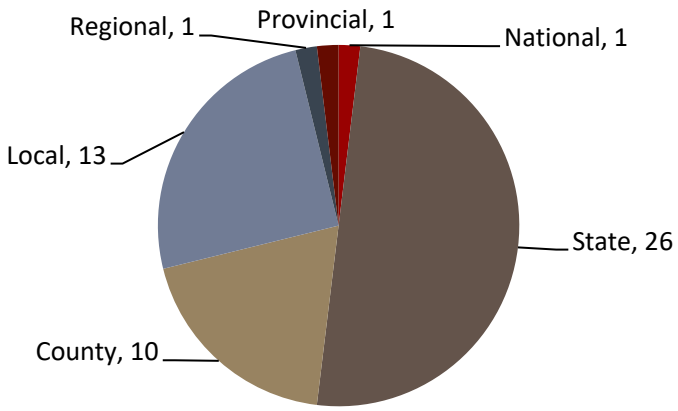
Two-Extract Workflow

Extraction of a sample for Y-screening, and if sufficient male DNA is detected, a second sampling, extraction and purification is performed for STR typing.

Survey Results

In May of 2019, the Y-screening survey was sent to the Technical Leaders of the approximately 200 NDIS-participating local, county, regional, state, and federal forensic laboratories in the United States that perform DNA testing, as well as, to the Royal Canadian Mounted Police (RCMP) in Ottawa and the Centre of Forensic Sciences (CSF) in Toronto. Responses to the survey were received from 103 different laboratories with 52 of the responding laboratories indicating that their laboratory currently performs Y-screening. Of these 52 laboratories, 38% are part of a multi-laboratory system and 62% are part of a single laboratory system. The jurisdictions served by the responding laboratories that currently perform Y-screening are detailed below.

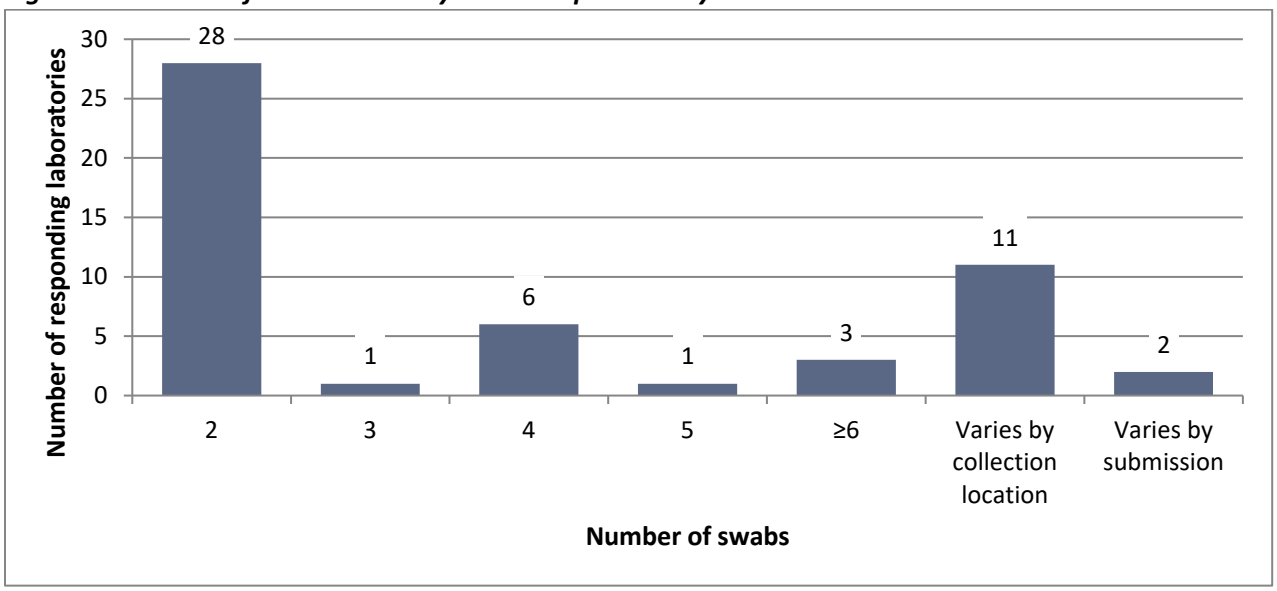
Figure 1. Jurisdictions served by laboratories performing Y-screening



Of the Y-screening laboratories, 44% have DNA and serology performed by the same individuals, while 56% have separate DNA and serology personnel.

SAEKs are designed to facilitate the standardized collection of evidence from a victim of an alleged sexual assault, and each SAEK typically contains orifice/body swabs that are collected by a Sexual Assault Nurse Examiner (SANE). For the laboratories currently performing Y-screening, the number of swabs routinely collected per item by the SANE personnel is detailed in Figure 2.

Figure 2. Number of swabs routinely collected per item by SANE



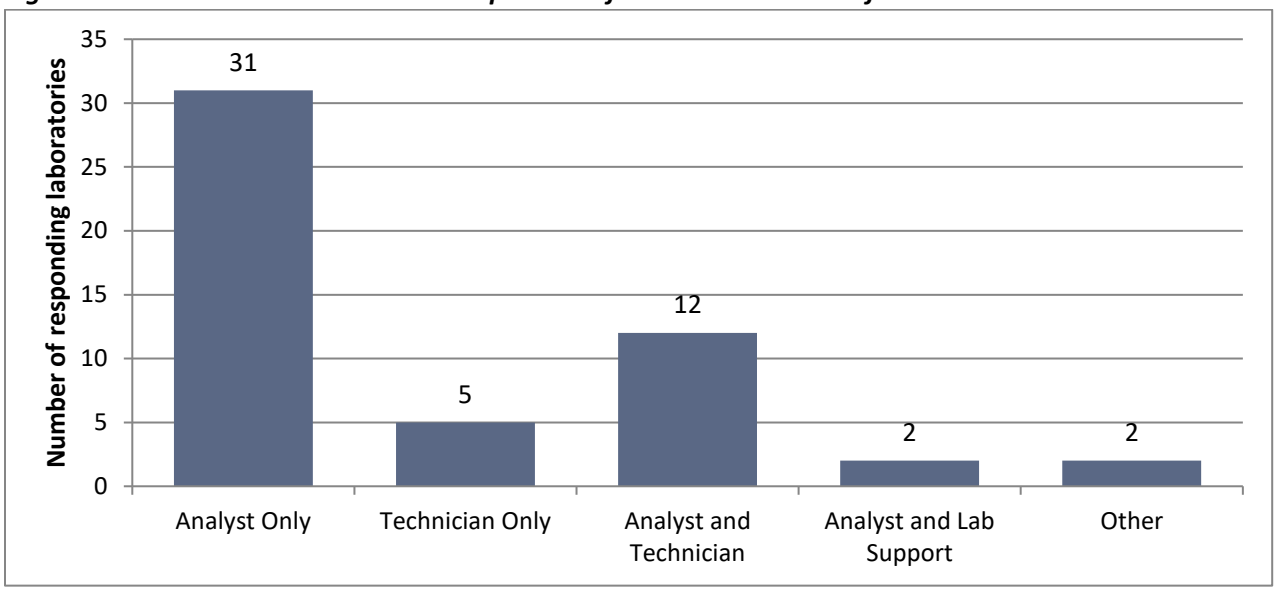
Of the 11 laboratories who responded that the number of swabs collected varied by location, generally four swabs were collected for the vaginal/cervical samples and two swabs were collected for all other locations.

Y-screening of SAEKs is typically implemented in a laboratory by using either a one-extract or two-extract workflow. Both workflows involve sampling of the items within the SAEK, extraction of the samples, and performing DNA quantification. A determination is made regarding which samples will be subjected to autosomal and/or Y-STR analysis based upon the DNA quantification result. In the one-extract workflow, the SAEK items are sampled and extracted in a manner that allows both the Y-screening procedure and STR analyses to be performed on the same extract. In the two-extract workflow, a smaller amount of each item is initially sampled and extracted for the Y-screening procedure, with a second sampling, extraction and purification performed for any item that will be subjected to STR analysis (autosomal or Y-STR). Of the laboratories currently performing a Y-screening procedure, 63% employ the one-extract workflow, while 37% employ a two-extract workflow. Note that some laboratories have both workflow options incorporated into their procedures.

Sampling and Extraction

The first step of any Y-screening workflow is to select and sample the items within the SAEK to perform Y-screening. For the laboratories currently performing Y-screening, the personnel who select and sample the items to be Y-screened is shown in Figure 3.

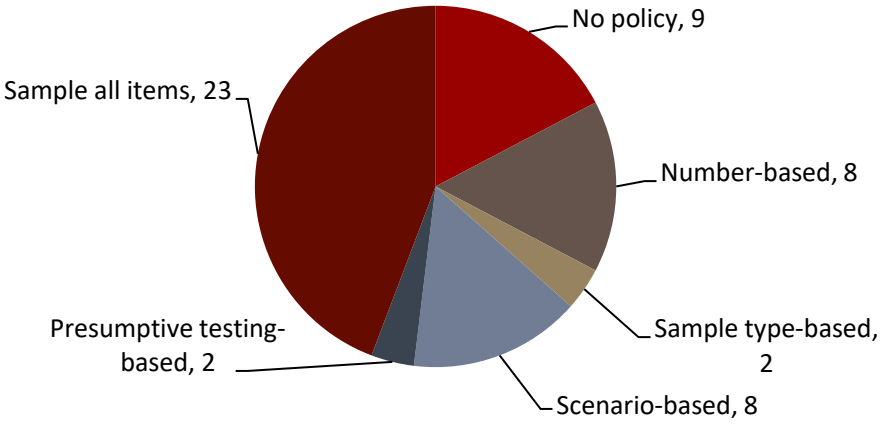
Figure 3. Personnel who select and sample items for the Y-screen workflow



The majority of Y-screening laboratories (86%) have analysts involved in the sampling of items in some capacity. Laboratories indicating "other" described personnel with specific titles such as "Screening Analysts" and "Criminalists."

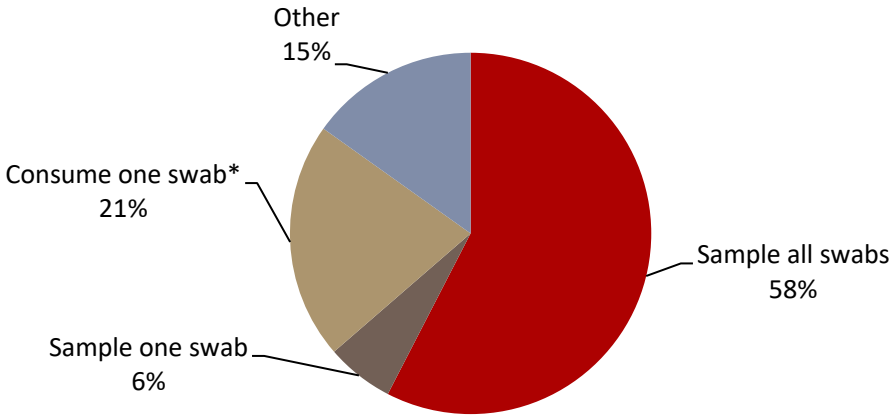
Policies on which items were sampled varied widely for laboratories currently performing a Y-screening workflow (Figure 4). Many Y-screening laboratories (44%) sample from all available items within the SAEK. Two Y-screening laboratories (4%) use presumptive testing to assist with determining which items in the kit will be selected and sampled for testing. For laboratories with a number-based policy, policies ranged from two items/kit to six items/kit, with the majority of these laboratories sampling three items/kit. Nine laboratories have no policy in place, allowing for total discretion in the number of items sampled. The remaining laboratories have either scenario-based or sample type-based policies in place.

Figure 4. Sample selection policies in responding laboratories



For laboratories that follow a one-extract workflow, 58% sample a portion of each swab collected within an item, while 27% sample only one swab per item. The remaining 15% of the laboratories employ a laboratory specific sampling approach, use presumptive test results, or allow analyst discretion to determine how many swabs from each item are sampled. In addition to variation between laboratories of the number of swabs sampled per item (Figure 5), the amount of each swab that is sampled varies as well. Sampling policies include sampling less than ¼ of each, ~ ¼ of each, ~ ½ of each, ~ ½ of one, ~ ¾ of one, and sampling from all to create one swab equivalent (e.g., for two swabs, sample ~ ½ of each; for four swabs, sample ~ ¼ of each).

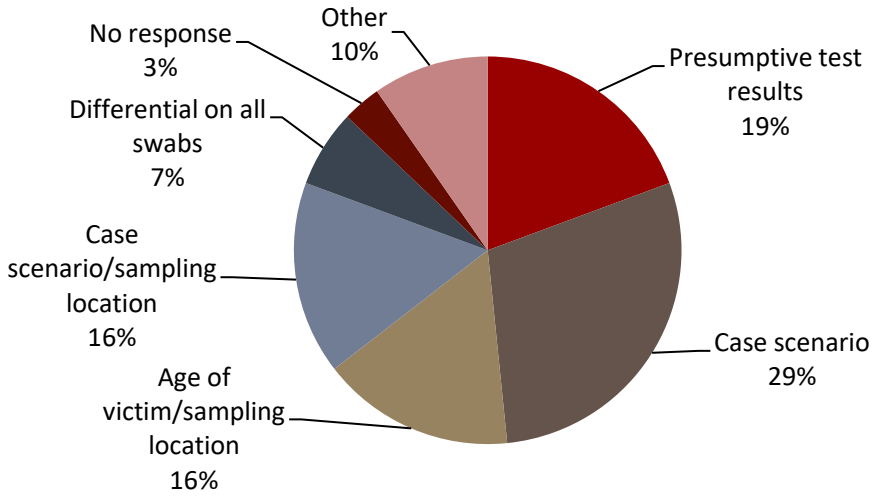
Figure 5. Number of swabs sampled per item for a one-extract workflow



**Laboratories consume one swab unless only one swab is submitted. If only one swab is available, the laboratory will consume ~½ of the swab.*

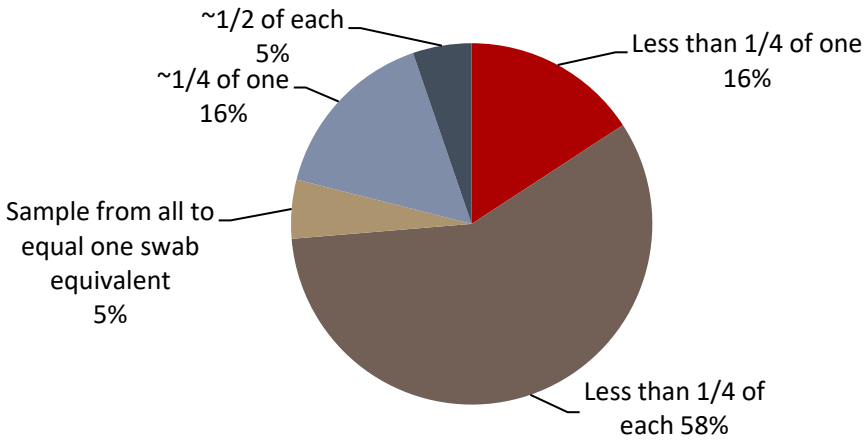
For laboratories that follow a one-extract workflow, approximately 81% do not perform presumptive testing prior to the Y-screening procedure. For these laboratories, different policies based on the case scenario exist for determining the appropriate DNA extraction method (i.e., non-differential extraction versus differential extraction) (Figure 6).

Figure 6. Policies on the determination of DNA extraction method for laboratories creating only one-extract



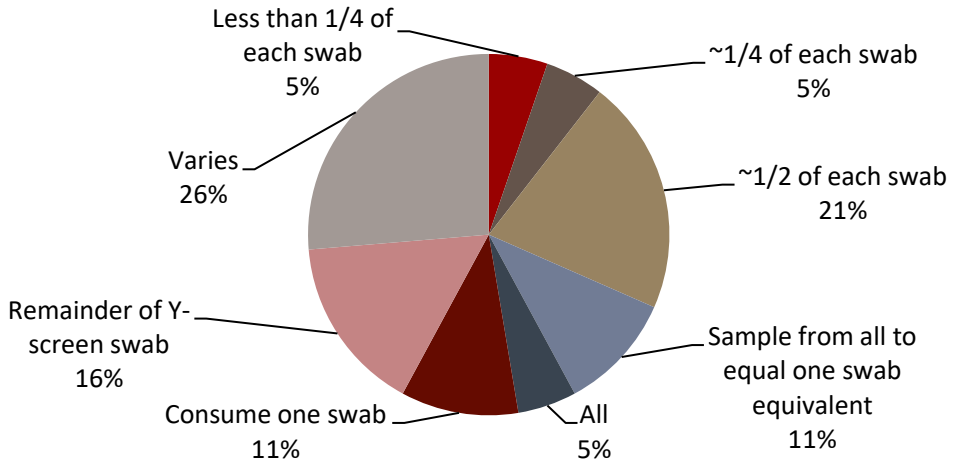
For the laboratories that follow a two-extract workflow, the first extraction is typically a non-differential extraction. Some laboratories have implemented an extraction method incorporating DTT into a non-differential protocol, while other laboratories are performing a direct quantification on a very small swab cutting. For the first extraction, the majority of laboratories sample less than ¼ of each swab in an item (e.g., four vaginal swabs submitted, less than ¼ of each vaginal swab is sampled and combined for the Y-screening extract). The remaining laboratories reported a variety of sampling strategies (Figure 7).

Figure 7. Amount of swab material sampled for the first extract in a two-extract workflow



In sampling for the second extract, an even broader range of sampling strategies is employed (Figure 8).

Figure 8. Amount of swab material sampled for the second extract in a two-extract workflow



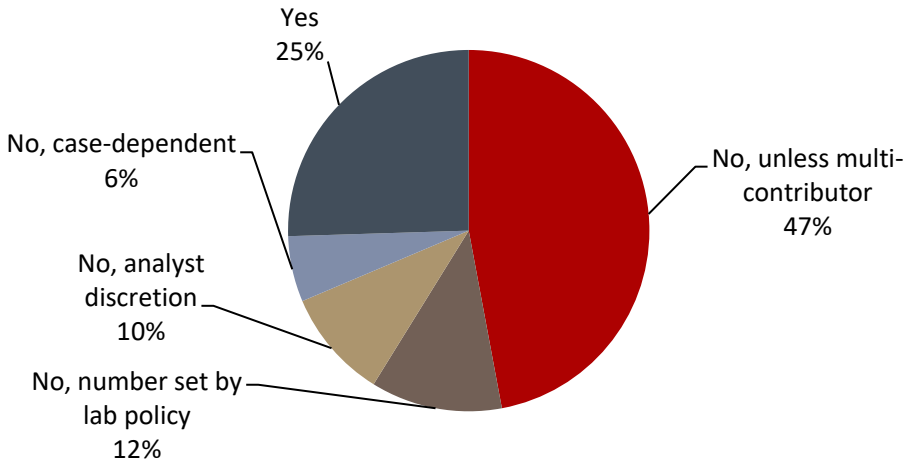
The "varies" category in the chart above consists of laboratories that use the quantification results from the first extract in a variety of ways to drive the sampling decisions for the second extract. For example, one laboratory utilizes the total male DNA detected and the ratio of that male DNA to the total human DNA to determine how much of the remaining swab(s) to sample.

Quantification and Amplification

Of the responding laboratories, 8% modified their conventional quantification procedure by either reducing the reaction volume or increasing the DNA input volume for Y-screening purposes.

Laboratories that perform Y-screening typically establish "stop-at-quantification" thresholds through internal validation studies. The thresholds typically applied include a minimum amount of DNA and/or minimum male:female or male:total genomic DNA value for autosomal STR amplification. Within a Y-screening workflow, qualifying samples are those samples that exceed the laboratory-specific "stop-at-quantification" thresholds. For participating laboratories, the approach to amplifying qualifying samples is described in Figure 9.

Figure 9. Survey responses to "Does your laboratory amplify all qualifying samples?"

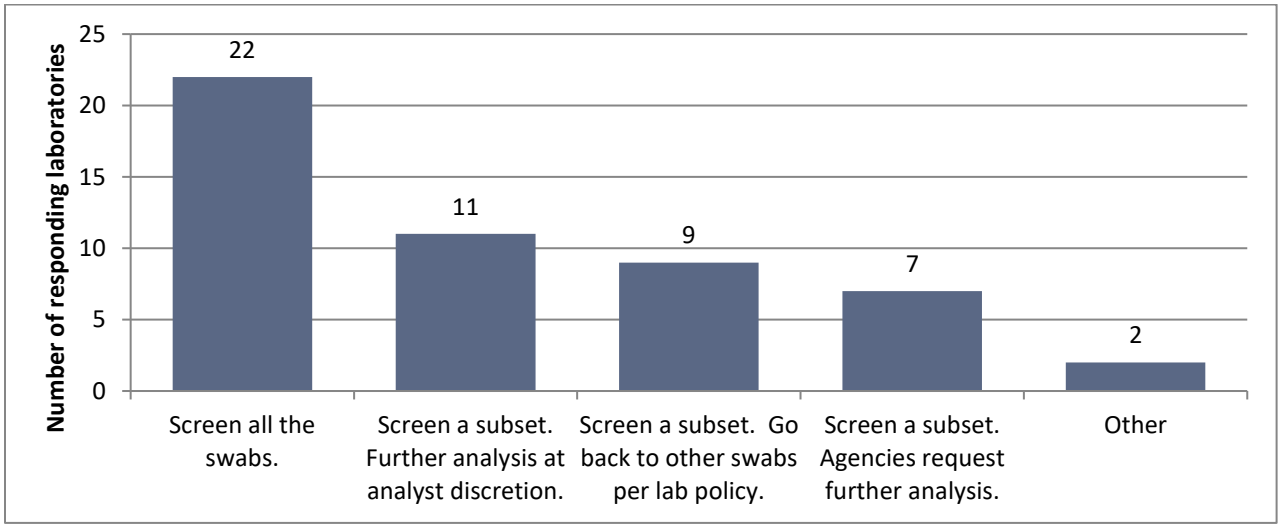


Multi-contributor cases are those in which there is more than one contributor foreign to the victim reasonably expected to be present based upon the case scenario. This may include cases in which there are multiple alleged assailants and also cases in which only a single assailant is alleged but there was recent consensual activity with another individual.

Scope of Initial SAEK Processing

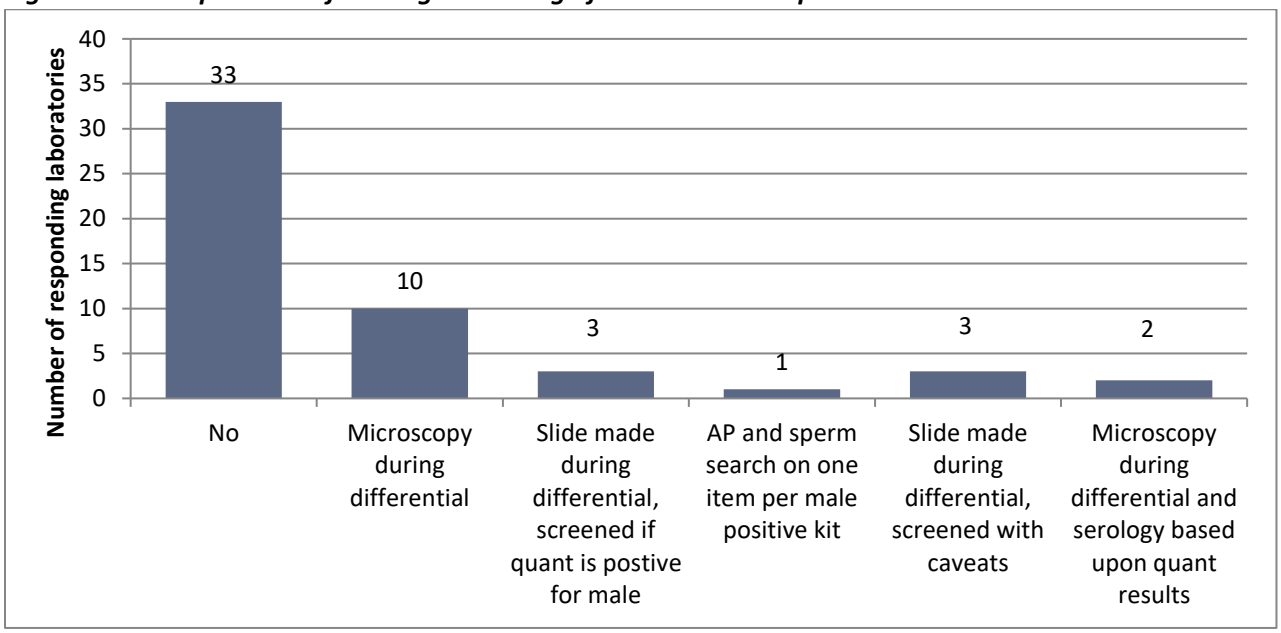
Of the responding laboratories (51 of 52), approximately 43% perform Y-screening on all the swabs within the SAEK. While the remaining laboratories initially perform Y-screening on a subset of the SAEK swabs with additional Y-screening performed by analyst discretion, laboratory policy, and/or by agency request (Figure 10).

Figure 10. SAEK processing strategies for Y-screening workflows



Laboratories employing Y-screening workflows determine whether serological testing will still be performed as part of this workflow. From the responding laboratories, 37% incorporate serological testing into their Y-screening workflow in some manner (Figure 11).

Figure 11. Incorporation of serological testing after Y-screen sample selection



Of the laboratories that do not routinely perform conventional serology after Y-screening, two conduct conventional serological testing prior to Y-screening. The remaining laboratories do not routinely use serological testing in SAEKs. For these laboratories, most report that serology is available if requested by law enforcement or an attorney; however, the laboratories responded that such a request was rare (less than 1% of cases analyzed).

Reporting

Thirty-six of the 52 responding laboratories replied that they employ separate personnel for sampling, serology, and/or DNA analysis. Of those 36, half (18) issue a single report containing both the results of the Y-screening and the STR analyses and half (18) issue two reports. The examples provided for the laboratories that issue two reports include the following:

1. one report for sample selection and a second report for the Y-screening and STR analyses,
2. one report for the Y-screening and a second report for the STR analyses, and
3. one report for the SAEK (Y-screening and STR analyses) and a second report for non-SAEK evidence.

Of the remaining 16 (of 52) laboratories, 12 laboratories issue a single report since the same personnel perform sampling, serology, and/or DNA analysis, and four laboratories did not respond to the question.

For the 31 laboratories currently performing a Y-screening workflow with no conventional serological testing, the reporting language used to describe the two fractions of a differential sample in the majority of the laboratories (17 of 31 labs, 52%) included the use of the terms “sperm and epithelial fractions” or

analogous terminology. Some laboratories include a caveat about the use of the term “sperm” (10 of 17 labs) while others do not (7 of 17 labs). Eleven of these 31 laboratories use other reporting language, such as abbreviations that do not include the term “sperm” (e.g., F1 and F2), or reporting the item as a whole, rather than fractions of the item. The remaining three laboratories provided no response to the reporting question.

Implementation Decision Points and Risk Considerations

Y-screening workflows have many benefits, largely related to efficiency and sensitivity [2]. Y-screening can detect male DNA when the associated body fluid(s) are present at a level that cannot be detected via conventional serological testing methods. Efficiency gains can be made with Y-screening workflows when implemented alongside automation and standardization of processes, and when maximizing the allocation of personnel. Limitations of Y-screening workflows are few, but center around whether the jurisdiction served by the laboratory can or will accept male DNA results without attempts at body fluid identification, and how laboratory personnel job functions will change under the new workflow. Laboratories may choose to do a pilot study in which the results obtained with conventional serology are compared to the results obtained with the Y-screening workflow as a means to optimize the Y-screening workflow and gather data to present to the necessary stakeholders.

Accreditation standards (ISO 17025:2017) require accredited forensic laboratories to assess their risk. Improving technology offers an alternative capable of mitigating risk; however, it comes at the cost of the investment in resources to investigate, select, validate, implement and train staff. Conventional serology used to process SAEKs (e.g., identification of sperm, enzymes or antigens prior to DNA analysis) has not changed markedly in several decades. There is an inherent risk of not detecting/visualizing sperm as the process is highly skills-based and challenges including low sperm count, sperm lysis, sampling issues and high levels of background cells hindering sperm searches can be encountered. The sensitivity and precision of DNA testing, more specifically DNA quantification, has improved significantly, and many forensic laboratories have determined that mitigating the risks associated with conventional serology outweigh the implementation barrier and are now successfully conducting a Y-screening regimen for SAEKs. Using these laboratories' experiences can be very helpful to laboratories that have yet to implement Y-screening; therefore, a goal of this document is to distill choices and best practices to streamline implementation.

While all laboratory methodologies have some inherent risk, points for laboratories considering the implementation of a Y-screening workflow include:

- Comparative risk of missing male-positive cases due to body fluid test limitations, to include the availability of presumptive testing versus confirmatory testing and sensitivity of said methods
- Sampling considerations regarding the number of swabs and the number of items
- Efficiency considerations, the procurement of equipment, and the use of automation in the laboratory
- Consistency from analyst to analyst
- Consumption of evidence, reagents, and analyst time
- The continued ability of the laboratory to answer the relevant investigative question(s) (i.e., the ability to follow Y-screen testing with conventional serology or other testing, as needed)

Diagrams of the two most common Y-screening workflows are included in Appendices A and B and involve either a one-extract or two-extract workflow. In the one-extract workflow, a single DNA extract is generated for the Y-screening process and possible STR typing. In the two-extract workflow, one DNA extraction is performed for the Y-screening process, followed by a second sampling and DNA extraction for STR typing.

Another general consideration for a laboratory planning a Y-screening workflow is whether to have staff dedicated to the Y-screening workflow, separate from the staff performing STR analysis, versus having staff who perform both the Y-screening and STR analysis workflows. A laboratory should consider the possible testimony implications of having multiple personnel involved in the Y-screening workflow. Additional factors to consider when planning a Y-screening workflow include training and proficiency requirements.

Additional decisions, and associated risk considerations, that a laboratory should address at each step when implementing a Y-screening workflow are detailed below.

SAEK Sampling

1) *Deciding what to Y-screen* – Y-screening is optimal for sexual assault evidence from a female victim in which a male perpetrator is alleged. Many laboratories have taken on Y-screening as the standard method for testing swabs collected as part of a SAEK, retaining conventional serology for non-swab evidence, while other laboratories have expanded the pool of evidence that they subject to Y-screening to include items such as underwear or bedding.

2) *Allocation of staff* – Both individual and team member approaches for sampling were identified in the survey. The majority of laboratories employing the individual approach utilized analysts; however, some laboratories used technicians. Team approaches consisted of analyst and technician or laboratory support personnel. Laboratories should consider utilizing appropriately trained technicians or support personnel for the sampling of SAEK swabs to improve laboratory efficiency. Technicians can also be used to operate the automated equipment used in a Y-screening workflow. Experience, training, and other accreditation requirements as well as the complexity (i.e., involving decisions/choices) of the task are important considerations in the staffing model to be employed.

3) *Determining items to be sampled* – The most common laboratory approach surveyed was to sample swabs from all locations collected. While this approach is more laborious, it alleviates the concern of missing potentially informative male DNA data in a SAEK when compared to using selective approaches (e.g., scenario/case history or time interval-based criteria) and eliminates the need for policies on revisiting kits when no informative results are obtained from an initial subset.

Benefits of Y-screening only a subset of items include case throughput (e.g., putting through three 2-item cases versus one 6-item case) and the benefit of limiting the analyses to those samples most likely to yield informative male DNA data, both in terms of analyst time and consumable costs [3]. Regardless of whether or not the laboratory chooses to sample all sets of swabs submitted as part of a SAEK, policies on other evidence types often submitted within a SAEK (e.g., slides, feminine hygiene products, underwear) will need to be developed by the laboratory.

4) *How many and amount of swab(s) to sample* – Laboratories will need to determine how many swabs from an item will be sampled and how much of the selected swab(s) will be sampled. In addition, if all swabs are not sampled, the laboratory should determine how the sampled swab(s) will be selected [4].

Laboratories that employ a two-extract workflow generally reported a small amount (i.e., approximately ¼ or less) of one swab used for the initial DNA extraction. The amount of the swab(s) used for the second DNA extraction was quite variable. For example, one approach was to base the amount to sample on the Y screening results, while another approach was to consume the remainder or a standard portion of positive swabs. Laboratories employing a one-extract workflow also reported variation in the amount of swab consumed for the Y-screening and downstream STR typing. The most common approach was to take a small portion from every swab. An alternative approach was to extract one whole swab, while another was to take just a portion of one swab (e.g., ½ of a single swab).

Regardless of the extraction workflow, a generalized sampling procedure makes the assumption that the swabs submitted are homogeneous; however this assumption may not be accurate. This survey showed that a majority of laboratories do not routinely perform any serology testing prior to sampling. However, sampling swabs based on the amount of visible staining and/or performing serology screening techniques (e.g., AP) can help with non-homogeneously distributed semen to identify the best swab and/or swab area for sampling and therefore may reduce the risk of missing informative male DNA data when all swabs are not selected for the Y-screen workflow. This approach would consume extra sample, resources, and time, therefore the best sampling approach for a laboratory should be carefully considered.

Laboratories should consider the amount of evidence that is necessary to sample under their workflow, given their quantification system, in order to maximize interpretable results while preserving evidence as much as practicable. During a pilot testing program, a laboratory may choose to examine whether there is one preferable method for sampling based upon their routine submissions. Alternatively, past results from conventional serological testing can be analyzed as a means to support policy decisions on sampling.

DNA Extraction

1) *Which extractions to perform* – Laboratories should decide what extraction method(s) (i.e., differential only or both differential and non-differential) will be incorporated into their Y-screening workflow. Many laboratories performing a one-extract workflow typically use case-specific information (i.e., case scenario or sampling location) to determine whether a non-differential or differential extraction will be performed, while a smaller number of laboratories perform a differential extraction on all samples. Laboratories performing a two-extract workflow typically perform a non-differential extraction (possibly with DTT) for the Y-screening extraction, and then use case-specific information, possibly in conjunction with the Y-screening result, to determine what type of extraction method to employ for the second DNA extraction.

Decisions regarding extraction workflows should take into account both technical and operational concerns. The two-extract workflow requires more bench time for those kits that proceed to a second extraction, but may reduce overall time when looking at the totality of kits tested (i.e., looking at the proportion of SAEKs for which testing is terminated after the results of the first extraction versus the kits that are carried forward). Performing a one-extract workflow minimizes the risk of missing the presence of male DNA inherent to testing only a small portion of the available swabs with the first extract of a

two-extract workflow, and minimizes contamination risks associated with opening an item of evidence repeatedly.

Regardless of whether the laboratory is using a one-extract or two-extract workflow, decisions must be made regarding when to perform a differential extraction. As previously described, some laboratories utilize conventional serology test methods in this determination (e.g., if swabs are positive with a presumptive test for semen, a differential extraction is performed), while others use case scenario to dictate extraction type. Still others perform a differential extraction on all swabs sampled. When making policy decisions surrounding differential extractions, laboratories should consider the benefits of performing differential extractions on samples that are believed to possibly contain semen based on case-specific information versus the drawbacks of unnecessary differential extractions.

2) *Who will perform the DNA extraction* – Laboratories employing a Y-screening approach must identify personnel to perform the DNA extraction. Per the SAFER document, "Laboratories should consider incorporating robotics and/or automation at each step of the DNA process for the most efficient high-throughput approach"[1]. In making this decision, laboratories should evaluate the automated processes available within their system and the available personnel in conjunction with their extraction workflow. For example, in a laboratory that performs a two-extract workflow, it may be beneficial to have a qualified analyst perform the initial extraction so they can quickly interpret the quantification results and either send the remainder of the SAEK on for further testing or terminate testing of the SAEK. In a laboratory with a one-extract workflow, technicians may be tasked with making the initial sampling decisions and taking the extracts up to the point that the quantification results can be interpreted by a qualified analyst.

Interpreting Quantification Data

Thorough validation of a quantification system is key to a successful Y-screening implementation. The validation should include a sensitivity assessment covering the limit of detection for male DNA and studies that address the point at which the ratio of human:male DNA detected precludes the successful autosomal STR analysis of a sample. When choosing a quantification kit for Y-screening, it is important to choose a kit that is sensitive enough to detect low levels of male DNA in samples that have high concentrations of female DNA, as some quantification kits can produce a false negative for male DNA when the female DNA is high [5]. These types of samples might not be suitable for obtaining male autosomal STR profiles but would be good candidates for Y-STR analysis. Quantification data could also be used to make an inference for the presence of spermatozoa in a sample [6]. A laboratory may choose to track casework data for a period of time after the implementation of a Y-screening protocol in order to refine decision points originating from validation of the quantification system.

1) *Who will perform the quantification and interpret the data* – Laboratories employing a Y-screening approach must determine who will perform the quantification and interpret the quantification data. The FBI Director's current Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS) details personnel and proficiency testing requirements for analysts and technicians [7]. A laboratory utilizing a team-based approach may choose to have quantification data interpreted by a different analyst than the STR reporting analyst. As with decisions centered around extraction, the benefits of

laboratory throughput should be weighed against the testimony liability associated with a single case involving multiple individuals within the lab.

2) *Which samples will be subjected to STR analysis* – Laboratories that employ a Y-screening approach typically validate a threshold for STR analysis (e.g., autosomal and/or Y). The threshold(s) may be based on the quantity of male DNA detected, the male:female or male:total ratio, and/or a combination of the quantity of human DNA and the male:female or male:total ratio. The laboratory should put in place policies that address the situation of multiple extracts from a single SAEK yielding quantification results qualifying them for autosomal STR analysis. Amplifying all qualifying samples may lead to an overabundance of data that does not further the investigation (e.g., finding the same unknown male on both the vaginal swabs and the cervical swabs, both profiles CODIS eligible). However, the "amplify all qualifying samples" approach reduces the risk of missing an informative or CODIS eligible profile and eliminates the necessity of designing a tiered system in which the results of an initial amplification are assessed prior to going back to additional qualifying samples if necessary.

While a small number of laboratories reported amplifying all qualifying samples, most Y-screening laboratories do not amplify all samples that contain male DNA unless there are multiple non-victim contributors suspected. In their planning, a laboratory should consider if there is a risk of not detecting additional contributors by not amplifying all qualifying samples, for example if the submitted case information was incomplete or inaccurate.

Reporting

How many reports will be issued – Similar to conventional serology screening, the Direct to DNA Y-screening approach also provides the option of two reports. The first report may contain the male DNA screening results and the second report may contain the genotyping results and conclusions. Alternatively, a single report may be issued containing the Y-screening and STR results and conclusions. When determining whether or not to author a separate Y-screening report, laboratories should consider what value is provided to the customer in receiving a report detailing the presence of male DNA against the allocation of personnel resources and time for reporting, technical review, and possible testimony. Regardless of the reporting policies ultimately adopted by the laboratory, reporting is a key topic to communicate with law enforcement partners when implementing a Y-screening workflow.

Other considerations

When considering the implementation of a Y-screening workflow, it is important to communicate with relevant stakeholders. The laboratory will need to be aware of any legal or investigative requirements which may necessitate the identification of semen and establish laboratory policy to identify when and under what circumstances it is appropriate to grant exceptions for conventional serology screening. Communicating the benefits of a Y-screening process should help alleviate concerns over discontinuing conventional serological examinations. Data obtained during a pilot testing period may assist in demonstrating the benefits of Y-screening by providing concrete examples of how the changes will directly impact the customer.

Laboratories should determine the scope of initial SAEK analyses and ensure this is communicated as described above. The most common approach reported was to screen all items. Alternative approaches reported included screening a further subset of items either according to analyst discretion, laboratory policy or agency request. While these alternative approaches conserve laboratory resources, the laboratory should evaluate the risk associated with not routinely screening all items against employing the resources necessary to test all items. In balancing these two considerations, laboratory policies on ceasing the analysis of a SAEK without screening all items should include the analysis of additional items or samples or the performance of additional tests (e.g., conventional serology) on a case-by-case basis as needed for investigative or court-preparation purposes [4].

Lastly, while the implementation of Direct to DNA Y-screening approach can increase efficiency and decrease the turnaround time for case analysis, it is important to consider communicating with the SANE community and other relevant law enforcement personnel to ensure that helpful information is passed on to the nurses doing the collection of evidence. It is critical that nurses performing the collection of evidence are aware of the capabilities of the laboratory to ensure the most effective collection.

QAS Implications of Y-Screening

As quantitative PCR (qPCR) is a DNA testing method, regardless of the workflow employed by the laboratory to utilize qPCR in testing sexual assault or other evidence, the relevant requirements in the Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS) apply.

Personnel

When implementing a Y-screen workflow a laboratory should consider how the QAS defines personnel (Standard 5), as the QAS requirements are only applicable to the portion of the Y-screen workflow pertaining to DNA. Table 1 shows procedural steps involved in Direct to DNA analysis and the personnel who can perform each task. Note that sampling/sample selection (e.g., inventorying sexual assault kit contents or taking cuttings of swabs for further testing) is not considered an analytical procedure governed by the QAS.

Table 1: QAS Applicability to Direct to DNA Staff and Process

	Sampling/ sample selection	Extraction/ lysis	Performing quantification	Interpreting quantification results	Reporting on quantification results
QAS not applicable	✔				
QAS-defined Laboratory Support	✔				
QAS-defined Technician	✔	✔	✔		
QAS-defined Analyst	✔	✔	✔	✔	✔

These analysts and technicians are subject to the relevant personnel requirements in Standard 5, training requirements in Standard 6, proficiency testing requirements in Standard 13, and professional development requirements in Standard 16. Personnel who perform technical review of quantification results or reports covering quantification results are subject to the applicable requirements of Standard 5.5, as well as those found in Standards 6 and 13.

Analytical Procedures and Equipment

Analytical procedures for extraction or sample lysis, performing qPCR and interpreting qPCR results are subject to the relevant requirements in Standard 9. Instrumentation used in these workflows shall be identified as critical in accordance with Standard 10.2.1 and other relevant requirements in Standard 10 must be met.

Reporting

It is not required that the laboratory issue a standalone report that covers qPCR testing; however, if it chooses to do so, the relevant requirements of Standard 11 must be met. If the qPCR testing results are incorporated into the final DNA testing report, there are no additional requirements.

Additional Y-Screening Applications

While this document has focused on the use of qPCR for Y-screening of sexual assault kit swabs, laboratories may also consider adapting Y-STR typing for use as a screening method. This would be useful in a case where bedding is being examined and there is reason to believe the semen of multiple males may be present. For example, a case where the assault occurred on bedding that potentially contains numerous stains from a consensual partner. A two-step extraction method can be employed to identify potential stains of interest. The first extraction would be a non-differential extraction with DTT which would be typed using Y-STRs. Stains of interest would be subjected to a second, differential extraction, and typed using autosomal typing if required. While more time and labor intensive than qPCR screening, this process saves time on the back end in cases where the person of interest is excluded during the first round of screening, or where only a limited number of stains are subjected to full differential processing and interpretation.

Conclusion

The survey provided valuable information regarding the Y-screening workflows that have been implemented within the forensic community for the testing of sexual assault cases. Responses were obtained from approximately half the CODIS laboratories (103 of approximately 200), and nearly half of the responding laboratories (52/103) had a Y-screening workflow in place. The information provided by the survey revealed that the Y-screening approach has been implemented in two different general workflows (one-extract and two-extract); however, decision point differences within each workflow exist from laboratory to laboratory.

The survey was helpful to highlight decision points that laboratories should address prior to implementing a Y-screening workflow, which include the following:

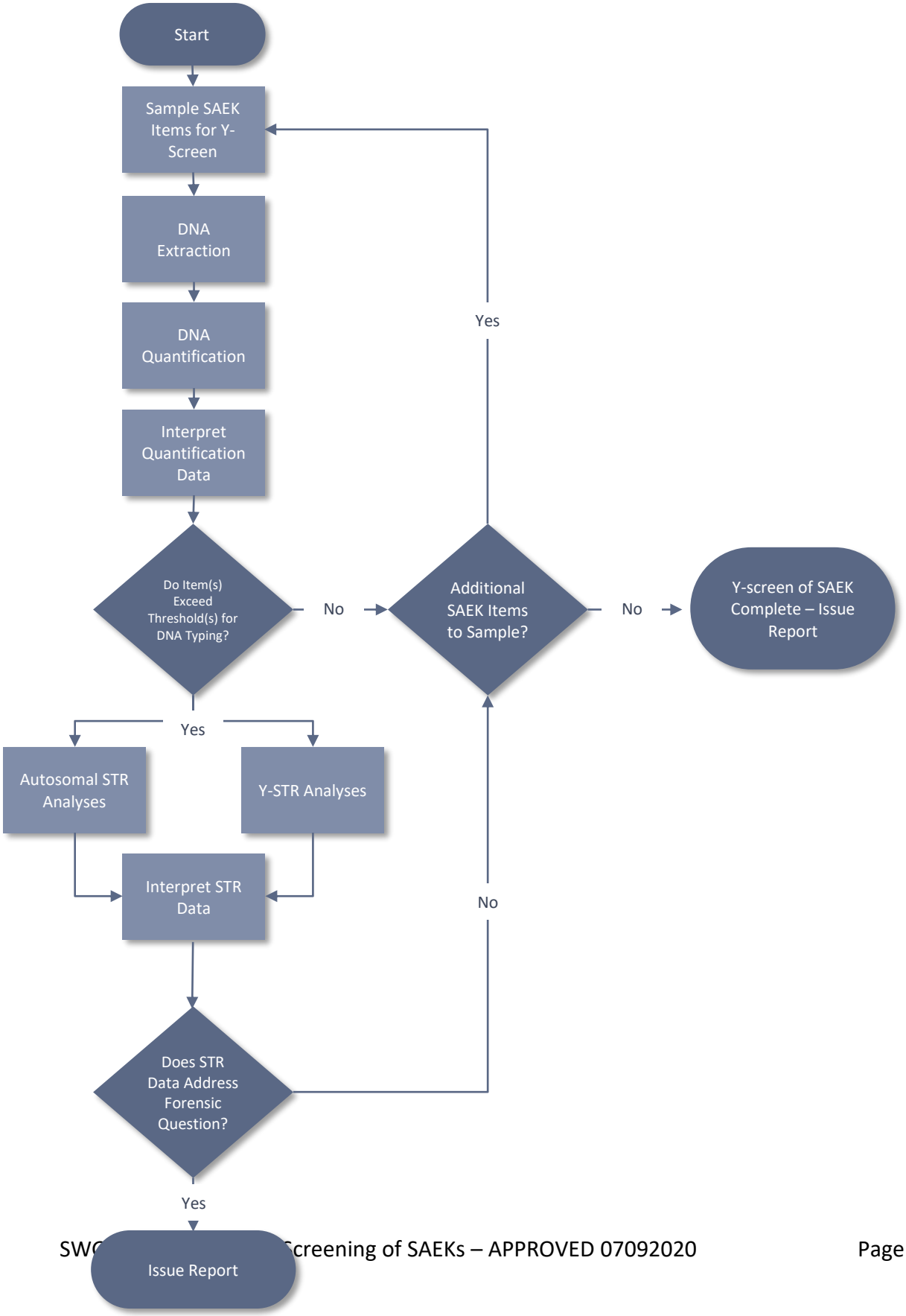
- number of swabs collected per orifice
- determining a strategy for SAEK processing (test all swabs or test a subset initially with additional testing performed as defined by the laboratory)
- swab sampling decisions (which swabs to sample and how much)
- if and how to incorporate any serological testing
- extraction decisions (one-extract or two; differential or non-differential)
- quantification decisions (92% of laboratories did not alter the quantification method)
- amplification decisions (which samples to amplify)
- reporting (one or two reports)

Y-screening is a Direct to DNA workflow which applies the sensitivity of DNA testing to sexual assault cases to augment or replace conventional serology. The impact of a Y-screening workflow is improved sensitivity, capacity, reproducibility and objectivity. As significant validation may be required, laboratories should consider which options best fit their cases, resources and stakeholders. Additional applications beyond SAEKs themselves should also be considered for the application of Y-screening. Further research is required to determine which of the multitude of options currently practiced should emerge as best practices.

References

1. National Institute of Justice. (2017) *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach*. p. xi; available at https://www.ncjrs.gov/pdffiles1/nij/250384.pdf.
2. Scientific Working Group on DNA Analysis Methods. (2016) *Recommendations for Efficient DNA Processing of Sexual Assault Evidence Kits*. Available at https://1ecb9588-ea6f-4feb-971a-73265dbf079c.filesusr.com/ugd/4344b0_4daf2bb5512b4e2582f895c4a133a0ed.pdf.
3. Peterson, J., Johnson, D., Herz, D., Graziano, L., Oehler, T. (2006) *Sexual Assault Kit Backlog Study Final Report*. NIJ# 2006-DN-BX-0094.
4. Wang, C. and Wien, L.M. (2018) *Analyzing Approaches to the Backlog of Untested Sexual Assault Kits in the U.S.A.* J Forensic Sci Vol. 63, No. 4, doi: 10.1111/1556-4029.13739.
5. Fregeau, C. and Laurin, N. (2015) *The Qiagen Investigator1 Quantiplex HYres as an alternative kit for DNA quantification*. Forensic Sci Intl: Genetics 16:148-162.
6. Alderson, G., Gurevitch H., Casimiro, T., Reid, B., Millman, J. (2018) *Inferring the presence of spermatozoa in forensic samples based on male DNA fractionation following differential extraction*. Forensic Sci Intl: Genetics 36:225-232.
7. Federal Bureau of Investigation. (2020) *Quality Assurance Standards for Forensic DNA Testing Laboratories.* Available at https://www.fbi.gov/services/laboratory/biometric-analysis/codis.

Appendix A – Example One-Extract Y-screen Workflow



Appendix B – Example Two-Extract Y-screen Workflow