DEVELOPMENT OF A HIGH-THROUGHPUT METHOD TO ISOLATE SPERM DNA IN SEXUAL ASSAULT CASES

FINAL REPORT

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DEVELOPMENT OF A HIGH-THROUGHPUT METHOD TO ISOLATE SPERM DNA IN SEXUAL ASSAULT CASES

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ABSTRACT

With the large number of sexual assault cases, the need for a fast and efficient method to process the more than 500,000 reported backlogged evidentiary cases is critical (1). Currently, the Federal Bureau of Investigation's national CODIS (COmbined DNA Index System) database contains over 3 million searchable profiles, which can be queried against evidentiary DNA profiles to identify rape suspects (2). In addition, the number of profiles within CODIS is quickly increasing. Following the approval of the "Sexual Assault DNA Backlog Reduction Act" of 2002, the major push to eliminate the large number of unprocessed rape kits began with the eventual goal of analyzing all backlogged samples (3). However, the processing of evidentiary swabs from these rape kits is labor intensive and the development of a more automatable method is highly desirable.

The standard technique for processing semen stains or swabs is known as "differential extraction". Typically, this is accomplished by first digesting the epithelial cells in a Proteinase K solution and then separating the digested cells from the intact sperm through centrifugation. The pellet is then resuspended and washed a number of times before being digested with a reducing agent, such as DTT, which breaks the sperm heads, releasing its DNA into solution. This is then followed by standard phenol-chloroform extraction to purify the DNA (4).

Recently, Bureco Corp. (Allschill, Switzerland), with assistance from Millipore Corp. (Bedford, MA), has created a double membrane filter designed to trap sperm while enabling the DNA from digested diploid cells to pass through. The filter consists of a stacked construction with

an 11 micron nylon net placed atop a 2 micron ISOPORE™ track-etch filter. This filter, following treatment with BackGround Quencher to prevent the binding of free DNA, was designed to resist filter flexing or pore size mutation under increased vacuum pressure (1). Given the initial evaluation and limited success of the filtration method (first presented at the 2004 National Institute of Justice Annual Grantees Meeting, Washington DC), alterations were made to the protocol that greatly increased the success rate for a variety of swab types and sperm counts. The following is an evaluation and optimization of the track-etch filtration method and a comparison of its performance to the standard differential extraction protocol using multiple amplification kits and sequencer platforms.

EXECUTIVE SUMMARY

Forensic laboratories in the United States currently have at least 500,000 unprocessed sexual assault cases. The standard method for purifying sperm from these swabs is to first resuspend all cells and to selectively digest the excess of the victim's epithelial cells. The intact sperm are then separated from the epithelial DNA by centrifugation, careful removal of supernatant, and extensive washing of the sperm pellet. Vacuum driven filtration is an alternative method for separating sperm from digested epithelial cells that is fast, inexpensive, and more amenable to automation.

Given the large number of unprocessed sexual assault cases, we have evaluated a modified filtration-based method to isolate sperm from digested epithelial cells that could significantly increase throughput and potentially overcome the problem of automating DNA sample preparation for sexual assault evidence.

Samples

Semen from healthy male volunteers was collected and an average number of sperm per microliter of semen was determined by use of a hemocytometer. Buccal swabs, provided by healthy female donors were collected in triplicate. Approximately 100,000 sperm were added to the swabs which were extracted using the track-etch filtration method with and without RNase treatment and compared to the standard differential method. Known dilutions of semen, ranging from 100,000 to 3,125 sperm, were also added to both buccal and blood saturated swabs from known female contributors, extracted using the RNase filtration protocol, and compared to the results of the standard differential method. In addition, 10 sexual assault evidentiary samples (5 vaginal swabs and 5 cuttings from semen stains) from nine previously adjudicated, non-probative cases were

collected and used to demonstrate the efficiency of the track-etch filtration method as compared to standard differential extraction.

ISOPORE™ Track-etch Filter

The ISOPORE™ track-etch filter is a stacked double membrane filter consisting of a single 11-micron nylon net filter placed on top of a 2-micron track-etch filter. The track-etch filter's distinctly defined and rigid pore size was designed to allow DNA from digested epithelial cells to pass through while trapping sperm. The track-etch filters are treated with a 10X BackGround Quencher (MRC, Inc., Cincinnati, OH) for 30 minutes to inhibit the binding of free DNA and placed within an empty Microcon-100 casing.

Extraction Methods

For comparison purposes, standard differential extraction was performed on all known swabs in parallel with the filtration method. Standard differential extraction was performed per Connecticut lab protocol by first incubating the above described swabs in a 450µl of extraction buffer and 15µl Proteinase K (20mg/ml), at 56^{o}C for 1 hour. The samples were pelleted and washed with dH_20 in order to remove any remaining female DNA from the sperm pellet. 450µl of extraction buffer, 15µl of Proteinase K (20mg/ml), and 20µl of DTT (36 mM) was then added to the pellet and incubated at 56^{o}C for 4-18 hours. A standard organic extraction was performed on all samples processed.

Samples were also incubated at 56^{o}C for 1-2 hours in the above described extraction buffer / Proteinase K solution along with 10µl of DNase free RNase (100mg/ml). The samples were pelleted and washed with dH_20. The pellets were then resuspended in extraction buffer and filtered through the track-etch filter at 350-400 mmHg vacuum pressure. Subsequently, the filters were placed in a Microcon-100 tube, and incubated in the presence of extraction buffer, Proteinase K, and beta-

mercaptoethanol (BME) at 56°C for 30 minutes. A standard phenol-chloroform / butanol organic extraction was then performed on the samples.

Quantitation, Amplification, and Gel Electrophoresis

Ethidium bromide stained 1% agarose gel and the Aluquant™ Human DNA Quantitation System (Promega, Corp., Madison, WI) were used to quantitate both the standard differential and filtration method samples. STR analysis was performed using the AmpF*l*STR® Profiler Plus™, CO*f*iler™, and Identifiler™ kits according to manufacture's protocols. Samples were amplified using GeneAmp® 9700 thermal cyclers. Amplification products were processed using ABI Prism™ 377 and 3100 DNA Sequencers and analyzed using Genescan® 3.1.2 and 3.7, Genotyper® 2.5 and 3.7, and Genemapper™ ID 3.1 software.

RNase Free versus RNase Swabs Experiment

Filter clogging was the most significant problem using the filtration procedure. Speculating that this could be the result of glycoproteins or RNA binding to clog the filter, samples were treated with DNase free RNase at 56 °C and extracted as described above. Swabs that underwent filtration without RNase demonstrated a low-level male profile ranging from ¼ to ½ of the major female profile, making it difficult to determine the complete genotype of the semen donor. However, when RNase was added to the swabs containing the same number of sperm, a full single-source male profile was routinely detected. With some samples, the RNase spiked swabs produced greater peak heights and cleaner profiles as compared to the swabs processed by the standard differential extraction method. This demonstrates that RNA released from the epithelial cells was binding to the filter, potentially trapping glycoproteins and cell debris.

Epithelial Cell/Semen Swab Experiment

Serial dilutions of epithelial cells (1 million-12,500) were added to cotton swabs in addition to 100,000 sperm, and extracted following RNase treatment but without removal of the epithelial-rich fraction after centrifugation. This was done in order to simulate swabs from sex assault cases while attempting to determine the effects of epithelial to sperm cell ratios on filtration. Only the mixture of 12.5K epithelial cells with the 100K sperm filtered effectively. With 250K (or more) epithelial cells, no filtration took place. The results demonstrate that the track-etch filters are not effective without first pelleting the sperm-rich fraction and removing the epithelial cells.

Blood/Semen Swab Experiment

Sexual assault cases may involve items containing blood-semen mixtures. For this experiment, serial dilutions of approximately 100,000 to 3,125 sperm were mixed with 250µl of female whole blood and extracted using both the standard and filtration method with RNase treatment. In all cases, the filtration samples produced the expected results with no allelic dropout or significant peak height imbalance. This also demonstrates that the addition of RNase does not affect the subsequent amplification or electrophoresis steps. In addition, the filtration profiles were concordant with those of the standard differential extraction method.

Non-Probative Casework Samples / Vaginal Swabs

Five non-probative cases with vaginal swabs of varying age and sperm counts (based on examination of vaginal smears) were processed in order to analyze the effectiveness of the filters under casework conditions. The results using track-etch filers were compared to those produced from the standard differential extraction during the original processing of the case.

The track-etch filters generally gave results comparable or superior to the original STR findings. In some cases, the filtration process removed all low-level female alleles from the sperm-rich fraction that were present in the original profiles.

Non-Probative Casework Samples / Semen Stains

Five semen stains were extracted using the filtration method and processed through gel electrophoresis. As with the vaginal swab cases, the filtered samples gave single source male profiles that matched that of the suspect. The peak heights and separation of male from female DNA of the filtered samples were equal to, or better than, the original casework profiles.

Conclusions and Future Analysis

We have shown that the ISOPORE™ track-etch filter can be effective for identifying the DNA profile of a semen donor from mixed body fluid samples. The efficiency of the separation using the track-etch filter is consistently equal or superior to the standard differential extraction procedure. Furthermore, more than two hours of hands-on bench time per experiment was saved using the track-etch filters. Automated sample processing is vital to the goal of reducing the thousands of backlogged sexual assault cases currently waiting to be processed. The problem of filter clogging has been greatly minimized by RNase treatment, but the removal of epithelial cell debris without a pelleting step must be resolved if the method is to be fully automated. The remaining issues of filter leakage and popping appear to be simple manufacturing problems that can be easily fixed.

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Background:

Forensic laboratories in the United States currently have over 500,000 unprocessed sexual assault cases. The standard method for purifying sperm from these swabs is to first resuspend all cells and to selectively digest the excess of the victim's epithelial cells with Proteinase K. The intact sperm are then separated from the epithelial DNA by centrifugation, careful removal of supernatant, and extensive washing of the sperm pellet. Subsequently, the sperm are digested with a reducing agent, such as DTT, which breaks the sperm heads, releasing its DNA into solution. This is then followed by standard phenol-chloroform extraction to purify the DNA (4). The standard differential extraction procedure has proven very effective and inexpensive, but the process is time consuming and many of the steps are difficult to automate. Given the very large backlogs facing the forensic community, the challenge is to substantively improve the selective lysis protocol. Vacuum driven filtration is possible alternative for separating sperm from digested epithelial cells that is fast, inexpensive, and more amenable to automation.

Previous Attempts to Improve Selective Lysis

A number of attempts have been made to improve on the method of isolating sperm DNA from sexual assault cases. For example, Y chromosome polymorphic markers can be amplified from unfractionated semen samples. However, this approach has limitations: the results cannot be searched against the CODIS STR database; Y-STRs do not improve the process when the rape victim is male, and males of the same paternal lineage usually have identical Y chromosome STR profiles. Another approach towards avoiding selective lysis is to physically separate sperm from intact epithelial cells. This has been done by flow cytometry (5). However, this technique is very

expensive and inherently slow due to the need to analyze and sort one cell at a time, and is unlikely to be applied to casework. Similar difficulties are associated with laser microdissection. Attempts have also been made to use anti-sperm antibody coated magnetic beads (6). Epitope stability, however, will likely be a problem with this approach when applied to casework because detergents such as Sarkosyl or SDS are required to efficiently elute sperm from the swabs, and these detergents destroy most of the epitopes recognized by the anti-sperm antibodies. Magnetic beads have been successfully used for many cell separation applications (7), but it remains to be seen if they can be used to separate human cells that have been dried onto an adsorbent substrate and then resuspended. Sperm can also be physically separated from the much larger intact epithelial cells by size using a 10 micron nylon weave filter (8). Unfortunately, the pores of these filters will expand under pressure, which mandates that only gravity be used to minimize epithelial cell contamination. In the absence of a strong driving force, capillary action on the filter surface competes with gravity flow through the filter and results in a large retention volume and difficulties with sample handling. Furthermore, DNA from epithelial cells lysed by the harsh detergent required for efficient cell re-suspension will pass through the filter along with intact sperm.

An ongoing problem with conventional filtration methods has been that of achieving complete passage of the epithelial cell debris and DNA through the filter. Standard filters consist of a chaotic mesh of fibers that generate pores of ill-defined size and can trap particles smaller than the nominal pore size. For example, a 2 micron polypropylene filter will have many pores less than 2 microns and will trap a significant amount of digested epithelial cells.

To overcome the limitations found with standard filters, track-etch filters were used for this project. Track-etch filters have precisely defined pores that are stable under pressure. The small pores of the 2 micron track etch filter are able to retain 100% of the sperm while allowing the

digested epithelial cell DNA to pass. However, some swabs have a significant amount of debris, including eluted cotton fibers that clog the 2 micron filter. In order to minimize the clogging problem, an 11 micron nylon pre-filter is placed directly on top of the 2 micron filter to keep large debris from clogging the 2 micron filter. During the filtration process, the 2 filters are used in series in a single step so that the operator (a technician or a robot) simply adds the solution to the top of the filter stack and allows a vacuum to draw the fluid through both filters at the same time. The addition of an RNase incubation step also significantly reduced filter clogging.

Project Goals

To address the large backlog of unprocessed biological evidence, the objective of this project was to evaluate the efficacy of the filtration-based method as a high-throughput tool for identifying the DNA profile of the semen donor from sexual assault evidence. We sought to develop a faster, more effective, less-labor intensive, and more cost-effective method to isolate sperm DNA from sexual assault samples to address the backlog of unprocessed biological evidence. Second, we compared our novel filtration based method to the standard differential extraction protocol in use at the Connecticut Forensic Science Laboratory on mock samples and non-probative casework.

We have evaluated a modified filtration-based method to isolate sperm from digested epithelial cells that could potentially overcome the problem of automating DNA sample preparation for sexual assault cases. The standard differential extraction protocol requires that intact sperm be centrifuged and the sperm pellet washed extensively in order to remove the victim's DNA after selective digestion of epithelial cells. Commercially available robotic systems have difficulty performing required operations such as centrifugation and supernatant removal. Therefore, the standard method for processing sexual assault cases is difficult to automate. With this problem in mind, we evaluated a combination of filters that collect intact sperm and allow digested epithelial cell DNA to pass

through when driven by vacuum. The filters are first treated with a blocking agent to eliminate binding of epithelial cell DNA and then placed in an empty Microcon-100 casing. The victim's DNA can be collected in the filtrate. Subsequently, the filter is washed extensively. Sperm DNA is captured on the filter and then released by digesting the sperm *in-situ* with a reducing agent and collected by vacuum. With our modified approach, sperm DNA yields are comparable to or better than yields obtained using the standard method. However, the filter clogging problem has not been fully resolved.

Samples

Semen from healthy male volunteers was collected and an average number of sperm per microliter of semen was determined by use of a hemocytometer. Buccal swabs, provided by healthy female donors were collected in triplicate. Approximately 100,000 sperm were added to the swabs which were extracted using the filtration method both with and without RNase treatment (see "Addition of RNase" section below). The sensitivity of the method and the efficiency of separating male from female DNA using the filters were compared to the standard differential method. Known dilutions of semen, ranging from about 100,000 to 3,125 sperm, were also added to both buccal and blood saturated swabs from known female contributors, extracted using the RNase filtration protocol, and compared to the results of the standard differential method. In addition, 10 sexual assault evidentiary samples (5 vaginal swabs and 5 cuttings from semen stains) from nine previously adjudicated, non-probative cases were collected and used to demonstrate the efficiency of the track-etch filtration method as compared to standard differential extraction.

ISOPORE™ Track-etch Filter

The ISOPORE™ track-etch filter is a stacked double membrane filter consisting of a single 11-micron nylon net filter placed on top of a 2-micron track-etch filter. The track-etch filter's distinctly

defined and rigid pore size was designed to allow epithelial cell DNA to pass through while trapping sperm. Unlike standard nitrocellulose filters, whose pore size varies from filter to filter, the track-etch filters were created by first subjecting a polycarbonate membrane to high-energy radiation and then, while soaking in an acid bath, distinct circular pores of well-defined size were etched through the filter (Fig. 1). The track-etch filters were then treated with a 10X BackGround Quencher (MRC, Inc., Cincinnati, OH) for 30 minutes, to inhibit the binding of free DNA and placed within an empty Microcon-100 casing.

Extraction Methods

For comparison purposes, standard differential extraction was performed on all known swabs in parallel with the filtration method. Standard differential extraction was performed per Connecticut lab protocol by first incubating the above described swabs in a 450µl extraction buffer (10mM Tris, pH 7.5-10mM EDTA-50mM NaCl-2% SDS) and 15µl Proteinase K (20mg/ml) solution, at 56^{o}C for 1 hour. The swabs were then placed in SPIN-EASE™ baskets and centrifuged at maximum speed for 5 minutes. All but 50µl of the supernatant was removed without disturbing the pellet, and placed in a tube labeled "A" fraction. The swab was then washed 2-3 more times in the SPIN-EASE™ basket with approximately 400µl of dH_20. The pellet was resuspended and washed an additional 4-5 times with 400µl of dH_20 in order to remove any residual female DNA from the sperm pellet. 450µl of extraction buffer, 15µl of Proteinase K (20mg/ml), and 20µl of DTT (36mM) was then added to the pellet and incubated at 56^{o}C for 4-18 hours (labeled "B" fraction). A standard phenol-chloroform / butanol organic extraction was then performed on both "A" and "B" fractions.

Addition of RNase

The filtration extraction protocol was slightly altered after the preliminary results were presented at the 2004 NIJ Grantees Meeting. The swabs/stain cuttings were incubated at 56°C for 1-2 hours in the above described extraction buffer / Proteinase K solution along with 10µl of DNase free RNase (100mg/ml). The swabs were spun down, the "A" fraction removed, and a single 500µl dH_20 wash was performed on the swab/cutting. The pellet was then resuspended and washed twice with 500µl of dH_20. The pellet was then resuspended into 500µl of extraction buffer and filtered through the track-etch filter at 350-400 mmHg vacuum pressure. Following filtration, the filter was vacuum washed with 1ml of extraction buffer. The filter was then removed, placed within a Microcon-100 tube, and a solution of 100µl of extraction buffer, 15µl of Proteinase K, and 10µl of stock beta-mercaptoethanol (BME) added to it. The filter was incubated at 56°C for 30 minutes. 400µl of extraction buffer was then added to the filter, spun at 5000 rpm's for 30 seconds, and the filter discarded. A standard phenol-chloroform / butanol organic extraction was then performed on both "A" and "B" fraction (Fig. 2).

Quantitation, Amplification, and Gel Electrophoresis

Ethidium bromide stained 1% agarose gels and the Aluquant™ Human DNA Quantitation System (Promega, Corp., Madison, WI) were used to quantitate both the standard differential and filtration method samples. Approximately 1ng of template was used for each amplification. STR analysis was performed using the AmpF*l*STR® Profiler Plus™, CO*f*iler™, and Identifiler™ kits according to manufacture's protocols and samples were amplified in a GeneAmp® PCR System 9700 (Applied Biosystems Inc., Foster City, CA). For comparative analysis, amplification products were separated on both the ABI Prism™ 377 and 3100 DNA Sequencers and analyzed using

Genescan® 3.1.2 and 3.7, Genotyper® 2.5 and 3.7, and Genemapper™ ID 3.1 software (Applied Biosystems Inc.).

Results & Discussion

RNase Free versus RNase Swabs Experiment

The recurring problem of filter clogging was the most significant issue during the initial study. Speculating that this problem could be due to either glycoproteins or RNA binding to clog the filter, the samples were treated with DNase free RNase at 56°C and extracted as described above. The swabs that underwent filtration without RNase demonstrated a low-level male profile ranging from ¼ to ½ of the major female profile, making it difficult to determine the genotype of the semen donor. However, when RNase was added to the extraction of swabs containing the same number of sperm, a full single-source male profile was routinely detected (Fig. 3). In some cases, the RNase-spiked swabs produced greater peak heights and cleaner profiles as compared to the swabs processed by the standard differential extraction method. This demonstrates that RNA released from the epithelial cells was binding to the filter, potentially trapping glycoproteins and cell debris.

Epithelial Cell/Semen Swab Experiment

100,000 sperm were mixed with serial dilutions of epithelial cells (1 million-12,500) on cotton swabs and extracted following RNase treatment but without removal of the epithelial-rich fraction after centrifugation. This was done in order to simulate swabs from sex assault cases while attempting to determine the effects of epithelial to sperm cell ratios on filtration. Only the mixture of 12.5K epithelial cells with the 100K sperm filtered effectively. With 250K (or more) epithelial cells, no filtration took place. The results demonstrate that track-etch filters are not effective without first pelleting the sperm-rich fraction and removing the epithelial cells.

Blood/Semen Swab Experiment

In addition to epithelial cells, sexual assault cases may contain evidence in which articles of clothing or swabs have semen stains mixed with blood. For this experiment, serials dilution of sperm (100,000-3,125) were mixed with 250µl of female whole blood and extracted using both the standard and filtration method with RNase treatment. In all cases, the filtration samples produced the expected results, with no allelic dropout or significant peak height imbalance. This also demonstrates that the addition of RNase does not affect the subsequent amplification or electrophoresis steps. In addition, the filtration profiles were concordant with those of the standard differential extraction method (Fig. 4).

Non-Probative Casework Samples / Vaginal Swabs

The goal of the ISOPORE™ track-etch filtration method is to increase casework throughput. Therefore, track-etch filters were evaluated using standard field samples. Of the five non-probative cases collected from the archives at the Connecticut lab, vaginal swabs of varying age and sperm counts (based on examination of vaginal smears) were processed in order to analyze the effectiveness of the filters under casework conditions (Table 1). Following filtration extraction, amplification, and gel electrophoresis, the profiles were analyzed and compared to those produced from the standard differential extraction during the original processing of the case. In four of five cases, the filtration took between 1-5 minutes to complete. However, in cases #1 and #4, the microcons appeared to have leaked during extraction. In case #5, the filter popped out of it's housing and was manually inserted back into the microcon-100 casing before completing filtration.

The results demonstrate that track-etch filters can be effective for processing semen-containing swabs. The two samples that leaked gave no results and the filter that popped produced a low-level partial male profile that matched the suspect within the case. However, for cases #2 and #3, there

were no problems with the filtration or extraction procedure and the profiles that were produced were single source male profiles that matched the suspect in the cases (Fig. 5). Most importantly, the filtration process removed all low-level female alleles from the "B" fraction that were present within the original profiles.

Non-Probative Casework Samples / Semen Stains

As with the previous non-probative vaginal swabs, five cutting from semen stains were extracted using the filtration method and processed through gel electrophoresis (Table #2). The profiles produced from the first cutting (case #1), and the cuttings from case #2 and #4 were either very low or demonstrated no results due to the fact that the remaining samples were very small. With case #4, no semen was apparent during UV analysis because most of the stain had been consumed during the original casework processing. However, for the second cutting from case #1 (Fig. 6) and for the cutting from case #3 (Fig. 7), the results were single source male profiles that match that of the suspect. The peak heights and separation of male from female DNA of the filtered samples were equal to, or better than, the original casework profiles.

Conclusions and Future Analysis

While the track-etch filtration method holds considerable promise, we have not been able to overcome filter clogging without the initial centrifugation step to pellet sperm cells followed by removal of the epithelial-rich fraction. Commercial production of the filters (in single-use and microtiter plate format) is also essential. The time to assemble the filter units manually is prohibitive and the forensic community is unlikely to adopt their use without commercial production.

We have shown that the ISOPORE™ track-etch filter can be effective for identifying the DNA profile of the semen donor from mixed body fluid samples. The efficiency of the separation using the track-etch filter is consistently equal or superior to the standard differential extraction procedure.

Furthermore, more than two hours of hands-on bench time per experiment was saved using the track-etch filters. While the problem of filter clogging has been greatly minimized by RNase treatment, removal of epithelial cell debris without a pelleting step must be resolved if the method is to be fully automated. Automated sample processing is vital to the goal of reducing the thousands of backlogged sexual assault cases currently waiting to be tested. The remaining issues of filter leakage and popping appear to be simple manufacturing problems that can be easily fixed.

Acknowledgments
We would like to thank Dr. Linda Strausbaugh (Center for Applied Genomics and Technology, University of Connecticut) and Todd Arnold (CUNO Inc.) for their valuable assistance in this project. In addition, we would like to thank the National Institute of Justice for funding this research.

Dissemination of Information

1. 2005 AAFS Annual Meeting, Poster Presentation.
2. NIJ Annual Grantees Meetings.
3. Manuscript for Journal of Forensic Science.

Table 1

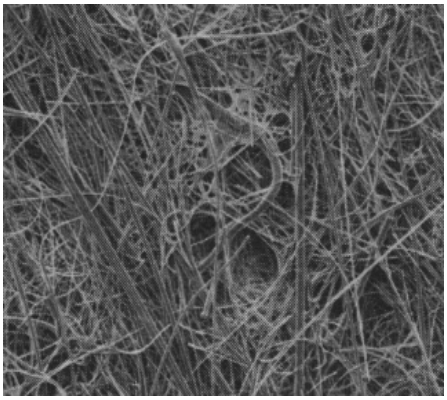
Year of Incident	Submitted Evidence	Designated Sperm Rating
1999	#2-2A-S2 (Cutting from sock)	?
1999	#2-2B-S2 (Cutting from sock)	Weak AP
2000	#2-2S2 (Stain from shirt)	2+
2000	#2-2R1 (Stain from towel)	1+ - 2+
2001	#3-3S1 (Cutting from crotch of panties)	3+

Table 2

Year of Incident	Submitted Evidence	Designated Sperm Rating
2002	#1C-3 (Vaginal Swab)	?
1999	#4C-1-3 (Vaginal Swab)	Weak AP
2001	#1C-3 (Vaginal Swab)	2+
2001	#1C-3 (Vaginal Swab)	1+ - 2+
2002	#1C-3 (Vaginal Swab)	3+

Figure 1

2 Micron Nitrocellulose



2 Micron Track-Etch

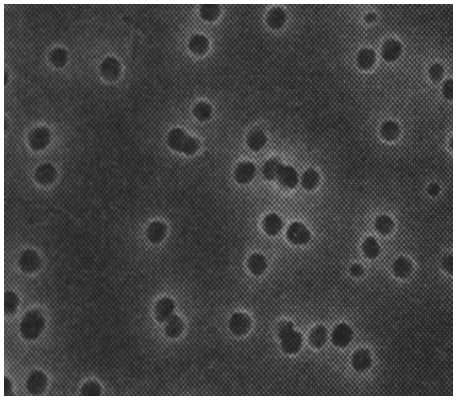
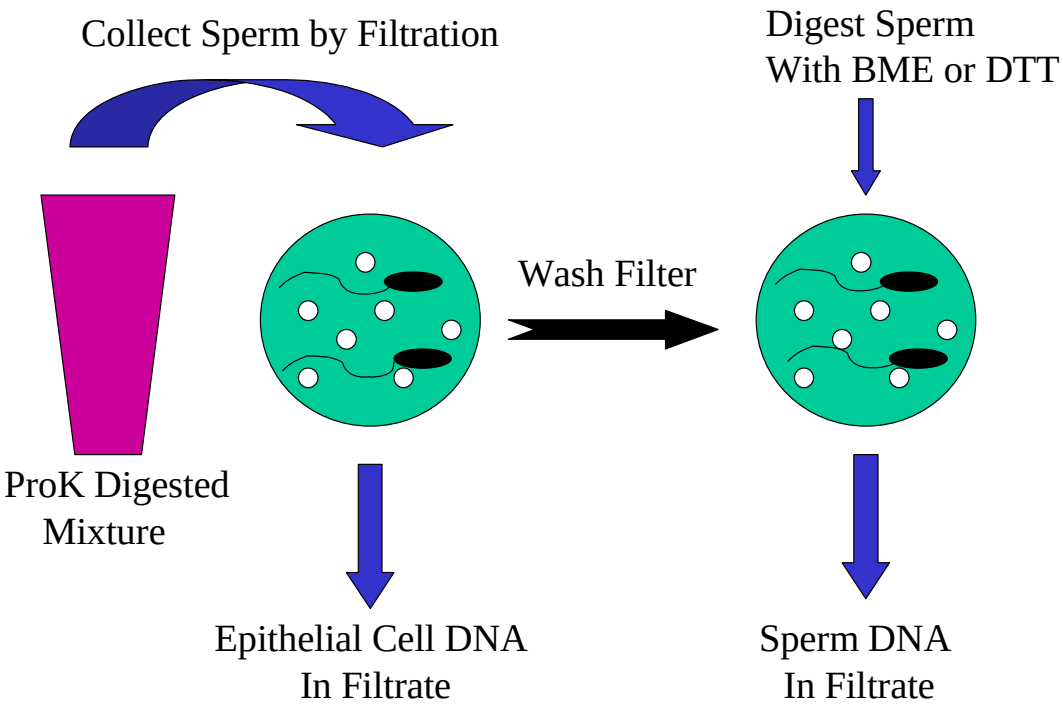


Figure 2



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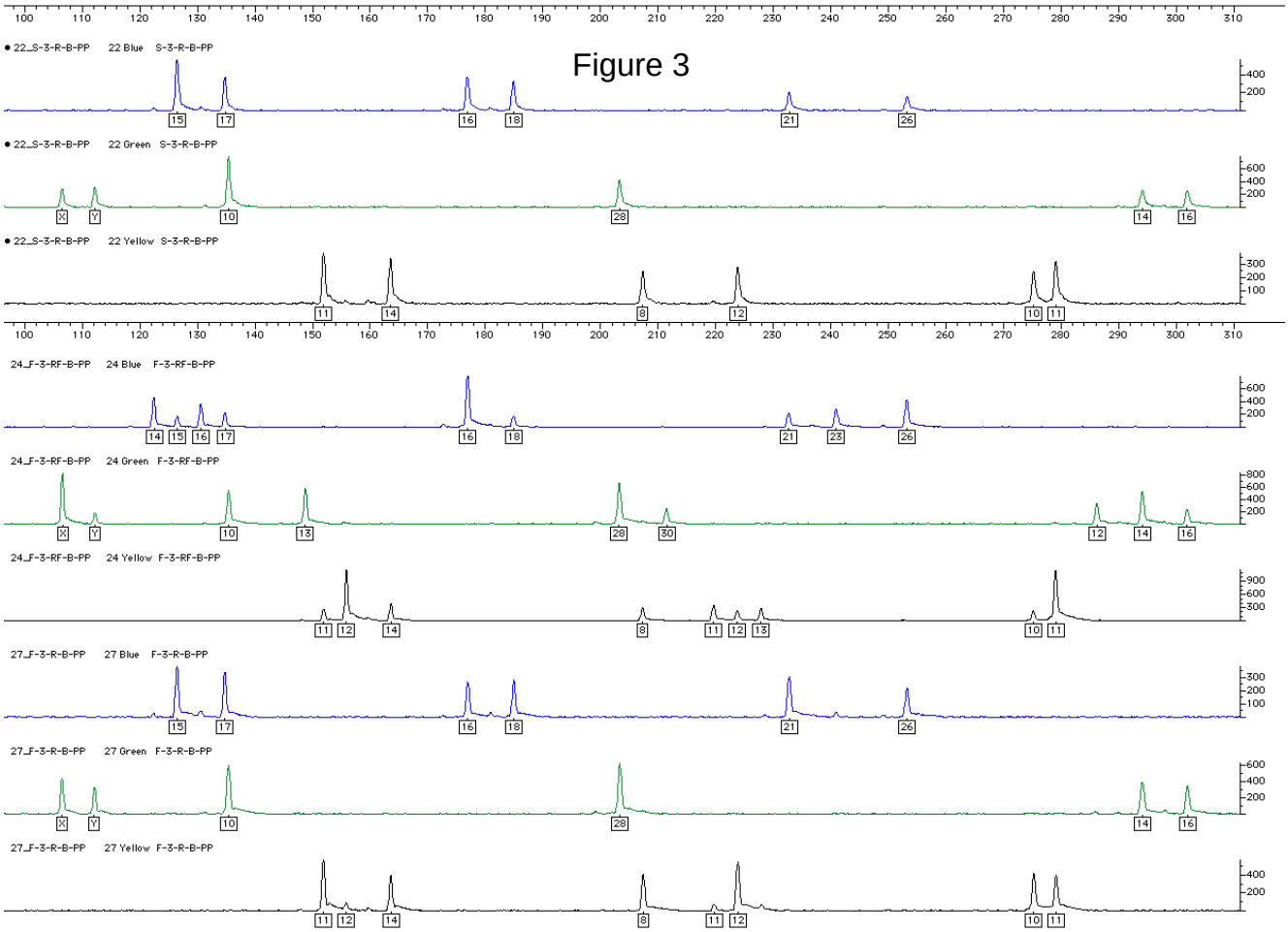

Figure 4

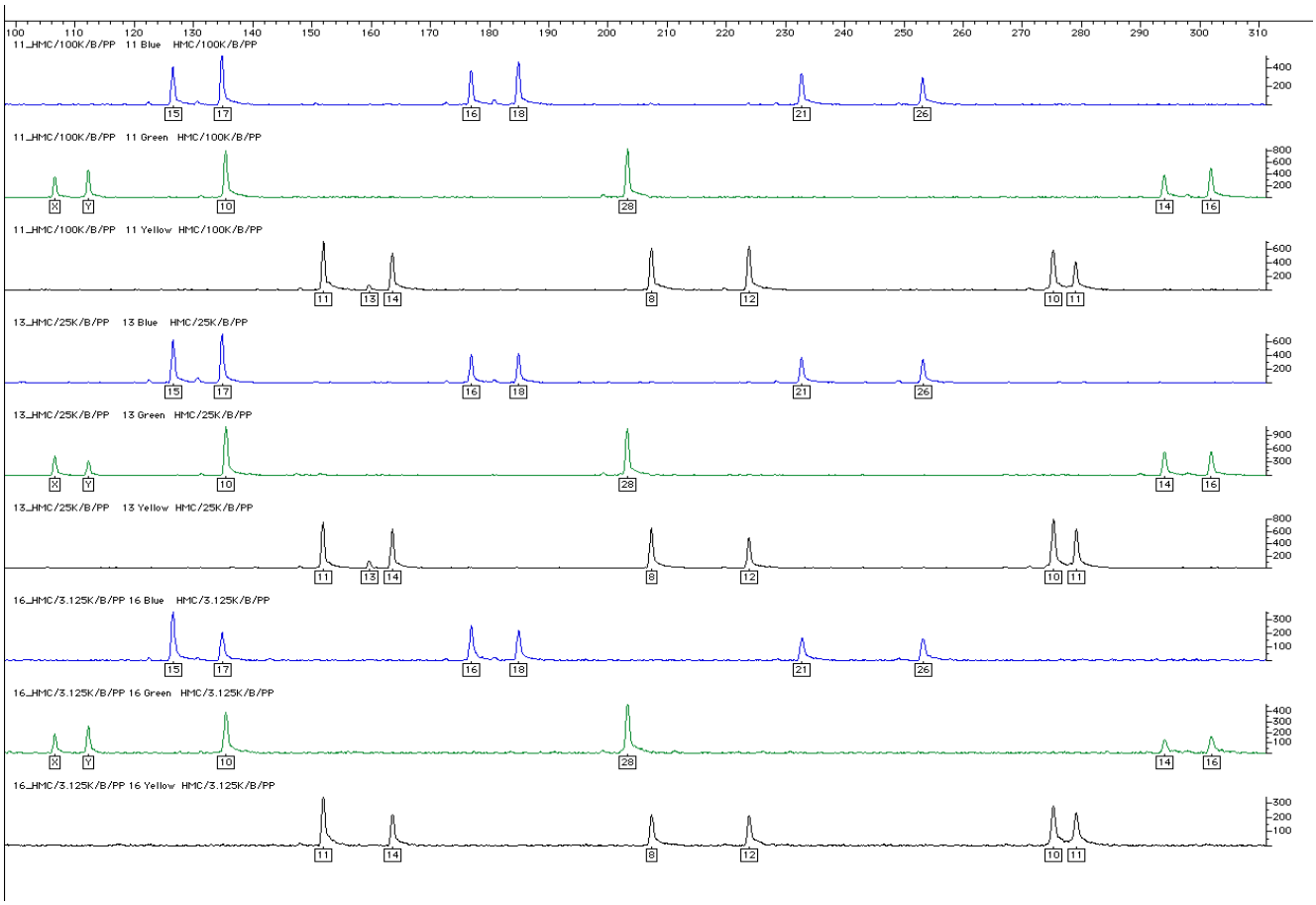

Figure 5

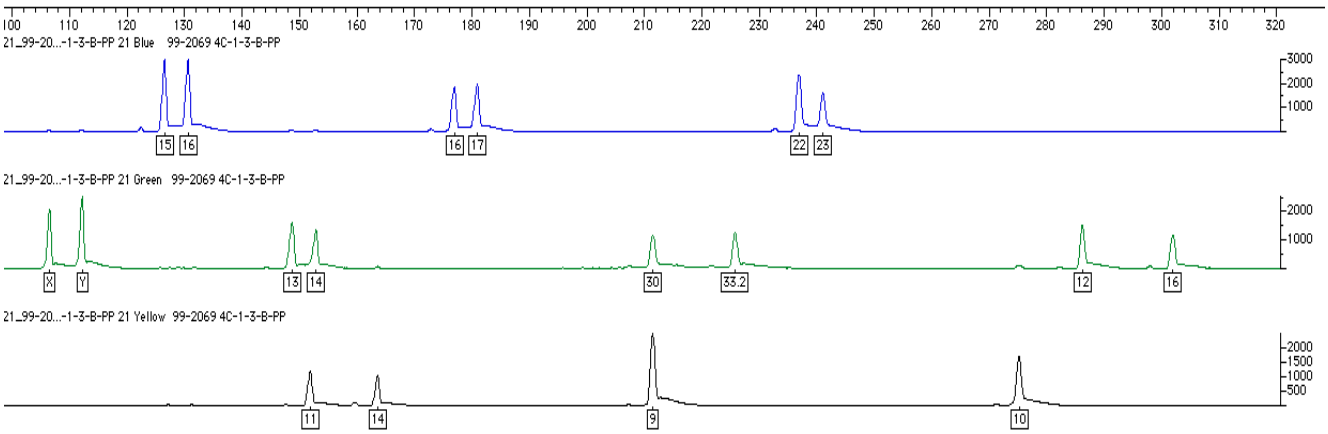

Figure 6

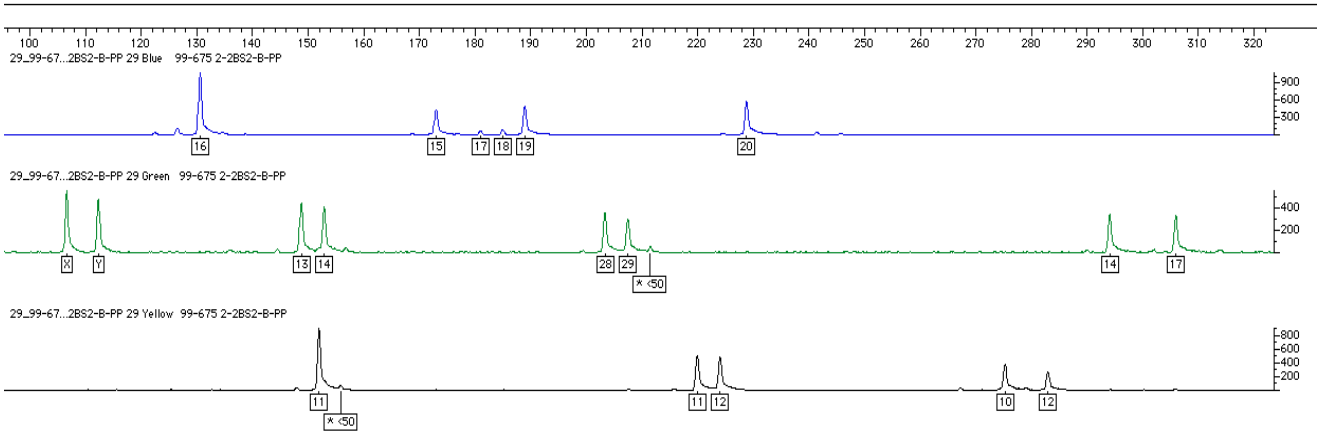

Figure 7

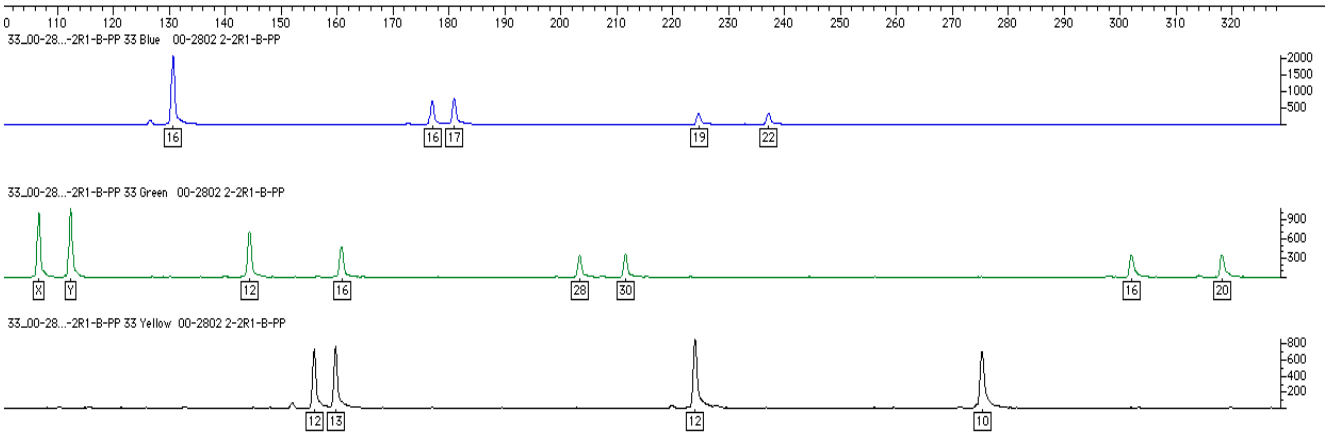

Figure Legends

Fig. 1
Comparative microscopic analysis of the mesh-like nitrocelloulose membrane and the ISOPORE™ track-etch filter with its fixed pore size.

Fig. 2
Diagram of the sperm filtration process. Sperm is trapped on the 2 micron ISOPORE™ track-etch filter while epithelial cell DNA is filtered through. The sperm is then digested with a reducing agent before being filtered and collected.

Fig. 3
Standard versus filtration extraction with and without the addition of RNase. The top profile (#22) is that of the standard differential extraction method. The middle profile (#24) represents the filtration process without the addition of RNase. The bottom profile (#27) consists of the same male and female contributors as the two previous profiles; however, RNase was added to the filtration method for this sample.

Fig. 4
Profiles of swabs processed with the RNase filtration method. Full profiles, matching that of the sperm donor, were produced from swabs ranging from about 100,000 to 3,125 sperm each mixed with 250µl of female whole blood (lane #11 = 100K sperm, lane #13 = 25K sperm, lane #16Z = 3.125K sperm).

Fig. 5
DNA profile of vaginal swab from a 1999 adjudicated sexual assault case. Using the RNase filtration method, a single-source male profile, matching that originally analyzing in the case, was produced. For this sample, low-level alleles matching that of the victim, which were in the original profile, were now eliminated using the track-etch filtration method.

Fig. 6
STR profile generated from a cutting of a semen stain on a sock from a 1996 adjudicated sexual assault case. Using the RNase filtration method a male profile was analyzed, matching that of the original profile generated in the case. Again, the filtration method was able to “clean-up” the “B” fraction by eliminating some of the victim’s alleles apparent during the original processing of the case.

Fig. 7

STR profile from a cutting from a semen stain on a towel from a 2000 adjudicated sexual assault case. The RNase filtration method was implemented and the resulting profile matches that of the suspect and is equal in quality to the original profile analyzed in the case.

Table 1
Summary table of non-probative vaginal swab casework samples from sexual assault cases processed with the sperm filtration extraction method. Designated sperm rating was determined by estimating sperm counts from smears made from the submitted evidence by serologists during the original evidence examination. Sperm rating is from greatest (4+) to least (1+), with "Weak AP" designating the absence of sperm on the smears but testing weakly for the presence of acid phosphatase.

Table 2
Summary table of non-probative semen stain cuttings from sexual assault casework samples processed with the sperm filtration extraction method. Designated sperm rating was determined by estimating sperm counts from smears made from the submitted evidence by serologists during the original evidence examination and the sperm rating is designated as described within table 1.

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