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A Landscape Study Examining Technologies and Automation for Differential Extraction and Sperm Separation for Sexual Assault Investigations

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Disclaimer

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Report Overview

The National Institute of Justice's (NIJ's) Forensic Technology Center of Excellence (FTCoE), led by RTI International, provides valuable resources that promote the use of technologies in the forensic community. Differential extraction is a technique that allows for the selective cell lysis and isolation of DNA from a mixture of sperm and epithelial cells, predominantly used with evidence collected from sexual assaults where a victim and suspect are not of the same sex. This method aids in the primary means by which a forensic laboratory processes evidence for sexual assault investigations. However, the manual conventional technique was developed 36 years ago,[1] is labor intensive, and may lead to poor DNA recovery. This landscape study reviews the conventional differential extraction methodology and its associated challenges, technology solutions that are commercially available and in development, and current research that will advance differential extraction and sperm separation techniques. The estimated pricing for products in this report is current as of 2021. Please contact the vendor directly for updated pricing.

Landscape Study Objectives

This landscape report provides crime laboratory directors, crime laboratory personnel, decision-makers, and end-users with the following:

- Background information about differential extraction methods and challenges posed by current techniques.
- A product landscape of commercially available chemical and filtration kits and automated systems.
- User profiles from end-users describing their experiences with differential extraction technologies.
- Insights into ongoing research areas for differential extraction technologies.

Landscape Methodology

To conduct this study, FTCoE used a process that included the following:

- Consulting peer-reviewed literature and secondary sources to obtain information about conventional methodologies, associated challenges, and solutions that are available or in development.
- Assessing the product landscape by contacting commercial vendors to gain insights about key product details.
- Contacting subject matter experts from academia and state and local crime laboratories across the United States to discuss the use and implementation of select marketed products compared with conventional techniques.

Subject Matter Experts

We would like to thank the following forensic science community subject matter experts, practitioners, and vendors who offered insight, analysis, and review.

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Executive Summary

A sexual assault kit (SAK) contains materials to collect samples (i.e., forensic evidence) from a victim of an alleged sexual assault. Foreign DNA that is recovered from the SAK evidence and is not associated with the victim can be entered into the Federal Bureau of Investigation's Combined DNA Index System (CODIS) to search for a possible suspect-offender match that can identify perpetrator(s), link cases together to identify serial perpetrators, or provide a promising investigative lead. Timely testing of SAKs ensures justice for victims and improves public safety.

In 2020, the Department of Justice's DNA Capacity Enhancement for Backlog Reduction Program[2] invested more than $80 million[3] in state and local jurisdictions, which helps enhance the ability of crime laboratories to process SAKs. This initiative is leading to improvements; however, forensic laboratories still feel the pressure to consistently improve their workflows and turnaround times to keep up with ever-increasing demands and caseloads. Moreover, this pressure has been magnified as many states have enacted laws to test all kits and to expedite the processing of SAKs—from addressing backlogs to requiring SAKs be tested within a certain timeframe. In 2019, 103 bills were introduced in 35 states and Washington, D.C.[4]

Sexual assault evidence may be collected using swabs from the body of a victim through the process of a medical forensic exam and submitted for testing in a sexual assault kit. Additional sexual assault evidence may be collected from clothing, bed linens, or other items that may contain genetic material. These items may be sampled by collecting the evidence on swabs or taking a cutting of the item. Although the technologies presented in this landscape study can apply to all sexual assault evidence, for the purpose of simplicity, our focus of discussion in this document will be sexual assault kits.

Many forensic laboratories still use the conventional differential method, or Gill method, when processing SAKs. In this method, the swab or cutting is added to a buffer solution that preferentially lyses epithelial cells to release DNA. The gentle lysis of epithelial cells does not target sperm cells. Intact sperm cells are centrifuged and pelleted. Subsequent washing removes any remaining epithelial DNA contained with the sperm pellet. The sperm pellet is then reconstituted and incubated in a new buffer solution to lyse the sperm cells releasing DNA.

The conventional method presents many challenges, as it is time intensive, requires skilled technicians, and can result in inefficient epithelial cell lysis separation—such as female DNA being present in the male sperm fraction. To address these challenges and meet new requirements for processing SAKs, improvements in workflows and turnaround times are critical. Advancements in technologies that increase efficiencies of male DNA recovery, automate SAK processing, and increase the potential of obtaining a CODIS-eligible DNA profile will have a positive impact in SAK processing, sexual assault investigations, and the administration of justice.

Several commercially available technology solutions address the challenges associated with the conventional method. Chemical and filtration kits offer modifications to reduce the number of manual steps, including wash steps, and automated systems enable rapid and more efficient sample processing. Each has its own unique advantages and challenges. Product pages in this landscape study provide an overview of these solutions, including specific advantages and key features for implementation. User profiles describe the user experience from a crime laboratory's perspective, with select products receiving specific feedback and considerations. This information may be helpful to other laboratories for implementation or consideration of a product.

Additionally, ongoing research efforts into novel differential extraction methods focus on improving the sperm cell recovery, using more selective isolation techniques and developing technology that is automated and user-friendly. All of these efforts aimed at improving conventional differential extraction offer the potential to reduce processing times and increase process efficiencies, by providing better separations that generate cleaner DNA profiles.

Background

In a sexual assault investigation, a forensic laboratory tests biological evidence for the presence of DNA; this evidence is typically collected from a SAK as part of a forensic medical examination. If the recovered DNA does not belong to the victim (i.e., foreign DNA), it can be entered into the appropriate index (e.g., Forensic Evidence Index) and uploaded into the State DNA Index System and the National DNA Index System, which are part of the Federal Bureau of Investigation's CODIS. If this new DNA entry matches the DNA of an existing entry, such as the DNA profile from forensic evidence in another case or the DNA profile from an arrestee or convicted offender, then this CODIS hit can provide a promising investigative lead, identify perpetrator(s), or link cases together to identify a serial perpetrator.

Timely Processing of All SAKs Is Important for Successful Investigation and Adjudication of Cases Involving Sexual Assault.

Possible outcomes from a CODIS hit put increased emphasis on SAK processing because these investigative leads are invaluable to supporting an investigation, ensuring justice for victims, and improving public safety. Several recent research efforts have demonstrated the importance of testing all SAKs in a timely manner—including data collected from Cuyahoga County, Ohio, and Detroit, Michigan. These results showed that case connectivity and serial perpetration are far higher than originally believed. In Cuyahoga County, more than 50% of 243 sexual assaults studied were linked to serial perpetration,[7] and a study of 449 SAKs tested in Detroit linked 60% of the resultant forensic hits to another sexual assault.[8] Moreover, NIJ-funded work by Dr. Rebecca Campbell et al. showed that there is an equal probability of obtaining CODIS hits from SAKs tested from stranger and non-stranger sexual assault offenses.[9]

Cost-benefit analyses demonstrate the value of testing and investigating **all SAKs**, compared with prioritizing certain subsets. Two studies reported significant cost savings using the following approaches:

- Spending ≈ $1,641 to test a SAK was reported to avert future sexual assaults costing ≈ $133,484 on average.[5]
- Cost savings to the community was shown to be ≈ $26.48 million (≈ $5,127 per SAK) from adverted future sexual assaults through convictions from investigating all SAKs.[6]

IMPACT

Sexual assault cases are complex and require a multidisciplinary approach to support victims.[10]

Timely laboratory testing of all SAKs can play an important role in a victim's post-assault experience, healing, and recovery.

Emerging technologies can decrease turnaround times for SAK processing, reduce the hands-on burden for forensic practitioners, and improve the ability to obtain a CODIS eligible profile.

This landscape study highlights and reviews:

- Differential extraction technologies that automate routine processing steps.
- Improving DNA recoveries that could lead to increased CODIS hits.

Legislation and Funding Efforts Have Provided Opportunities for Many Agencies to Address SAK Processing Inefficiencies.

Many states have enacted laws to expedite SAK processing, from addressing backlogs of untested SAKs to requiring that SAKs be tested within a certain timeframe. In 2019, 103 bills were introduced in 35 states and Washington, D.C.[4] (The End the Backlog website provides an updated overview of this work via an interactive map.)[11]Although this legislation supports sexual assault response reform, these laws impact crime laboratory operations by requiring laboratories to keep up with caseload demands and expedite laboratory processing of SAKs.

The Debbie Smith Act was enacted in 2004 and authorizes funding to support forensic laboratories for SAK testing and capacity-building activities, among other initiatives.[12] This includes the Department of Justice's DNA Capacity Enhancement for Backlog Reduction Program (CEBR)[2] that supports state and local jurisdictions to enhance the ability of forensic laboratories to process SAKs. Under NIJ direction (prior to 2020) and currently under Bureau of Justice Assistance direction, the CEBR program supports efforts related to improving laboratory workflows, efficiencies, and results—including the procurement of supplies, chemicals, and equipment. Additionally, NIJ supports research and development efforts related to sexual assault, and many of the technologies discussed in this report were supported through NIJ's research and development programs.

Differential Extraction Technologies Represent an Opportunity to Improve DNA Recovery and SAK Workflows in Sexual Assault Investigations.

To meet SAK processing requirements, improvements to laboratory workflows and turnaround times are critical. Advancements in technologies that automate SAK processing and improve DNA recovery are having a positive impact. Oftentimes, these advancements focus on one step in the SAK testing workflow, and cumulative improvements at each step can greatly improve overall sample processing from SAK to DNA profile (**Exhibit 1**). In a SAK workflow, a differential extraction step isolates epithelial and sperm cell types in a biological sample and can lead to improved DNA recovery from the alleged assailant. Although most sexual assault cases are female victim and male perpetrator cases, which can present biological samples where the presence of epithelial female DNA can complicate obtaining the perpetrator male DNA profile, there are a variety of sexual assault case types. These include cases with (1) victims and perpetrators of the same sex, (2) male victims and female perpetrators, and (3) one victim with multiple perpetrators, all of whom have an impact on the biological evidence available for analysis. Differential extraction is most effective for sexual assault cases that involve a victim and a perpetrator who are not of the same sex or certain cases that involve same-sex male victim and perpetrator (e.g., forensic evidence containing epithelial cells from the body of the victim and perpetrator sperm cells). However, sexual assault cases that involve a victim and a perpetrator who are both female are not suitable for differential extraction. Cases with one victim and multiple perpetrators can also impact the overall SAK processing workflow where DNA profile interpretation and mixture deconvolution can be valuable in addition to differential extraction (e.g., if there are multiple male perpetrators).

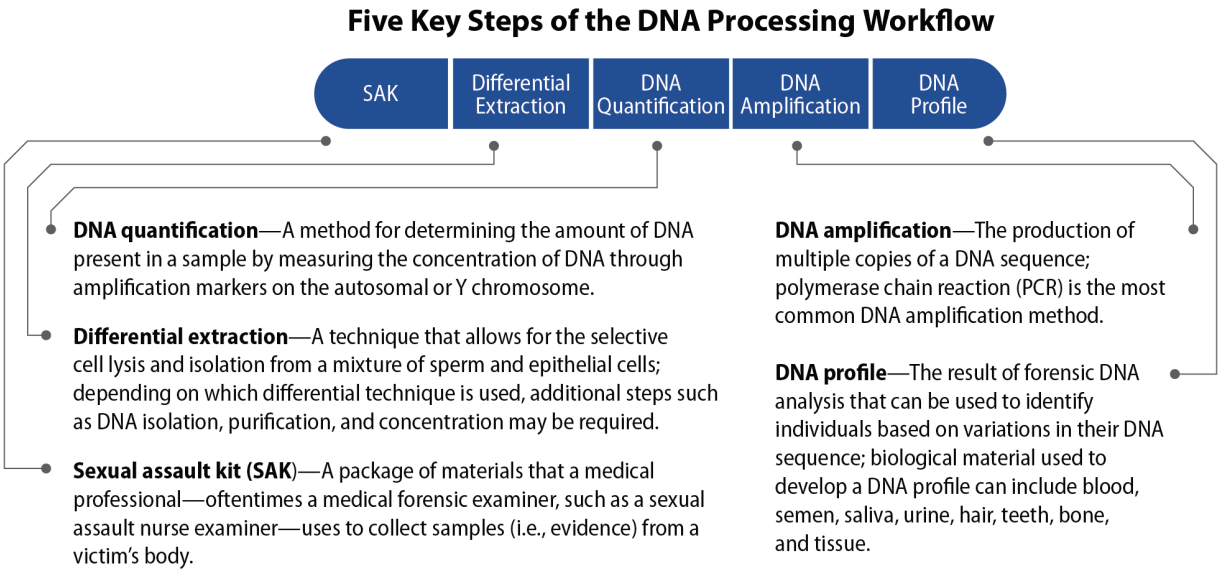


Exhibit 1: The DNA processing workflow from SAK to DNA profile that includes differential extraction for samples containing both sperm and epithelial cells begins with the differential extraction step, followed by DNA quantification, DNA amplification, and finally, analysis to generate a DNA profile. A DNA isolation, purification, or concentration step may be required, depending on the differential extraction technique used.

Most Laboratories Use the Gill Method for Differential Extraction

In 1985, Gill et al. first published a differential extraction method for a sample containing both epithelial (nonsperm) cells and sperm cells.[1] This method used preferential chemical lysis for the separation of epithelial DNA from a sample prior to sperm cell lysis. Since the introduction of the Gill method, slight modifications and advancements—including wash and centrifugation steps for improved separation—have been made to streamline the differential extraction process and increase the recovery of the sperm cell fraction.[13] However, forensic laboratories still use the core of the Gill method, preferential chemical lysis, to separate and extract sperm and epithelial DNA in a sample. This method remains at the forefront of separating sperm and nonsperm DNA because it is relatively simple and inexpensive and does not require any costly laboratory equipment.

For the purposes of this report, the following terms are commonly used interchangeably when discussing differential extraction methods:

- Conventional method, traditional method, organic method, and Gill method

The most common differential extraction procedures consist of several manual steps, chemical reagents, and routine laboratory equipment (e.g., centrifuge, pipettes). **Exhibit 2** highlights the different steps of a traditional differential extraction procedure.

First, a SAK sample (routinely a swab used to collect a biological sample) is added to a buffer solution that contains proteinase K (ProK) and surfactant (e.g., sodium dodecyl sulfate [SDS], sarkosyl) for incubation. The epithelial cells are preferentially lysed to release the DNA during this step; this is considered a gentle lysis, as the epithelial lysis buffer does not target the sperm cells. The sample swab, also referred to as *substrate*, is then removed from the buffer solution, followed by centrifugation to pellet intact sperm cells, with subsequent removal of the supernatant that contains the released epithelial DNA. Oftentimes, multiple wash steps are then performed (≈ 3–5 washes) by adding more buffer solution, centrifuging, and removing the supernatant to collect any remaining epithelial DNA contained with the sperm pellet. At this point in the differential extraction procedure, the sperm cell fraction (as a pellet) should be separated from the epithelial cell fraction (removed as supernatant). The sperm pellet is then reconstituted and incubated in a new buffer solution (sperm lysis buffer) containing ProK, SDS, and dithiothreitol (DTT) to lyse the sperm cells releasing DNA. The addition of DTT, or a different reducing agent, is required to reduce the disulfide bonds within the sperm cell head for sperm cell lysis. **The Virginia Department of Forensic Sciences offers an example differential extraction method procedure on its website.[14]**

Differential extraction is also applied to sexual assault evidence samples aside from swabs in SAKs—including various clothing items, bed linens, and so on.

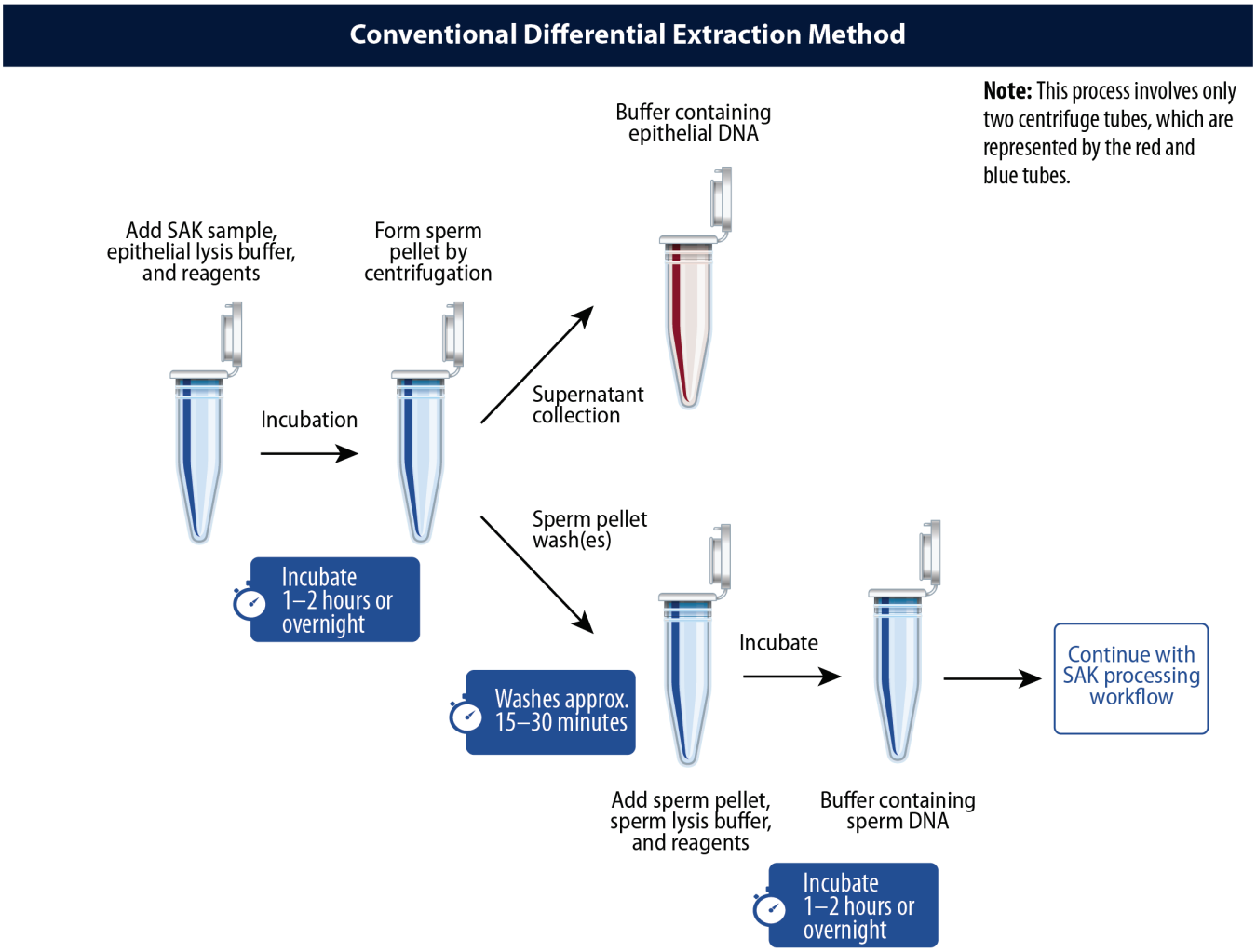


Exhibit 2: Conventional differential extraction is a time-consuming, manual procedure.

Multiple Factors Can Limit Typical Differential Extraction Methods, Impacting Perpetrator DNA Recovery and Isolation from Victim DNA.

Evidence samples from SAKs are often unevenly balanced with more of the victim's epithelial cells than the foreign sperm material from a perpetrator. This unfavorable ratio of sperm to nonsperm DNA in SAK samples makes recovering and isolating the sperm cells during the differential extraction procedure critical for collecting enough male DNA to generate a CODIS-eligible profile. Consequently, reduced recovery of the sperm fraction can lead to a partial DNA profile with missing data or below a defined threshold, leading to an ineligible DNA profile for CODIS upload. **Exhibit 3** highlights several key factors within the procedure that can impact the resultant DNA profile.

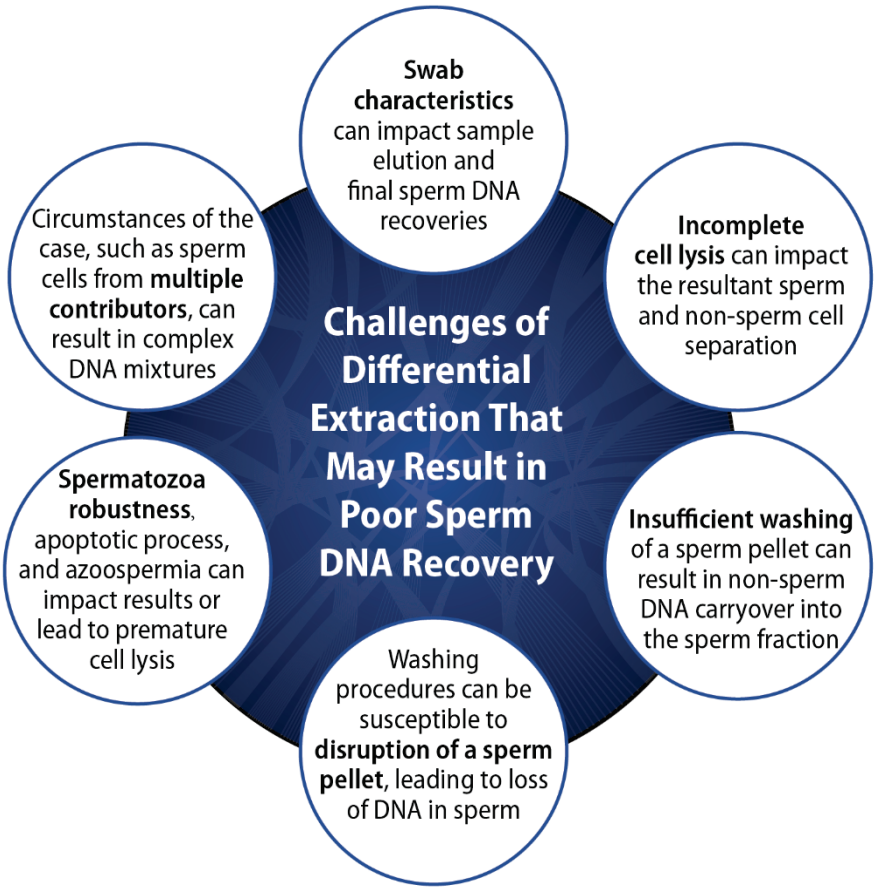


Exhibit 3. Performing consistent differential extraction using conventional methods requires careful attention to steps such as lysis and wash steps.

Sample Collection Material

The efficiency of the initial step in the differential extraction procedure can subsequently impact final sperm DNA recoveries. SAK samples can consist of different materials (e.g., cotton or nylon swabs with woven or flocked swab fibers) for the collection of biological material, and each material can have different characteristics for sample collection and extraction. Biological material can be both absorbed (i.e., permeated) and adhered to these different materials. Although this allows for biological material to be collected effectively for storage and transfer to the laboratory, these properties can also impact the ability to release the biological material from the swab, resulting in some loss of sample.[15-18] With a limited amount of foreign biological material present, sample loss to the sample collection material during extraction can impact the ability to generate a DNA profile.

Inefficient Epithelial Cell Lysis

The initial gentle lysis of epithelial cells step, prior to centrifuging the sperm cell pellet, can also impact final recoveries and isolation of the sperm DNA. The epithelial cells can remain in the sperm fraction if the initial lysis is too gentle and does not open all the epithelial cells to release the DNA. Alternatively, an initial lysis that is too strong can result in the lysis of both the epithelial cells and some of the sperm cells, leading to the presence of male DNA in the nonsperm fraction and decreased recovery of the male DNA in the sperm fraction. However, a study showed

that, without DTT, variations in incubation time and temperature or ProK and SDS concentrations exhibited minimal impact on premature sperm cell lysis, demonstrating the robustness of the differential lysis method.[19]

Multiple Wash Steps

Ideally, a differential extraction procedure would lead to complete recovery and isolation of both the sperm and nonsperm fractions from a SAK sample. However, as with most procedures, each manual step is susceptible to carryover from the other cell fraction, leading to the presence of sperm and nonsperm DNA within each separated solution or the incorrect fraction. After the initial lysis of epithelial cells, several wash steps are performed to clean the sperm cell pellet and remove any residual epithelial DNA, although the number of washes varies among laboratories. This procedure can be tedious and is susceptible to disrupting the sperm cell pellet, which can result in the loss of sperm cells to the supernatant solution. Automating this process may provide increased or more consistent recoveries and decreased hands-on burden and sample processing time (≈ 15–30 minutes, depending on the number of washes).

External Influences

Aside from the differential extraction procedure itself, accounting for outside factors can be challenging and can impact DNA recoveries. These factors can include the circumstances of the case such as samples with biological material from multiple contributors, storage conditions of the SAK samples, and the time that elapsed between the sexual assault and collection of a SAK.[20-23] These potential outside factors can often lead to a lowered quantity or quality of DNA present in a SAK sample, significantly impacting the ability to generate a CODIS-eligible DNA profile. For example, factors impacting the integrity of the sperm cells—such as aging or drying that leads to sperm cell degradation or weakened cells—have been shown to impact premature sperm cell lysis during the epithelial cell lysis step.[19] Additionally, characteristics that vary between individuals—such as spermatozoa robustness, apoptotic process, and azoospermia—can impact the quantity and quality of DNA present.[24] Samples containing multiple male contributors can result in a complex DNA mixture wherein the individual male DNA profiles cannot be determined or deconvoluted from the mixture.

Landscape of Differential Extraction and Sperm Separation Products

Technology Solutions Could Address Current Limitations in Differential Extraction Methodology.

There are several commercially available differential extraction products on the market, most of which employ a different approach to address the challenges associated with the conventional method (**Exhibit 4**). Some techniques offer modifications to reduce the number of manual steps, including wash steps, and other techniques automate the method for more efficient sample processing. **The commercially available products described in this section have been categorized into (1) chemical and filtration kits and (2) automated systems.**

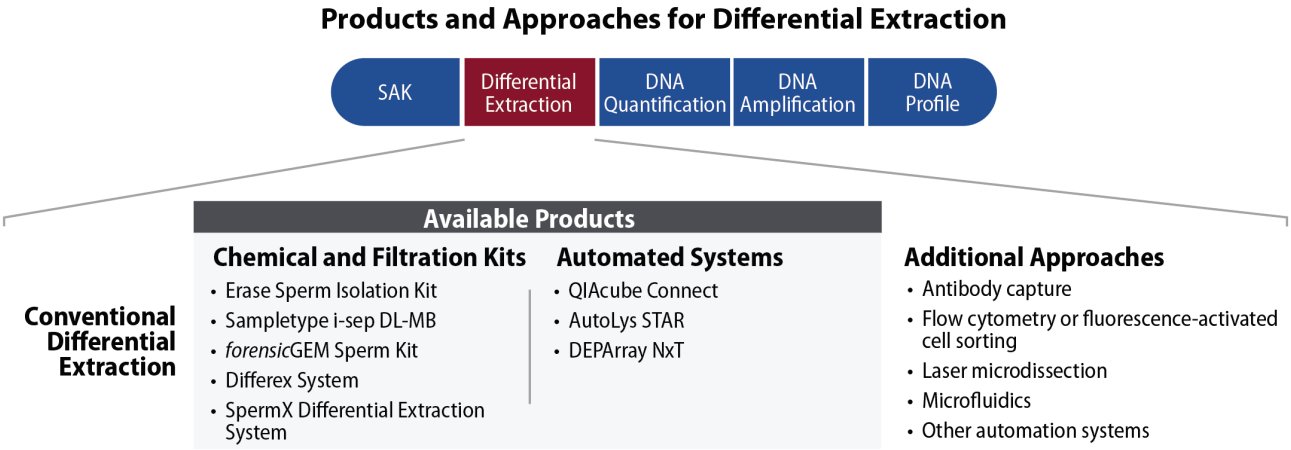


Exhibit 4: Several methods and technology solutions are available for differential extraction and sperm separation from SAK samples. This landscape study focuses on technology solutions that are commercially available and in development for differential extraction of sperm from SAKs. Several methods and technology solutions are available for differential extraction and sperm separation from SAK samples. This landscape study focuses on technology solutions that are commercially available and in development for differential extraction of sperm from SAKs.

The following sections present ***product pages*** and ***user profiles*** for **chemical and filtration kits** and **automated systems** that are currently on the market for differential extraction.

Product pages highlight the available products and technology, including specific advantages and key features for implementation.

User profiles describe the user experience from the crime laboratory's perspective, with select product pages providing specific feedback and lessons learned during the implementation and validation of these products. This information may be helpful to other laboratories with the product's implementation or consideration in mind.

Product pages and user profiles are not intended as recommendations or endorsements of a specific technology, product, or equipment by any organization or agency named within this report—nor is the information intended to imply that any technology, product, or equipment is the best available or only available for the purpose.

Additionally, some laboratories and personnel who participated in the user profiles preferred to remain anonymous.

Chemical and Filtration Kits

In general, chemical and filtration kits are consumable-type kits that contain chemicals or filters needed to perform sperm isolation for differential extraction. These kits need to be routinely repurchased because they can only be used for a finite number of samples, including quality control checks prior to use. Although chemical and filtration kits may seem to be limited by their lack of automation and required manual steps, these kits are often more easily implemented because of their relatively low costs, simple protocols provided by vendors, and use of routine laboratory equipment. Adapting a different chemical or filtration kit for differential extraction of SAK samples also requires less commitment for a laboratory because of the minimal startup costs. Additionally, many of these techniques can still be applied to more generic automation options, such as robotic liquid handlers. Product pages are provided for the following chemical and filtration kits:

Automated Systems

Automated systems are implemented to decrease the number of hands-on steps for differential extraction and can vary in cost based on their complexity and utility. Although these automated systems are a one-time purchase, routine procurement of consumables and necessary chemicals is still required. Automated systems increase throughput, reduce variability and errors in differential extraction workflows, and enable analysts to focus on higher-order tasks. **Additionally, automated systems can decrease the chance for cross-contamination; however, this requires careful contamination studies for the automated script (protocol) and sample deck layout.** Some systems are dedicated to one particular assay kit or workflow step, and others are agnostic such that they may be used with a wide variety of assay kit chemistries or methods and across the entire workflow. Select product pages and user profiles are provided for the following automated systems:

Exhibit 5 provides an overview of the advantages and challenges associated with various commercial differential extraction techniques, including chemical and filtration kits and automated systems. **Exhibit 6** provides an overview of key features—such as cost, number of manual steps, and number of washes—of the technology solutions profiled in this landscape study. **Exhibits 7** and **8** provide a more in-depth comparison of these different techniques.

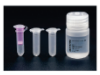
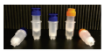
Exhibit 5. Advantages and challenges are associated with chemical and filtration kits and automated systems.

Product Overview

Products	Advantages	Challenges
Chemical and filtration kits	• Reduced upfront costs • Training comparable to traditional method • Protocol available from vendor	• Manual hands-on method • Cost associated with routine purchase of kits
Automated systems	**Increased** • Sample throughput • Reliability and consistency **Decreased** • Run-to-run variability • Risks of human error and cross-contamination • Hands-on time so that analysts can work on other tasks	• Several upfront costs • Reagent use and procurement are still required

Exhibit 6. Commercially available differential extraction products on the market offer several different options in pricing, manual steps, and analysis times.

Currently Available Technology Solutions for Differential Extraction

Chemical and Filtration Kits		Number of Manual Steps	Number of Washes Per Run	Run Time
Erase Sperm Isolation Kit		5	None	2 hours
Sampletype i-sep DL-MB		5	None	2 hours
*forensic*GEM Sperm Kit		6	None	>1 hour
Differex System		19	4	2 hours
SpermX Differential Extraction System		14	3	4.5 hours
Automated Systems		**Number of Manual Steps**	**Number of Samples Per Run**	**Run Time**
QIAcube Connect		None	6–12	**1 hour** (6 samples) **1.5 hours** (12 samples)
AutoLys STAR		1	Up to 48	Depends upon number of samples and lysis method chosen
DEPArray NxT		Sample preparation; approximately 4–6 washing steps and 2–3 sample transfer steps	1; recovering up to 48 single cells	2–3 hours

The following are technology adoption considerations for examining the products highlighted throughout the ***product pages*** and ***user profiles***:

- Cost constraints—startup versus continued costs
- Laboratory throughput needs from caseload demands
- Training requirements
- Technology validation
- Technology uptake and changing overall DNA workflows

https://www.ptclabs.com/erase/

Erase Sperm Isolation Kit by PTC

The Erase Sperm Isolation Kit (Paternity Testing Corporation, PTC Laboratories; Columbia, Missouri) uses DNase to selectively digest remaining epithelial DNA in the sperm cell pellet. Selective digestion occurs after the first steps of the conventional differential extraction method when the epithelial cells are lysed using proteinase K, followed by centrifugation to pellet the intact sperm cells and removal of the supernatant that contains the epithelial DNA. PTC found that DNase does not digest sperm DNA present in the intact sperm heads but selectively digests soluble epithelial DNA. Therefore, DNase is used to remove remaining epithelial DNA present in the sperm cell pellet, eliminating the need for the additional wash steps and increasing the isolation of the sperm DNA. Replacing SDS with Triton X-100 is essential for this process because SDS inactivates DNase.

PTC developed the Erase Sperm Isolation Kit with partial funding by NIJ grant 2009-DN-BX-K039, *Automated Processing of Sexual Assault Cases Using Selective Degradation* https://www.ncjrs.gov/pdffiles1/nij/grants/241332.pdf.

Erase Sperm Isolation Kit in the Literature

A 2017 study reported side-by-side comparison experiments of the Erase Sperm Isolation Kit and the standard differential extraction method using mock sexual assault swabs. Results showed that, although the DNase approach had lower sperm recoveries than the standard method, the female DNA carryover was significantly lower with the Erase Sperm Isolation Kit (capacity for four-fold improvement in sperm isolation due to reduction of nonsperm DNA carryover).[25]

Advantages of the Erase Sperm Isolation Kit

The kit is a simple and effective method that uses a selective DNA degradation process, eliminating multiple wash and dilution steps, decreasing processing time and epithelial DNA contamination in the sperm DNA fraction. Additionally, the Erase Sperm Isolation Kit procedure can be performed as a single-tube method or a 96-well plate with automation. This kit was specifically designed for compatibility with multiple liquid handling platforms and existing laboratory equipment.

Implementation and Use

- Requires routine laboratory equipment (e.g., microcentrifuge, pipettes).
- Includes all lysis buffers, reagents, and tubes needed for the protocol in the kit.
- Involves a 2-hour procedure.
- Might require less training for the manual protocol than the traditional method.
- Costs approximately $500 for 50 reactions.

A Large Public Laboratory System in Texas Validated the Erase Sperm Isolation Kit to Help Process Sexual Assault Cases.

Select laboratories within a laboratory system in Texas validated the Erase Sperm Isolation Kit aimed at replacing the traditional differential extraction method. Both the Erase Sperm Isolation Kit and the traditional method are manual differential extraction procedures that can be adapted for increased automation if a laboratory has automated liquid handling equipment available. However, if procuring automation equipment (e.g., Hamilton and Tecan liquid handlers) is not feasible, the Erase Sperm Isolation Kit offers several advantages for improving differential extraction workflows.

The kit allows for the elimination of wash steps, thereby significantly reducing the time needed to complete the protocol. Traditional differential extraction can take 1 day or longer to complete, depending on the number of samples. For example, starting the protocol in the early afternoon would get samples ready for overnight incubation to digest the sperm. The next morning would be spent cleaning up the samples and preparing for DNA analysis. The Erase Sperm Isolation Kit reduces the time required to prepare samples for DNA extraction to less than half a day.

One challenge with the traditional differential method is the learning curve required to hone the technique. Performing the wash steps takes practice so that the sperm pellet is not disrupted, which would result in sample loss to the supernatant. Using enzyme digest with the Erase Sperm Isolation Kit (1) removes the wash steps, resulting in a smaller chance of pellet disruption or contamination of the sperm fraction with epithelial DNA, and (2) enables a cleaner single-source profile from the sample. As a result of the cleaner sperm cell fractions, the laboratory was able to deduce mixtures more confidently from multiple male contributors by using the kit. In addition—in collaboration with another division that has an NIJ grant for conducting genealogy testing—the laboratory used the Erase Sperm Isolation Kit to achieve better separation of the sperm and epithelial cells for samples in the project.

> "The Erase Sperm Isolation Kit is a viable option for reduced differential extraction times in laboratories that have limited funding for more automated equipment."
>
> *—Laboratory Representative*

In 2019, the Texas legislature passed House Bill 8, requiring sexual assault kits to be tested within 90 days.[26] Purchasing automated instruments can provide benefits for complying with this new law. As a result, the laboratory is looking to implement QIAcube workstations (QIAGEN, Hilden, Germany) at all of their forensic laboratories. The laboratory system still believes the Erase Sperm Isolation Kit is a viable option over the traditional differential extraction method for laboratories that have limited funding for automated extraction equipment. For interested laboratories that have the equipment, the Erase protocol can be automated on instruments such as Hamilton or Tecan liquid handlers for the additional benefit of a decreased protocol time.

Key Considerations for the Erase Sperm Isolation Kit

- Offers a convenient, time-saving method for manual differential extraction.
- Allows for possibility of achieving cleaner DNA profiles.
- Supports automated workflow; if automation is desired, an agency may need to work with PTC Laboratories or automated liquid handler vendors to adapt the protocol.

https://www.biotype.de/en/products/sample-preparation/sampletype-i-sepr-dl

Sampletype i-sep® DL-MB by Biotype GmbH

The Sampletype i-sep® DL-MB uses a proprietary self-sealing filter that enables a stepwise lysis and separation of DNA to be performed without additional pipetting. The Sampletype i-sep® DL Filter Column is placed into a 2 mL Sampletype i-sep® Collection Tube and then sample and epithelial lysis buffer are added to the column. Epithelial cells are lysed and the collection tube with Sampletype i-sep® DL is centrifuged. The lysate comprising the lysed female cells passes through the filter, whereas the intact sperm cells remain trapped. The Sampletype i-sep® DL Filter Column is transferred to a new collection tube, and the Sampletype i-sep® MB lysis buffer is used to lyse the remaining sperm cells. Ethylene oxide sterilization of tubes and columns ensures there is no RNase and DNase to affect samples. This filtration technique reduces the risk of cross-contamination, as there is no manual substrate transfer.

Additional information about the Sampletype i-sep® DL-MB:

Efficient and reproducible separation of DNA fractions during differential lysis.

Fast and easy to perform DNA preparation in the same device without extra pipetting steps.

Minimizes risk of cross-contaminations and mix-ups.

Superior lysis using Sampletype i-sep® MB buffer for 90% lysis efficiency.

Advantages of the Sampletype i-sep® DL-MB

The Sampletype i-sep® DL-MB filtration method can effectively isolate sperm cells without the formation of a sperm cell pellet. This reduces the risk of disturbing the pellet during washing steps and possible loss of sperm cells. Additionally, this technique can decrease the risk of cross-contamination, as there is no manual substrate transfer during the procedure because it remains within a closed system.

Implementation and Use

- Requires routine laboratory equipment (e.g., microcentrifuge and pipettes).
- Sampletype i-sep® DL 2 mL Collection Tubes and Sampletype i-sep® MB lysis buffer included.
- 2-hour procedure.
- Minimal training required—augments conventional differential extraction procedure.
- Costs approximately $4,700 for 250 reactions.

www.microgembio.com/product/forensicgem-sperm-dna-extraction-kit/

*forensic*GEM™ Sperm Kit by MicroGEM

The *forensic*GEM™ Sperm Kit (MicroGEM US; Charlottesville, Virginia) employs a mixture of temperature-controlled enzymes to rapidly extract sperm DNA from sexual assault samples. First, a low-temperature mesophilic cell wall–degrading enzyme breaks down sperm heads. Then, sperm cells are lysed and DNA is striped of nucleosomes by a thermophilic proteinase at 75°C. Finally, the proteinase is deactivated at 95°C, resulting in single-stranded DNA suitable for quantitative polymerase chain reaction (qPCR) and short tandem repeat (STR) amplification. This temperature-controlled DNA extraction process involves decreased hands-on time, does not require any purification steps, and does not use reducing agents that can inhibit DNA analysis.

MicroGEM Offers the *forensic*GEM Universal Kit for Differential Extraction.

The *forensic*GEM Sperm Kit can be used with the *forensic*GEM Universal Kit and a nuclease to perform differential extraction from sexual assault samples. This differential extraction protocol takes less than 30 minutes to quickly separate the epithelial and sperm cell fractions, which are then ready for STR profiling without the need for laborious extract clean-up, thereby minimizing DNA loss and maximizing sample throughput.

MicroGEM is currently working with partners to validate this protocol.

Advantages of the *forensic*GEM™ Sperm Kit

Sperm cells are a difficult type of cell to lyse, often requiring qPCR and STR-inhibiting chemicals—such as SDS, mercaptoethanol, and DTT—for lysing. These chemicals are toxic to downstream processes and must be removed via purification steps. Additionally, these steps are time-consuming and result in a loss of DNA. The *forensic*GEM™ Sperm Kit augments the traditional differential extraction method to result in sperm DNA suitable for PCR and is qPCR-ready without additional purification steps.

Working with a limited number of cells is challenging because DNA can be lost at every step; however, the *forensic*GEM™ Sperm Kit allows for sperm extraction in small volumes with very few sperm present because DNA is not lost from purification steps.

Implementation and Use

- Requires routine laboratory equipment (e.g., microcentrifuge, thermal cycler).
- Requires epithelial cell lysis reagents or the *forensic*GEM™ Universal Kit for differential extraction.
- Takes less than 15 minutes for sperm cell lysis.
- Might require less training for the manual protocol than the traditional method.
- Costs approximately $242 for 50 reactions.

www.promega.com/differex

Differex™ System by Promega

The Differex™ System (Promega; Madison, Wisconsin) provides both manual and automated (see callout box) methods for differential extraction. For the manual method, samples are incubated in an aqueous digestion solution (with proteinase K added by the user) to lyse epithelial cells and then centrifuge to pellet the sperm cells. A nonporous, DNA-binding, magnetic DNA IQ™ resin is added to the sperm pellet, and a magnet is applied, causing the resin to cap the pellet. The epithelial fraction is removed from the well and set aside for further analysis. The sperm pellet is then washed twice with digestion solution. A third wash removes the resin and resuspends the sperm pellet. The suspension is then centrifuged again, DNA IQ™ resin is readded, and the magnet is reapplied. A nonaqueous separation solution is added to the well, and a final wash is performed. The separation solution is denser than the wash solution, allowing for efficient removal of the residual digestion solution wash. The sperm pellet is then resuspended and ready for DNA extraction. Watch the Automated Differex System video for more information.

Promega Supports the Application of the Differex™ System for Automation

The Differex™ System is used in an automated format, in combination with Promega's DNA IQ™ System for DNA purification, the Slicprep™ 96 Device, and the MagnaBot Flat Top magnet with 96-well plates.

Promega offers a technical manual for using the Differex™ System on the Beckman Coulter Biomek® 4000 automated liquid handler.

Advantages of the Differex System

The Differex™ System offers similar recovery to the traditional method for differential extraction and works with challenging, new, and old samples typically seen in SAKs.

When combined with the DNA IQ™ System and Slicprep™ 96 Device on robotic platforms, the Differex™ System can process up to 48 differential extractions in less than 5 hours. This timeframe includes incubation time and less than 1 hour of hands-on time.

Implementation and Use

- Requires routine laboratory equipment (e.g., microcentrifuge and pipettes).
- Includes all lysis buffers, reagents, and tubes needed for the protocol in the kit.
- Involves a 2-hour manual procedure.
- Might require less training for the manual protocol than the traditional method.
- Costs approximately $500 for 50 reactions.

https://InnoGenomics.com/products/sperm-x/

SpermX™ Differential Extraction System by InnoGenomics

The SpermX Differential Extraction System (InnoGenomics, New Orleans, Louisiana) uses a nanotechnology-derived polymer membrane that functions as a separation medium to effectively trap sperm cells while enabling efficient flow through of the digested epithelial cell DNA. A SAK sample is first processed with a preferential epithelial cell lysis, similar to traditional differential extraction. Sperm cells are captured on the membrane within the SpermX device's consumable-type tube system. A sperm digest is performed with the swab or substrate (e.g., cotton, silk, polyester, denim, carpet) in the device for collection of the final sperm fraction.

InnoGenomics developed SpermX with partial funding by NIJ grant 2015-DN-BX-K072, *Highly Efficient Sperm Cell Separation from Sexual Assault Samples for DNA Analysis Using Novel Polymer Filter Technology* https://nij.ojp.gov/funding/awards/2015-dn-bx-k072.

SpermX™ Differential Extraction System

InnoGenomics engaged several public laboratories to help validate the SpermX Differential Extraction System, including the Utah Bureau of Forensic Services, which is in the process of validating a customized SpermX automated method for processing 95 samples simultaneously. Hands-on analyst time has shown to be reduced 3-fold.[27]

See user profile from Utah BFS on pg. 22 for additional information.

Advantages of the SpermX Differential Extraction System

The SpermX system captures approximately 6-fold more male DNA in the sperm fraction and can yield results with very low sperm amounts from valuable crime scene evidence compared to what normally is needed for traditional methods. This is a rapid, reproducible system that is easy to implement manually and amenable to high-throughput automated workflows, such as the AutoLys (Hamilton) robotic workstation.

Implementation and Use

- Requires routine laboratory equipment (e.g., microcentrifuge and pipettes).
- Involves a 4.5-hour manual procedure for 10 samples.
- Involves a 4-hour automated procedure with the Hamilton AutoLys STAR for 95 samples, including 2-hour preparation of reagents, substrates, labelling and opening of tubes prior to deck loading.
- Might require less training for the manual protocol than the traditional method.
- Costs approximately $2500 for 100 reactions.

Utah Bureau of Forensic Services is validating InnoGenomic's SpermX for automated processing of sexual assault kits on the Hamilton AutoLys STAR.

Emily Simek, Forensic Scientist Manager DNA Technical Team
Lies Janssens, Senior Forensic Scientist

Since 2018, Utah Bureau of Forensic Services (UBFS) has been conducting semi-automated differential extraction using the QIAcube (QIAGEN) and is currently in the process of validating a fully automated differential extraction method using InnoGenomics' SpermX on the Hamilton AutoLys STAR liquid handler. UBFS implemented the AutoLys in 2017 for other forensic laboratory processing (e.g., reference samples and property crimes) and chose to explore full automation of the SAK processing workflow to increase throughput, minimize variation in sample processing, reduce possible sample handling errors, and assess cost savings. Additionally, whether UBFS uses the QIAcube or AutoLys STAR for differential extraction automation, both separations are followed by extraction on the Hamilton STARlet.

During initial validation studies, the SpermX filters consistently recovered more male DNA than UBFS' current method; however, samples also contained higher carry-over of female epithelial DNA into the male fraction. UBFS plans to explore method optimization during continued validation studies to reduce the amount of DNA carryover. Current supply chain delays have limited the availability of necessary laboratory consumables to complete this optimization and the SpermX validation.

> "InnoGenomics' SpermX requires less manipulations, and when paired with the AutoLys for automation, greatly reduces analyst hands-on time and increases throughput."
>
> *— Emily Simek, Forensic Scientist Manager DNA Technical Team*

In terms of time savings, the ability to run 95 samples at a time with SpermX on the AutoLys offers considerable advantages over the 36-sample limit from using multiple QIAcubes simultaneously. UBFS was able to maximize hands-free processing with the AutoLys STAR liquid handler to allow for analysts to tend to other duties. The SpermX and AutoLys automated protocol provides a total run time for differential extraction of 13 hours with only 4 hours of manual, hands-on time compared to 7.5 hours of hands-on time using the QIAcube semi-automated method (all times do not include extraction on the Hamilton STARlet). For laboratories facing a large caseload, the time and labor savings are significant.

UBFS recommends conducting an analysis to determine possible cost and labor savings for laboratories considering the SpermX or increased automation in SAK processing workflows. For example, UBFS conducted a thorough cost analysis on its currently used semi-automated method compared to the fully automated SpermX method to best determine the possible cost improvements. Due to the novelty of this method, UBFS created its own scripts and deck layouts for using SpermX with the AutoLys and are happy to share with other forensics laboratories. UBFS is also willing to share its experiences with other forensics laboratories to help them determine the best strategy for SAK processing.

Key Considerations for SpermX

- Shown to increase male DNA recovery; however, optimization is still needed to reduce potential DNA carryover of female DNA into the male fraction.
- SpermX on the AutoLys allows for a fully automated method that increases throughput, reduces variations among analysts, and has the ability to save time and costs when processing large batches of SAKs.
- Working with vendor contacts, and considering budgeting for Application Support from Hamilton, if adopting custom liquid handler scripts and deck layouts can greatly assist with validation.

Product Discussion—Chemical and Filtration Kits

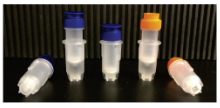
Each commercial differential extraction kit has associated advantages and disadvantages, and **Exhibit 6** provides a comparison of specific key features. The more traditional chemical reagent kits discussed included the Erase Sperm Isolation Kit and *forensic*GEM Sperm Kit. These two kits provide buffers and chemical reagents needed for their intended procedures aimed at decreasing the hands-on time and number of wash steps, and their main differences come from the proprietary solutions that make up the lysis buffers. The Erase Sperm Isolation Kit incorporates a DNase to eliminate the need for multiple wash steps. Conversely, the *forensic*GEM Sperm Kit uses alternative temperature-controlled enzymes for sperm lysis to eliminate the need for downstream DNA purification steps for PCR-ready DNA.

Other commercially available kits include the Sampletype i-sep DL, the Differex System, and the SpermX Differential Extraction System. These kits aim to improve the selective isolation of sperm cells through sperm cell capture or filtration. The Sampletype i-sep DL-MB and SpermX Differential Extraction System are consumable-based kits, an alternative to a chemical kit, and include collection tubes with proprietary polymer membranes that collect the sperm cells and allows the epithelial debris to pass through the membranes. These filtration methods reduce the risk of sperm cell loss during wash steps and limit cross-contamination from multiple transfer steps. The Differex System also targets sperm cells; however, the basis of this method is a chemical interaction rather than the physical barrier from a filter. This system (1) uses paramagnetic beads with a proprietary coating to capture sperm cells selectively and (2) uses a magnet during wash steps to reduce the risk of sample loss.

All chemical and filtration kits discussed augment established differential extraction protocols and may require the procurement of additional chemicals and buffers. For example, all kits require the procurement of ProK (**Exhibit 7**). Each commercial kit aims to reduce the overall time required for differential extraction, simplify the number of manual steps, and reduce the chance for sperm cell and DNA loss. These chemical and filtration kits are relatively less expensive than automated technologies and are therefore seen as easier on procurement based on these costs alone. However, considerations outside of procurement costs—including the costs associated with labor, time, and resources required for validating new techniques—may lead to an automated platform that is more advantageous.

Chemical and Filtration Kits Overview

Exhibit 7. Specifications, costs, and operational considerations for chemical reagent and filtration kits on the market.

Chemical and Filtration Kits					
Reagent Kits	**Erase Sperm Isolation Kit**	**Sampletype i-sep DL-MB**	***forensic*GEM Sperm Kit**	**Differex System**	**SpermX Differential Extraction System**
					
Manufacturer	PTC Laboratories	Biotype GmbH	MicroGEM	Promega	InnoGenomics
Cost	~$500	~$4,700	~$225	~$500	$2,499
Number of samples per kit	50	250	50	50	100
Cost per sample	~$10	~$19	~$4.50	~$10	$24.99
Technical manual	Not available online; comes with kit	Technical flyer	Quick-Start guide	TM331	User guide
Run time	2 hours	2 hours	< 1 hour	2 hours	4.5 hours
Number of manual steps	5	5	6	19	14
Number of washes	No washing of sperm pellet	No washing of sperm pellet	No washing of sperm pellet	4	3
Additional consumables required	Extra tubes, Extraction Buffer, Proteinase K	Proteinase K, DTT	Routine extraction tubes (e.g., Eppendorf tubes)	Proteinase K; ClickFit Microtubes, 1.5ml; DNA IQ Spin Baskets; Barrier tips; DNA IQ System; Ethanol; Isopropanol; DTT, molecular grade	Proteinase K, DTT
Laboratory equipment needed	Microcentrifuge	Microcentrifuge	Microcentrifuge; heat block or bath	Microcentrifuge; heat block or bath; Manual Differex Magnet	Centrifuge; oven or thermomixer/block

Notes:

For the Differex System from Promega TM331 user guide, please see here: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/differex-system-for-use-with-the-differex-magnet-tprotocol.pdf?la=en

For the MicroGem Quick-Start Guide, please see here: https://microgembio.com/wp-content/uploads/2019/03/QSG_004_190531_forensicGEM-Sperm.pdf

For a technical flyer on the Sampletype i-sep DL-MB, please see here: https://www.biotype.de/fileadmin/user/Flyer/Sampletype-i-sep-DL-DNA-separation-SATDLFL01v1en.pdf

Contact InnoGenomics directly for a copy of the SpermX Differential Extraction System user guide.

https://www.qiagen.com/bw/products/discovery-and-translational-research/dna-rna-purification/instruments-equipment/qiacube-connect/?clear=true#orderinginformation

QIAcube Connect by QIAGEN

The QIAcube Connect (QIAGEN; Hilden, Germany) automates the wash steps in differential extractions. Initial sample preparation is performed by lysing epithelial cells. Samples are then added to the system, and a traditional differential extraction technique is employed. Sperm cells are pelleted, the nonsperm fraction is removed, wash buffer is added, and sperm cells are pelleted three times. Sperm lysis buffer is added to obtain the sperm DNA fraction. The QIAcube Connect can process up to six samples in 1 hour or up to 12 samples in 90 minutes. Downstream automated extraction platforms such as the EZ1 Advanced instruments or QIAsymphony instruments using the QIAamp DNA Investigator chemistry is compatible with this platform.

QIAcube Connect Washing Station in the Literature

A 2020 study used mock sexual assault swabs to compare the automated QIAcube Connect to manual differential extraction methods.[28] Results showed similar human-to-male DNA ratios for the washing station and conventional differential extraction (approximately 1 to 1). Additionally, results from this study also showed that only two of six sperm fraction samples resulted in mixture DNA profiles with the QIAcube Connect, whereas five of six resulted in mixture profiles for the conventional manual method.

Advantages of the QIAcube Connect

The QIAcube Connect provides a system for automated differential separation and wash steps. The provided automation reduces the run-to-run variability and assists in standardizing the differential extraction procedure.

Additionally, with proper cross-contamination studies of the procedure, the decreased hands-on time can reduce the risk of cross-contamination between steps and sample loss.

Implementation and Use

- Includes automated system that replaces the need for routine laboratory equipment (e.g., microcentrifuge).
- Requires lysis buffers, microcentrifuge tubes, and pipettes.
- Involves an approximately 1–1.5 hour procedure (can vary depending on the number of samples).
- Requires minimal training—automates conventional differential extraction procedure.
- Costs approximately $30,000.

North Carolina State Crime Laboratory Uses Qiagen's QIAcube Connect for Automated Processing of Sexual Assault Kits.

Mackenzie DeHaan, Forensic Scientist Supervisor

Like many crime laboratories across the nation, the North Carolina State Crime Laboratory (NCSCL) desired methods that consistently test SAKs in a timely manner. In 2019, the state passed the Survivor Act (H.B. 29),[29] impacting SAK processing. This law requires medical facilities and other agencies that collect SAKs to notify law enforcement within 24 hours of collection; law enforcement is required to pick up the SAK within 7 days and submit it to the crime laboratory within 45 days for testing.

Several factors contributed to NCSCL selecting the QIAcube Connect. At the time, NCSCL used other QIAGEN products, including both the QIAgility System and QIAamp DNA Investigator Kit. Therefore, implementing the QIAcube would complement these other platforms and allow continued work with established QIAGEN contacts and support staff. Another reason for NCSCL to explore the QIAcube was its desire to find equipment that (1) does not cause significant changes to current workflows and (2) would be readily accepted and adopted by analysts. Together, an experienced analyst and a novice analyst from NCSCL provided a comprehensive validation for the QIAcube Connect. This validation confirmed that automation of differential extractions on the QIAcube Connect increased consistency and decreased the potential for human errors during SAK processing.

With 25 analysts in the NCSCL Raleigh laboratory and seven in the Western laboratory, reducing variabilities among analysts using a manual differential extraction method was an important factor in the decision to implement automation technology. Prior to considering the QIAcube Connect, one of the most common challenges NCSCL encountered with traditional differential extraction was that this manual method could result in inconsistencies in the amount of carryover between sperm and nonsperm fractions. Once brought online, the QIAcube Connect reduced these inconsistencies. Another advantage emerged by enabling batch processing, which can be challenging using the manual method. The QIAcube Connect also freed up analysts' time to work on other tasks and responsibilities. The Raleigh laboratory now has four QIAcube systems, and the Western laboratory has two.

> "The manual method is labor-intensive and partially an art form. We needed a way to achieve more consistent results with a higher volume of sexual assault kit processing."
>
> *—Mackenzie DeHaan, Forensic Scientist Supervisor*

With additional automation integrated into the SAK processing workflow, one new consideration NCSCL had involved balancing the shift in throughput for different workflow steps. Using automation to move additional samples through the differential extraction process can create potential bottlenecks in other areas, such as data interpretation and report writing. Therefore, more expensive high-throughput techniques—such as automated liquid handlers—were not considered because not all workflow steps allowed for the same amount of throughput. NCSCL developed a Lean Six Sigma process to determine how to best manage an efficient workflow that prevents analysts from putting more cases into the system than can be completed in a given timeframe.

Key Considerations for QIAcube Connect

- Enables more consistent results from all users; the ability to batch samples sped up SAK processing.
- Supports automation of differential extraction, which frees up analysts' time to pursue other tasks—but the shift in workflow and possible bottlenecks should be considered.
- Allows for already-established relationships with vendor contacts to support validation of new technology; these relationships can greatly assist in the ease and speed of implementing new technology.

https://www.hamiltoncompany.com/automated-liquid-handling/assay-ready-workstations/autolys-star

AutoLys STAR by Hamilton

The AutoLys STAR is an automated workstation from the Hamilton Company (Reno, Nevada). This workstation includes on-deck robotic components that handle samples without the need for manual interventions. The AutoLys STAR comes with preprogrammed software that provides a fully automated standard differential extraction workflow for processing up to 48 SAK samples in a single automation run using specially designed and barcoded AutoLys tubes. Using this AutoLys system for automated differential extraction has been principally proven effective in generating male DNA profiles in samples up to 96 hours after a sexual assault.[30]

The AutoLys STAR Is LIMS-Compatible

At the end of each run, a file containing details about sample type, location, and action can be sent to the LIMS. This information may be output in a standardized form for inclusion in case file reports. Barcoding allows for chain-of-custody tracking of samples and blanks correlated with item numbers, case files, and assigned analysts. Additionally, password-protected protocols prevent accidental changes during operation.

—Kevin Miller, MS, PhD; Senior Market Segment Leader, Hamilton

Advantages of the AutoLys STAR

The AutoLys STAR provides an automated sample processing system for complete automation of the differential extraction procedure. Automation reduces run-to-run variability, aids in standardizing the differential extraction procedure, and reduces the risk of cross-contamination and sample loss.

Additionally, the AutoLys system is fully customizable, allowing for multipurpose use for additional SAK processing protocols (e.g., modified differential extraction methods or plate preparation for subsequent workflow steps).

Implementation and Use

- Is a fully automated system that includes necessary laboratory equipment (e.g., microcentrifuge).
- Requires the purchase of necessary reagents and consumables for the given protocol.
- Provides sample tracking through barcoded tubes.
- Involves a procedure that lasts approximately 3.5 hours (can vary depending on the number of samples).
- Requires minimal training—automates conventional differential extraction procedure.
- Costs approximately $280,000.

The California Department of Justice Has Invested in the AutoLys STAR to Fully Automate Differential Lysis of Sperm in SAK Samples.

Sonja Klein, Senior Criminalist, Method Development; Gunther Scharnhorst, Senior Criminalist, Method Development; Chris Tanforan, Senior Criminalist, Casework; Jeanette Wallin, Criminalist Supervisor, Method Development

The California Department of Justice (DOJ) operates 11 forensic laboratories in the state and has been processing SAKs in a direct-to-DNA high-throughput format since 2005. In 2011, the California DOJ adopted an internally developed high-throughput, 96-well alkaline-based differential extraction method.[31] This method used DNase to separate epithelial and sperm fractions and was excellent at providing clean sperm fractions; however, yields were not as high as with the traditional differential extraction method. Additionally, this modified method was not automated, requiring hands-on time and skill despite the 96-well plate format. Because of these shortcomings, the California DOJ returned to the Gill method and focused internally on improvements.[32, 25]

In 2017, the California DOJ considered the AutoLys STAR as an automated liquid handler to address the need for more automated and consistent SAK processing. Various features—including robotic movement of samples using a unique tube-within-a-tube design—convinced laboratory analysts of the potential to fully automate differential extraction. The laboratory developed an automated version of the standard differential extraction method using a Hamilton demonstration model.[30] Support through NIJ DNA CEBR program grants 2016-DN-BX-0030 and 2017-DN-BX-0030 allowed for the purchase of AutoLys systems, and the State of California is providing additional funding to purchase remaining instruments for all laboratories within the California DOJ laboratory system. The goal is to have two AutoLys systems per laboratory.

> "The Hamilton AutoLys provides full automation that includes substrate removal. The only manual step is adding sample to the AutoLys tube."
>
> —Jeanette Wallin, *Criminalist Supervisor*

Implementing the AutoLys system reduces labor costs, provides more consistency and throughput for sample analysis, and communicates more efficiently with LIMS (see callout box on pg. 27 for additional information). The system enables the laboratory to perform differential extractions on 24 samples at a time, which provides sufficient capacity to manage the number of SAKs each laboratory receives weekly. These improvements adhere to a state law that became effective in January 2019, requiring laboratories to process SAKs within 120 days of receipt.[33] Laboratories should assess their collective SAK process to determine whether the scale that AutoLys provides is worth the investment. The initial cost is considerable; however, saving money on labor costs—such as those associated with manual differential extraction, the speed at which samples are processed, and the ability for analysts to process other evidence on the platform—may pay for the system over time. The California DOJ is also looking to develop other processes on the AutoLys system, including generating lysate and removing substrate of non-differentially extracted samples, pairing the system with an external DNA extraction robot, and developing a method for direct amplification of case reference samples.

Key Considerations for AutoLys STAR

- Offers a method for automating the manual differential extraction protocol for decreased hands-on sample processing time and increased sample throughput.
- Provides more consistency in sample analysis and enables coordination with LIMS.
- Can be used beyond differential extraction and applied to additional sample processing steps (e.g., nondifferential DNA extractions) for increased workflow automation.

https://www.siliconbiosystems.com/deparray-nxt

DEPArray™ NxT by Menarini Silicon Biosystems

Menarini Silicon Biosystems (Bologna, Italy) developed the DEPArray™ NxT to automate identifying and sorting single cells or groups of cells. This technology uses immunofluorescence and bright field imaging for accurate cell identification and combines microfluidics and microelectronics to manipulate cells for separation and recovery. A semiconductor chip controls an array of microelectrodes within a flow cell to generate up to 8,000 dielectrophoretic "cages" to isolate single or groups of cells. Once identified and sorted, the desired cells are moved into a recovery chamber upstream of a standard microcentrifuge collection tube through automated software. Although this technique was initially developed for oncology applications, it has been found to be highly effective for sorting sperm cells from epithelial cells for differential extraction.

The DEPArray™ Forensic Sample Prep Kit provides all reagents for the immunofluorescent labeling of epithelial, sperm, and blood cells. Once cells are isolated and recovered, the DEPArray™ LysePrep Kit can be used for cell lysis for direct downstream genetic analysis.

The development of this technology was partially funded by NIJ grant 2015-NE-BX-K002, *An automated dielectrophoretic-based single cell separation technique to improve laboratory efficiency, mixture deconvolution and combat sample inhibition*.

DEPArray™ in the Literature

One study reported single-source male profiles were obtained from 26 of 27 (96.3%) samples processed with the DEPArray™ NxT as opposed to only 9 of 28 (32.1%) samples with traditional differential extraction.[34]

Advantages of the DEPArray™ NxT

The DEPArray™ NxT provides a system for automated differential separation of epithelial and sperm cells, with increased precision, using a single-cell sorting technique. The ability to identify, separate, and recover single cells or groups of cells prior to genetic analysis reduces the need for centrifugation and wash steps and can resolve challenges inherent to mixed biological evidence.

Implementation and Use

- Is an automated system for separation of epithelial and sperm cells.
- Requires the purchase of DEPArray™ reagent kits for use—DEPArray™ Forensic Sample Prep Kit, DEPArray™ Lyse Prep Kit.
- Involves a procedure lasting approximately 6 hours, including sample preparation.
- Requires minimal training—automates cell separation and requires manual lysis.
- Costs approximately $200,000.

Syracuse University Examined the Use of the DEPArray™ System to Assess Challenging Sexual Assault Kit Samples.

Michael A. Marciano, PhD, Research Assistant Professor and Director of Research, Forensic & National Security Sciences Institute (FNSSI), Syracuse University

Dr. Michael Marciano and colleague Victoria Williamson (Forensic Biologist, Erie County Central Police Services Forensic Laboratory) recently hosted a FTCoE webinar[35] during which they described findings of a study that incorporated the DEPArray™ NxT into a standard forensic workflow—DNA extraction, PCR amplification using the PowerPlex® Fusion 6C Human DNA amplification kit, and detection using the Applied Biosystems™ 3500xL Genetic Analyzer.

This method separates the epithelial and sperm cells prior to extraction; therefore, a major workflow difference with the DEPArray NxT is that it requires cell staining with different antibody-based stains (e.g., epithelial, sperm, nucleic acid) for downstream sorting. Menarini Silicon Biosystems offers the cell-staining kit and stepwise protocol. Batch preparation of multiple samples (e.g., four, five) simultaneously may be used during cell staining—and the workflow often includes staining cells in one afternoon and running samples back-to-back on the DEPArray NxT the next day. The full workflow may take several hours to complete for staining (2–3 hours), instrument preparation (0.5 hour), running samples (2 hours), and post-processing (0.5 hour). Translating the cell staining for use on an automated liquid handler for increased automation of this step is possible.

Although this automated cell sorting system requires front-end preparation, there are several unique advantages. Because of the DEPArray NxT's cell-staining, imaging, and sorting process, the number of total cells and sperm cells is known and the amount of DNA can be calculated directly, thus not requiring DNA quantification using quantitative PCR. Additionally, because the cells are sorted and collected, possible impurities (i.e., inhibitors) are removed, resulting in cleaner samples for downstream PCR. Finally, the DNA profile that is developed can be directly traced to the individual cells that were used to develop the profile. Although this landscape study explores the use of DEPArray NxT for differential extraction, this technique is advantageous for mixture deconvolution and special cases. For example, Dr. Marciano is currently working with the Acadiana Criminalistics Laboratory to explore the DEPArray NxT's applicability for special casework in which there are potential mixtures and exceedingly low levels of sperm (NIJ 2020-DQ-BX-0019). By labeling and isolating each sperm cell using the DEPArray NxT, the team can identify the individuals involved.

> "We are currently engaged in method development activities for Y-chromosome labeling and using the DEPArray to selectively collect male cells from same cell mixtures if there is limited to no sperm present in a sample."
>
> *—Michael A. Marciano, PhD, Professor and Director of Research, FNSSI, Syracuse University*

The DEPArray system requires specialized training; however, the advantage of cell sorting with the DEPArray NxT is that this technique could be applied to other DNA casework, in addition to situations in which differential extraction would be typically used for special casework scenarios and mixed samples with multiple contributors.

Key Considerations

- Eliminates the need to do mixture analysis and deconvolution for single-cell or targeted-cell analysis, potentially solving cases that had difficulty establishing the number of contributors and their identities.
- Does not require assessing a standard curve for DNA quantification; the DEPArray can move single cells into a collection tube so that the analyst knows exactly how much DNA is available based on the number of collected cells. For example, 10 sperm cells would yield 33 picograms of DNA.
- Has a method that allows DNA profiles to be attributed to specific cell types.

Product Discussion—Automated Systems

Available technology for increased automation either targets the differential extraction protocol specifically or is more broadly focused on technologies adapted for this application. Additionally, when assessing technology that improves workflow automation, considering which technologies are fully automated and those that are semiautomated can be beneficial. Fully automated technologies require no user intervention during completion of the sample processing protocol. Alternatively, semiautomated technologies may automate a step in the protocol such as cell separation but require manual addition of lysis buffers or the transfer of samples manually for incubation.

Commercial differential extraction, or cell separation, automated technologies include the QIAcube Connect and the DEPArray NxT. Although both technologies are considered more specialized, the cell separation techniques are very different. The QIAcube Connect strictly automates the conventional differential extraction procedure to allow for minimal manual intervention during the protocol and washing steps. Alternatively, the DEPArray uses a more sophisticated cell sorting technique that can isolate individual cells—stained as epithelial or sperm—for downstream recovery. However, both technologies can be considered semiautomated, as some user intervention is required to complete all differential extraction steps through sperm cell lysis.

An alternative method for increased automation is a robotic liquid handler that incorporates robotic pipettors and can include other routine equipment (e.g., centrifuge) into the workstation setup. Although there are various workstations, the AutoLys STAR is preprogrammed for the conventional differential extraction protocol. The AutoLys STAR workstation allows for complete automation of the differential extraction protocol (for either conventional or modified method) without manual intervention (fully automated) due to the on-board laboratory equipment. Additionally, the AutoLys STAR workstation can be customized for use with some of the chemical reagent kits discussed (e.g., SpermX Differential Extraction System) by implementing custom scripts and decks. Additionally, the AutoLys STAR can also be adapted for multipurpose use (e.g., reference samples, other SAK processing steps) if desired or as throughput allows.

The differences in procurement costs are greater between the automated technologies and commercial kits. For automated technologies, the QIAcube Connect is the least expensive of the commercial automated methods (by a factor of about 10) and has a throughput of 6–12 samples at a time. Depending on budget and processing demand, laboratories account for limited throughput by purchasing multiple QIAcube Connect. Although the AutoLys STAR and DEPArray NxT are more expensive than the QIAcube Connect, these technologies vary in throughput—48 samples and one sample at a time, respectively. Due to the precision and mechanism of cell sorting for the DEPArray NxT, multiple samples can be prepped at once, but only one sample can be run at a time using this instrumentation. **Exhibit 8** provides a comparison of additional key features.

Considerations for Laboratory Automation

New legislation, combined with the impact DNA evidence has in solving criminal cases, has made automation a key factor in increasing capacity while maintaining quality. However, implementing laboratory automation can be an expensive endeavor, so careful consideration should be made for laboratory implementation.

Automation of laboratory processes can generally be broken down into two categories:

Semiautomated – Semiautomated technologies may be used to improve the automation of one or more manual steps in a laboratory protocol, but still require significant manual intervention to complete a protocol from start to finish.

Fully automated – Automated technologies may be used to completely automate a laboratory protocol, requiring limited or no user intervention during completion of a sample processing protocol.

Whether a laboratory is looking to increase automation or fully automate a workflow can have differing considerations based on a laboratory's need.

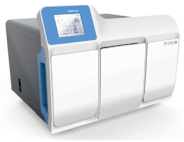
Additionally, transitioning from a fully manual process to a fully automated process can be significant. Although automation offers several improvements, the following factors should be considered for implementing automation:

- Up-front and continued costs, throughout requirements, possible integration with other related laboratory workflows, defining validation targets throughout the protocol, establishing vendor relationships, creating or modifying software scripts, and having the ability for LIMS integration.[36]
- When determining which technology to implement to improve laboratory automation, it may also be beneficial to perform an internal cost-benefit analysis (CBA) to evaluate the impact of implementing a various automation-supporting technology.

CBA is a process used to measure the benefits of a decision or take action, minus the costs associated with taking that action. CBA tools are freely available on the internet, such as one provided by Smartsheet. Manufacturers or vendors may also assist in CBA as part of the sales process. Generally, CBA can be divided into the following five basic steps:
Step 1: Understand the cost of maintaining the status quo or currently used protocols and workflows.
Step 2: Identify all relevant upfront, direct, and indirect costs, including labor, associated with the new procedure.
Step 3: Identify benefits in terms of time and cost. Factor in other impacts that automation may provide for the laboratory.
Step 4: Assign a monetary value to the costs and benefits. If needed, consult with others to determine the value you will assign to intangible benefits, such as maintaining employee satisfaction.
Step 5: Create a timeline for expected costs and revenue. Determine the length of time needed to reach your break-even point or the timeframe in which costs and benefits equal out.

Automated Systems Overview

Exhibit 8. Table of specifications, cost, and operational considerations for automated systems on the market.

Automated Systems			
Instrument	**QIAcube Connect Washing Station**	**AutoLys STAR**	**DEPArray NxT**
			
Manufacturer	QIAGEN	Hamilton	Menarini Silicon Biosystems
Cost	~$30,000	~$280,000	~$200,000
Differential extraction steps required before running samples	1 step; epithelial cell lysis	None	Swab or punch cell resuspension in buffer cell permeabilization, staining, and fixation
Run time	1 hour (6 samples) 1.5 hours (12 samples)	Depends upon number of samples and lysis method chosen	1.5 hours for loading, cell identification, and transfer to parking chamber; then 2 minutes per cell delivery. Max 48 cells = 96 min
Number of samples per run	6 to 12	Up to 48 samples at a time, which will yield up to 96 samples (48 epithelial and 48 sperm fractions) per run	1 sample, recovering up to 48 single cells or 5 pools, up to 85 cells each
Number"of manual steps	None	Placement of samples into tubes; no other manual steps	Sample preparation takes about half day and an overnight incubation; approximately 4–6 washing steps and 2–3 sample transfer steps
Additional consumables required	Tips and tubes ~$1.50 per sample	Pipette tips, AutoLys tubes, FlipTubes or microcentrifuge tubes, 96 well plates, waste bags	Nylon swabs, short tandem repeat polymerase chain reaction amplification kit
Other laboratory equipment needed	None	This depends upon the workflow chosen	Centrifuge, cell counter countless, thermal cycler

Alternative Differential Extraction Techniques in Development

Several additional methods have been applied to isolate sperm cells from epithelial cells; these other methods have not been discussed thus far because they are currently only being used for research, have not been fully commercialized yet, or are still being developed. **Products profiled throughout this next section are categorized as (1) ongoing research or (2) emerging technology.** Appendix A highlights various cell separation and differential extraction techniques, including those discussed to this point (e.g., chemical lysis, filtration, laboratory robotics). This list is not exhaustive of all ongoing research and products undergoing commercial validation related to differential extraction; however, the work highlighted here is intended to provide examples of various ongoing and promising research efforts. **Exhibit 9** provides recent (from 2015–2020) NIJ-supported research and development related to differential extraction and sperm cell separation.

Exhibit 9. NIJ-supported research and development (2015–2020) related to differential extraction.

Title	Award No.
Automation of Differential Extraction with Sperm Quantitation Using Microfluidic-Integrated Shadow Imaging System for Forensic Applications	2015-DN-BX-K037
Highly Efficient Sperm Cell Separation from Sexual Assault Samples for DNA Analysis Using Novel Polymer Filter Technology	2015-DN-BX-K072
An Automated Dielectrophoretic-Based Single Cell Separation Technique to Improve Laboratory Efficiency, Mixture Deconvolution and Combat Sample Inhibition	2015-NE-BX-K002
Optimized, Semi-Automated Differential DNA Extraction (Denver PD Crime Laboratory)	2015-NE-BX-K005
A Rotational Platform-Driven Microdevice for Differential Separation, Purification, & Amplification of Sexual Assault Forensic Samples	2016-NE-BX-0002
Automation of a Sperm DNA Capture Assay for Forensic Applications	2016-DN-BX-0156
Rapid Extraction of Sperm from Sexual Assault Kits	2016-DN-BX-0161
Capillary Zone Electrophoresis Automated Fraction Collection for the Forensic Analysis of Sexual Assault Evidence	2017-IJ-CX-0003
A Confirmatory Test for Sperm in Sexual Assault Samples Using a Microfluidic-Integrated Cell Phone Imaging System	2017-NE-BX-0004
Autosomal DNA-STR Profiling of Directly Captured Spermatozoa from Post-Coital (3–10 Days) Cervico-Vaginal Samples	2018-NE-BX-0001
Assessment of Sexual Assault Kit (SAK) Evidence Selection Leading to Development of SAK Evidence Machine-Learning Model (SAK-ML Model)	2019-NE-BX-0001
Bio-inspired Material-Integrated Beads for Differential Extraction of Sperm in Forensic Applications	2019-NE-BX-0003
Adaptation of the DNAse I Procedure to the Biomek NXP Robotic Platform for More Efficient and Automated Sexual Assault Sample Processing	2019-NE-BX-0002
Completion of the SONIC-DE 2.0 System for Implementation in Forensic Laboratories	2019-NE-BX-0004
An Affinity-Free, Centrifugal Microfluidic System for Rapid, Automated Differential Extraction	2020-DQ-BX-0024
Sex-Based Targeted Recovery of Cells in a Heterogeneous Mixture: Separating Male and Female Like Cells	2020-DQ-BX-0019

Previous and Ongoing Research

Due to challenges with conventional differential extraction (e.g., the number of manual steps, efficiencies, and susceptibility for male DNA loss to the epithelial fraction) and some limitations with alternative products on the market (e.g., cost and the lack of complete automation), ongoing research is important for generating alternative approaches that address these challenges. Several research groups have aimed to address various aspects of differential extraction to improve the method. Two notable approaches include methods aimed at improving the sperm cell recovery using more selective isolation techniques and research aimed at developing automated, user-friendly technology. The following sections describe several promising research efforts and prototype technology demonstrations, many of which NIJ has supported; however, this information is not exhaustive of all ongoing research efforts for differential extraction.

Selective Isolation Methods

Selective cell separation methods can enhance the traditional differential lysis method by using capture methods for isolation and enrichment prior to cell lysis to target sperm cells. Nori and McCord first used a method called pressure cycling technology to apply cycles of hydrostatic pressure, which induced mechanical stress on cells to destabilize the cell membrane to cause lysis.[37] Cotton swabs were added to an alkaline solution of sodium hydroxide and 5 minutes of pressure cycling technology was applied to lyse the epithelial cells, following an incubation at 95°C in the alkaline solution without pressure to lyse the remaining sperm cells. This procedure resulted in 104 ± 6% recovery of the epithelial DNA and 69 ± 6% recovery of the sperm DNA from mock sexual assault kit samples with a one-to-one ratio. This method was later modified in 2017 to include a pretreatment method using a commercial immunomagnetic cell capture kit for epithelial cells.[38] This pretreatment step improved the isolation of sperm cells using ratios up to 200:1 of excess epithelial cells.

This research was funded by NIJ grant 2011-NE-BX-K550, *Rapid and Selective Extraction of Male DNA from Rape Kits and Other Forensic Evidence Using Pressure Cycling*.

An alternative example of a selective isolation method using antibodies was published for the use of magnetic beads coupled to anti-PH-20 antibodies to specifically target PH-20 located on the head of sperm cells.[39] Prior to sperm cell lysis, the immobilized antibody-sperm were retained magnetically to allow for DNase washes to remove female DNA. Using this method, sperm cells were successfully isolated from a mixture ratio of 10^3 to 10^5 for the amount of sperm to epithelial cells. Additional antibody methods were applied in this work and in additional work by researchers to selectively target sperm cells (e.g., CD52[40] and MOSPD3[41] antibodies).

Automated Technology Developments

Research efforts focused on circumventing the traditional chemical lysis method for a more automated separation of epithelial and sperm cells to reduce the chance for mixed fractions. Wright et al. used capillary zone electrophoresis to separate intact sperm cells, epithelial cells, and cellular debris into discrete bands within a capillary based on differing electrophoretic properties.[42] The capillary exit was coupled to a fraction collector to deposit the separated bands into a 96-well plate for an automated approach to cell separation. The overall separation and fraction collection were performed within 15 minutes and the complete sample elution, cell separation, and cell lysis protocol, resulting in purified DNA could be completed within 30 minutes with the use of a robust enzyme for complete cell lysis. The same robust enzyme was used to lyse both epithelial and sperm cells because the cell separation and fractionation were performed upstream. This described procedure does not require any wash steps or centrifugation, reducing the sample processing time and manual steps required. Because of the small volume of sample injected into the capillary, applying an initial centrifugation step to

This research and development work from Wright et al. involved engagement with the Nappanee Police Department (Indiana) and Palm Beach Police Department (Florida) public laboratories.

concentrate sperm cells into a decreased volume prior to injection could significantly increase the chance of fractionating sperm cells. **This research was funded by NIJ grant 2017-IJ-CX-0003, *Capillary Zone Electrophoresis Automated Fraction Collection for the Forensic Analysis of Sexual Assault Evidence*.**

A technology-based approach for the automated isolation of sperm cells has been demonstrated by microfluidic-based acoustic separation techniques. These techniques exploit the inherent differences in size, density, and compressibility of epithelial and sperm cells to selectively isolate sperm cells using forces from acoustic waves. Successful demonstration of acoustic differential extraction was first shown in 2006[45] and was later adapted and tested using a range of mock SAK samples.[46, 47, 43, 44] Clark et al. developed and built an acoustic differential extraction prototype system that included a user-friendly interface for operation, and all necessary hardware and electronic components were fully integrated into the system toward a robust and compact technology.[17] The acoustic differential extraction technology was used to demonstrate sperm cell capture from mock samples that contained a 40-fold increase in female epithelial cells compared to sperm cells.[19] The authors also evaluated how other biological components—including blood, yeast, and bacteria (*E. coli*)—affected the sperm cell trapping and isolation; all of these components resulted in negligible effects. **This research was funded by NIJ grants 2013-NE-BX-K027, *Delivery of a Microfluidic Acoustic Sperm Cell Trapping Prototype for Rapid Processing of Sexual Assault Evidence* and 2019-NE-BX-0004, *Completion of the SONIC-DE 2.0 System for Implementation in Forensic Laboratories*.**

This research and development work from Clark et al., involved engagement with the following public laboratories: Palm Beach County Sheriff's Office, Florida; U.S. Army Defense Forensic Science Center; Virginia Department of Forensic Science; New York City Office of Chief Medical Examiner; Texas Department of Public Safety Houston Regional Crime Laboratory.[43, 44]

Technology Toward Commercialization

Unlike the previously described research efforts, some products progress further toward commercialization, such as the NGDE™ system. This technology received NIJ funding during the initial research and development phases.

DxNow (Gaithersburg, Maryland) is developing the NGDE™ system, a microfluidic device that performs on-chip differential extraction.[48, 49] This platform aims to improve the capture and isolation of sperm cells and DNA, in addition to reducing the time required to perform differential extraction. The microfluidic platform selectively captures sperm cells by using a unique oligosaccharide sequence (Sialyl Lewis^X or SLeX), which is a major carbohydrate ligand for sperm-egg binding and resulted in an approximately five-fold higher sperm capture efficiency.[50] Cells are eluted from collection material (e.g., swabs), and the sample solution is then added to the NGDE system. Sperm cells are captured with SLeX, whereas the epithelial cells do not bind and are removed during washing. Wash steps are performed within the microfluidic system, and isolated sperm cells are then lysed with a lysis buffer. Once the sperm DNA is extracted, the sample solution is collected from the system and used for downstream processing. The differential extraction procedure is performed within 80 minutes compared to approximately 5+ hours for the conventional differential extraction method. Additionally, this system allows for on-chip quantitation by counting the number of sperm cells and then calculating the amount of DNA based on the number of cells. SLeX has multiple binding sites on the sperm's surface; therefore, using this method with aged samples was advantageous and achieved 70%–92% capture efficiencies with forensic mock sample storages for over a decade. **Initial research and development of this microfluidic device was funded by NIJ grant 2015-DN-BX-K037, *Automation of Differential Extraction with Sperm Quantitation using Microfluidic-Integrated Shadow Imaging System for Forensic Applications* and 2017-NE-BX-0004, *A Confirmatory Test for Sperm in Sexual Assault Samples using a Microfluidic-Integrated Cell Phone Imaging System*.**

Training and Validation for Emerging Technologies

- Driving advancements in forensic workflows and research to practice is a community effort. Support from practitioners, vendors, and other subject matter experts helps researchers develop value-adding innovation. Please consider how you may be able to assist researchers and NIJ grantees to advance their work.

Potential Support Avenues (Other than Grants)

- Hosting graduate students through internship and academic research programs to support the validation of new or emerging forensic methods and technologies.
- Working with vendors to receive a demo instrument to trial their technologies (e.g., early adopter, beta-tester) without the financial commitment.
- Connecting your laboratory to a vendor contact or partnering laboratories for validations and help coordinate to meet your laboratory's needs—FTCoE can help.
- Contacting us—reach out to forensiccoe@rti.org if you are interested in working with emerging technologies and do not already have a vendor contact. FTCoE can facilitate connecting your laboratory to a vendor contact or partnering laboratories for validations and help coordinate to meet your laboratory's needs.

Future Perspectives

Advancements within the field of forensic DNA methodology may impact how differential extraction methods and technologies are used in the future because mixture deconvolution techniques can be used downstream of the SAK processing workflow once a DNA profile is generated. Due to DNA mixture deconvolution, there may be less emphasis on continuing to explore methods of improving differential extraction efficiency and more emphasis on throughput and automation.

Direct amplification (or direct polymerase chain reaction) is another example of an advancement in forensic DNA methodology. This technique allows for DNA amplification without upstream DNA extraction and purification steps. For samples with low levels of DNA, direct amplification has been suggested as a method for circumventing the DNA quantification step to limit the amount of DNA loss that can occur at additional workflow steps (e.g., extraction, purification, quantification).[51]

Although forensic practitioners routinely discuss these advancements and several laboratories have begun validating these methods, these techniques have not yet been widely implemented in forensic laboratories for processing forensic DNA evidence. Delaying the implementation of advanced techniques in forensic laboratories is often due to several factors, including prerequisite training, validation, hiring personnel, and regulatory guidelines that may require the inclusion of certain workflow steps (e.g., quantification).

Conclusion

Implementing advanced SAK processing workflows is one approach to addressing SAK processing requirements and increasing caseload capacities. Advancements within these workflows include improving efficiencies to increase the probability to obtain CODIS-eligible profiles and reducing the hands-on time required to perform the workflow

protocols. Because differential extraction is one of the first steps in the SAK workflow, poor separation of epithelial and sperm cells can have a downstream effect on the remaining workflow and resulting DNA profile. Challenges that can impact differential extraction include poor isolation of sperm cells, loss of sperm cells during the wash steps, quality and quantity of the male DNA, and attributes of the sperm cells due to differences among individuals.

Two approaches are often considered for advancing conventional differential extraction—improving the isolation efficiency for sperm cells or automation of the workflow. The ***product pages*** and ***user profiles*** highlighted these two techniques for the technologies shown in **Exhibit 10**.

Exhibit 10. Available technologies by approach.

Available Products	
Chemical and Filtration Kits • Erase Sperm Isolation Kit • Sampletype i-sep DL-MB • *forensic*GEM Sperm Kit • Differex System • SpermX Differential Extraction System	**Automated Systems** • QIAcube Connect • AutoLys STAR • DEPArray NxT

Overall, the chemical and filtration kits are relatively less expensive than automated technologies to begin working with, and the kits may be a more cost-effective approach to exploring methods for improved differential extraction results. Alternatively, if cost is less prohibitive, automated systems can be helpful in reducing hands-on time for analysts so that they can work on other tasks. The QIAcube Connect is an advantageous option if an automated system with less throughput than an automated liquid handler is needed because the cost is much lower. Ongoing research and emerging technologies suggest that more products for improving the separation of epithelial and sperm cells may be available in the future.

Several of the advancements highlighted in this landscape study have the potential for greater impact on the forensic community, and a combination of improved efficiencies and automation may be the ultimate objective. These advancements positively impact sexual assault victims by helping to provide case resolution and forensic practitioners by decreasing assay times and increasing productivity.

Key Takeaway from User Experiences

Several laboratory personnel were interviewed about their experiences with activities related to the operation, implementation, and validation of various differential extraction technologies, including the Erase Sperm Isolation Kit, QIAcube Connect washing station, AutoLys STAR, and the DEPArray™ system.

- Each person provided some suggestions about lessons learned during these activities, and an overlapping theme was **vendor relationships**. Key takeaways and considerations from the user profiles related to establishing vendor contacts can be applied to the implementation of any new technology, including consumable kits or automated systems.

Establishing Relationships with Vendor Contacts Can Have Many Benefits:

- Seeing quicker response times when requesting assistance or additional information.
- Receiving engagement and support during validation that can result in a smoother and more rapid validation.
- Receiving tailored assistance if changes can be made to allow a technology solution to fit your laboratory's specific needs (e.g., writing or updating code for automated liquid handler protocols).
- Having opportunities to acquire demonstration instruments, more commonly referred to as *demoing new technologies*, free of charge.

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Glossary of Commonly Used Terms

Automated systems—Systems that decrease the number of hands-on steps for a manual laboratory procedure; automated systems can increase throughput, reduce variability and errors from manual workflows, and enable analysts to use the additional time to work on other tasks.

Carryover—Contamination from one cell fraction to another, resulting in the presence of sperm and nonsperm DNA within each separated solution or the incorrect fraction.

Chemical and filtration kits—Consumable kits that contain chemicals or filters needed to perform laboratory procedures.

Combined DNA Index System (CODIS)—A national DNA database created by the Federal Bureau of Investigation (FBI); CODIS is also a generic term that typically represents both the FBI's program of support for databases, including the State DNA Index System and the National DNA Index System, and the system software.

Differential extraction—A technique that allows for the selective cell lysis and isolation of DNA from a mixture of sperm and epithelial cells.

DNA amplification—The production of multiple copies of a DNA sequence. Polymerase chain reaction (PCR) is the most common DNA amplification method.

DNA isolation—A process that separates DNA from other cellular components through certain procedures that can be chemical or physical methods for isolation.

DNA profile—The result of forensic DNA analysis that can be used to identify individuals bases on variations in their DNA sequence. Biological material used to develop a DNA profile can include blood, semen, saliva, urine, hair, teeth, bone, and tissue.

DNA purification—A process often used after DNA extraction and DNA isolation whereby DNA is further separated from any possible contaminants that could inhibit downstream PCR or other processing methods.

DNA quantification—A method for determining the amount of DNA present in a sample by measuring the concentration of DNA through amplification markers on the autosomal or Y chromosome.

Gill method—A differential extraction method that selectively lyses and separates epithelial cell DNA from a sample prior to extracting DNA from sperm cells.

Mixture Deconvolution- In DNA analysis, the process of separating unknown profiles from samples containing DNA from multiple contributors.

Sexual assault kit (SAK)—A SAK is a package of materials that a medical professional—often a medical forensic examiner, such as a sexual assault nurse examiner—uses to collect samples (i.e., evidence) from a victim's or suspect's body.

Throughput—The amount of material or items passing through a system or process.

Workflow—A series of steps required to accomplish a task; SAK workflows may change as a laboratory incorporates new instruments.

Appendix A

The following are techniques and associated descriptions for differential extraction methods.

- **Preferential chemical lysis:** The most frequently used technique to preferentially break down or compromise sperm and epithelial cell membranes using selective degradative chemicals.
- **Filtration:** Physical separation of epithelial and sperm cells based on inherent differences in cell size using filtration.
- **Antibody capture:** Sperm cells are captured using antibodies to target biomolecules specific to sperm cells. These antibodies are often bound to magnetic beads, allowing the captured cells to be magnetically retained during wash steps.
- **Laser microdissection:** A precise technique using microscopy to identify cells followed by laser manipulation to remove the cells of interest from the substrate.
- **Flow cytometry or fluorescence-activated cell sorting:** A technology that uses specific membrane proteins or fluorescent labeling to mark and sort single cells through a flow cell.
- **Laboratory robotics:** Robotic liquid handlers or equipment used for automating routine laboratory sample processing protocols. Robotic liquid handlers integrated with other laboratory equipment (e.g., centrifuge, heat block) are considered automated workstations.
- **Microfluidic devices and systems:** Devices that have at least one micron-sized dimension and use the differing physical and chemical properties of epithelial and sperm cells for separation on a microscale.

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