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TECHNICAL NOTE

CRIMINALISTICS

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Automation of the Differential Digestion Process of Sexual Assault Evidence*,†

ABSTRACT: Sexual assault evidence samples require the use of a specific process known as a differential digestion to separate sperm from nonsperm cells prior to DNA extraction. An automated differential digestion process was developed using a selective degradation technique, which uses DNase I to digest the remaining nonsperm DNA in the sperm fraction. The use of DNase on pristine samples, as well as aged and degraded samples, was assessed to ensure that the quantity and quality of the sperm DNA were not compromised or adversely affected. Samples processed using the selective degradation technique yielded comparable DNA yield and DNA typing data to the conventional differential digestion process. The automated process utilized 96-well plates for high throughput and incorporated microscope slide preparations for confirmation of sperm. It reduced processing time by about sixfold and was paramount in the elimination of the Oakland Police Department Criminalistics Laboratory's sexual assault kit backlog.

KEYWORDS: forensic science, DNA analysis, differential digestion, differential extraction, selective degradation, DNase, automation, robotics, VERSA 1100, sexual assault evidence kit

Many forensic laboratories are faced with an increasing demand for analysis of sexual assault evidence. The time-consuming and labor intensive work that is required for DNA analysis, combined with an insufficient number of analysts to meet annual case requests in many laboratories, quickly result in an overwhelming backlog. This high demand is leading laboratories to transition to automation as a solution for processing a large number of cases in a highly efficient manner. Although many areas of DNA analysis have adopted automation (1–3), the process which separates sperm DNA from nonsperm DNA in sexual assault evidence samples remains a time-consuming manual task.

Evidence items from sexual assault cases often involve mixtures of DNA from the victim and another male. To effectively interpret the DNA profiles obtained from evidence items, the victim's nonsperm cellular material, which is often predominantly epithelial cells, must be separated from the male donor's sperm cells in a process called the differential digestion. First introduced in 1985 (4), the differential digestion process takes advantage of the presence of disulfide bonds which protect the sperm heads, making them resistant to lysis by sodium dodecyl sulfate (SDS) and proteinase K (pro K). Nonsperm cells are preferentially lysed by SDS and pro K while the sperm heads remain intact. The supernatant, which contains the lysed epithelial cells and other nonsperm cellular material, is removed into a separate tube; this is termed the nonsperm fraction. The remaining pelleted material termed the sperm fraction, which contains the intact sperm heads, is cleansed of any residual nonsperm cell DNA by a series of wash and centrifugation steps. Finally, the sperm heads are lysed by addition of SDS, pro K, and dithiothreitol (DTT), which disrupts the disulfide bonds protecting the sperm cell membrane. The differential digestion is an effective process for separation of DNA from sperm cells and DNA from nonsperm cells. However, it is a time-consuming and laborious manual process, and complete separation of the nonsperm DNA from the sperm cell DNA is not always achieved.

Slight modifications have been made to the differential digestion since its introduction (5–9); however, the process has remained relatively unchanged. A technique termed "selective degradation" has been proposed as a modification to the differential digestion process (10). This approach uses a degradative enzyme to degrade any residual nonsperm cell DNA remaining in the sperm fraction after lysis; the unlysed sperm cells remain unaffected by this enzyme. Selective degradation eliminates the need for multiple centrifugation and wash steps, making this process amenable to automation (10). Other versions of the selective degradation process have been introduced (11). In this article, DNase I was utilized as the degradative enzyme.

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The first portion of this study focuses on optimization of the selective degradation differential digestion process. The areas that were evaluated include selection of a nonionic detergent, concentrations of DNase, $CaCl_2$, and $MgCl_2$, and DNase inactivation. The emphasis on this study is to examine the use of DNase on forensic samples for its effects on DNA yield and STR DNA typing. In addition, the effect of DNase on aged and environmentally compromised samples is also examined. The final portion of this study focuses on the transition of the optimized selective degradation differential digestion process to an automated platform, how this automated process was implemented into casework, and the impact of the automated differential digestion on casework.

Materials and Methods

Samples

All semen and vaginal swab samples were voluntarily donated. Sperm samples were prepared by removing the seminal fluid after centrifugation and reconstituting the remaining sperm cells in phosphate-buffered saline (PBS). Epithelial cells from sperm-free vaginal swabs were recovered by soaking the swabs in PBS and then removing the swabs from the solution. This allowed for consistent aliquots of cells for sample comparison. The DNA concentration of the sperm and epithelial cell samples was determined by DNA quantitation using real-time PCR. Neat epithelial cell samples were used in all experiments, while dilutions of the neat sperm samples were made to target the desired concentrations for various experiments.

Mock sexual assault samples were prepared by combining 15 μL of each of the prepared sperm and epithelial cell samples. Initial manual experiments were performed using liquid samples to eliminate the variability factor of cells sticking onto swabs. After achieving optimal parameters, the process was transitioned from a manual single-tube format to an automated 96-well plate format. Samples were prepared in the same way as the manual experiments with the exception of using sterile cotton swabs as the substrate for sperm and epithelial cells.

Eleven semen stains spotted onto fabric swatches between the years of 1952 and 1991 were obtained from Forensic Analytical Specialties, Inc (Hayward, CA). Six of the stains had been stored at room temperature while the other four had been stored frozen at −20°C.

Environmentally compromised samples were simulated by subjecting sterile cotton swabs containing epithelial cells and sperm cells through ten freeze/thaw cycles. Another set of swabs were kept in a humidity chamber for five days at 75°C and 80% relative humidity.

Samples that were processed on the automated platform using the VERSA 1100 liquid handler (Aurora Biomed, Vancouver, B.C., Canada) included replicate mock sexual assault samples (comprised of liquid cell preparation samples and swab samples), aged semen stains, and environmentally comprised samples.

Reagent Selection and Optimization Experiments

The following nonionic detergents were evaluated for compatibility with the selective degradation differential digestion process: Triton X-100, Tween 80, and IGEPAL CA-630. Multiple experiments were conducted with varying concentrations of DNase, $CaCl_2$, and $MgCl_2$ to optimize the protocol to achieve clean separation of sperm and nonsperm DNA, high DNA yield, and good quality STR DNA typing data. The following concentrations were evaluated:

Reagent	Concentrations
DNase	1, 2, 5, 10, 15, 18, 360 Kunitz units (U)
$CaCl_2$	1, 2, 3, 4, 5, 10, 25, 125 mM
$MgCl_2$	10, 25, 45, 90, 180 mM

Microscopic Examination

Microscope slides were prepared on an eight-well microscope slide (Fisher Scientific, Hampton, NH) prior to and after the initial nonsperm digestion step. Aliquots of the samples, consisting of re-suspended cellular material, were stained with the nuclear fast red and picroindigocarmine dyes (Christmas Tree stain) (12) (Serological Research Institute, Richmond, CA) and examined with brightfield microscopy. The expected concentration of sperm-derived male DNA per sample was calculated using 10% of the total sample for microscopic examination, assuming 80 fields of view at 400x magnification, eluting into a final DNA extract volume of 50 μL, and expecting ~3.5 pg of DNA per haploid sperm cell.

Biological Material Digestion

Biological material was removed from the substrates by cutting a portion of the fabric or swabs and placing them into either microcentrifuge tubes or 96-well plates. The samples were digested with 510 μL of Tween 80 buffer solution (20 mM Tris–HCl, 1 mM EDTA, 2% Tween) and 10 μL of pro K (20 mg/mL) and incubated at 56°C for 30 min using a dry block heating and shaking incubator (Eppendorf Thermomixer® R). Following incubation, the samples were centrifuged for 3 min at 2000 × *g*. The supernatant containing lysed epithelial cell material was removed and transferred to a separate tube, now called the nonsperm fraction. DNase digestion of the remaining sperm fraction was completed with 290 μL of the Tween 80 buffer solution, 25 μL of a solution containing $CaCl_2$ (5 mM) and $MgCl_2$ (90 mM), and 15 μL of DNase I solution (1U/μL DNase I, 40% glycerol, brought up to volume with DEPC treated water) and incubated at 56°C for 15 min to destroy any residual nonsperm DNA. The DNase was inactivated with 20 μL of EDTA (0.5 M) for 10 min at room temperature. The sperm fractions were then digested with an additional 10 μL of pro K (20 mg/mL) and 20 μL of DTT (1 M) and incubated at 56°C for 15 min.

DNA Extraction and Quantitation

The nonsperm and sperm cell fractions were extracted on the EZ1 Advanced XL BioRobot® (Qiagen, Hilden, Germany) using the Investigator Kit Large Volume Protocol which utilizes a silica bead-based extraction method. DNA extracts were quantitated using the Plexor® HY kit (Promega, Madison, WI) on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) and were analyzed using the Plexor Analysis Software v1.5.4.18. The Plexor® HY Kit provides simultaneous quantitation of autosomal (total human) and Y-chromosomal (male) DNA (13). Sperm fraction DNA concentrations were evaluated based on the male DNA yield. The sperm fraction autosomal DNA yield was not used because nonsperm DNA carryover may be present, which would provide a less accurate reflection of the recovered sperm cell DNA.

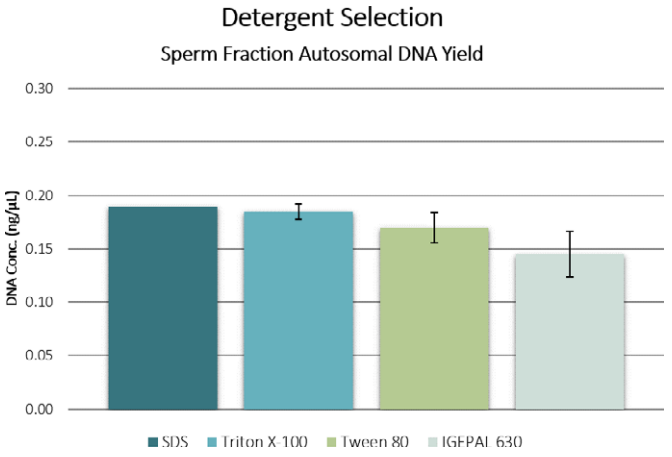

FIG. 1—*Autosomal DNA yield of the sperm cell fraction of samples digested with SDS and the three nonionic detergents. The control sample (SDS) has a sample value of* n = *1.*

DNA Amplification and STR Genotyping

Samples were normalized to a concentration of 0.15 ng/μL of total human DNA in 10 μL and were amplified using the AmpF*l*STR® Identifiler® Plus PCR Amplification Kit on a GeneAmp® 9700 PCR thermal cycler (Applied Biosystems) for 28 cycles. PCR products were separated by capillary electrophoresis using an ABI® 3130 Genetic Analyzer (Applied Biosystems) using 5-sec injections. STR DNA typing data were analyzed using GeneMapper® ID 3.2.1 software (Applied Biosystems) with a 50 RFU analytical threshold and 150 RFU stochastic threshold. The acceptable peak height ratio was 60% based on internal laboratory validation study across all loci.

Results and Discussion

Validation Study

The selective degradation technique as outlined by Garvin (10) was tested to evaluate the process of using DNase to eliminate residual nonsperm DNA and to determine whether it would be amenable to automation. The addition of DNase to the differential digestion process required changing the detergent in the lysis buffer from SDS (ionic detergent) to a nonionic detergent. The sodium ions in SDS inhibit DNase by competing with divalent ions (such as Mg^{2+} and Ca^{2+}) for a site that either directly or indirectly affects the active site on DNase (14–17). Three nonionic detergents, Triton X-100, Tween 80, and IGEPAL CA 630, were selected and evaluated for the following criteria: compatibility with DNase, cell lysis efficiency as compared to SDS, and compatibility with microscope slide sample preparation steps. Replicate samples containing both epithelial and sperm cells were differentially digested with the three nonionic detergents and with SDS. Microscopic examination of postepithelial cell digestion cell pellets confirmed the absence of intact epithelial cells and the presence of the sperm cells in all samples. All three nonionic detergents exhibited lysis ability that was comparable to SDS based on DNA yield (Fig. 1) and were compatible with DNase as demonstrated by clean, single source DNA profiles in the sperm fraction. Only Tween 80 was able to heat dry on microscope slides, thus it was selected as the detergent for the lysis buffer in this process.

The initial experiments of the selective degradation technique using reagent concentrations published in the Garvin paper resulted in lower DNA yield and poorer STR DNA typing data in the sperm fractions as compared to the conventional differential digestion. Only 30% of the male DNA from the sperm fraction was recovered compared to the same sample digested with the conventional differential digestion process. STR DNA typing data of samples amplified with 1.5 ng of DNA resulted in low peak heights between 100 and 900 RFU and significant peak height ratio imbalances (Fig. 2). The only difference in reagents between the conventional and selective degradation differential digestions was DNase, $CaCl_2$, $MgCl_2$, and Tween 80. Potential degradation of DNA may be caused by DNase. The activity of DNase is directly affected by the Ca^{2+} and Mg^{2+} divalent cation activators (14,16,18,19). Therefore, varying concentrations of DNase, $CaCl_2$, and $MgCl_2$ were tested to improve the DNA yield and DNA typing results. Decreasing the $CaCl_2$ concentrations resulted in an increase in the sperm fraction DNA yield (Fig. 3). Similarly, decreasing the DNase concentration led to significantly improved STR DNA typing data (Fig. 4). It was observed that the ratio of $MgCl_2$ to DNase concentrations affected the activity of DNase and its ability to eliminate all residual nonsperm DNA. Decreasing the DNase concentration required increasing the $MgCl_2$ concentration to maintain single source DNA profiles in the sperm fraction (Fig. 5). Optimization of the reagent concentrations for the selective degradation differential digestion process resulted in sample DNA yield and STR DNA typing comparable to the conventional differential digestion samples (Fig. 6).

Inactivation of DNase prior to sperm cell lysis is important to prevent the loss of sperm cell DNA. DNase can be inactivated by the addition of reducing agents, SDS, or metal chelators such as EDTA (20,21). DNase may also be heat inactivated at temperatures above 65°C for 5–10 min (22). To determine the best and simplest method for complete DNase inactivation, experiments were performed where EDTA was added to the sperm fractions of replicate samples after the DNase digestion step. Half of the samples were left at room temperature, and the other half was incubated at 65°C. Results showed no difference in the DNA yield or STR DNA typing data quality between DNase inactivation with or without heat. DNase inactivation by EDTA was confirmed by adding the same amount of extracted DNA to a blank sample that has been subjected to the selective degradation process up until after EDTA was added and to a sample containing only TE^{-4}. Quantitation results showed no statistical difference in the DNA yield, indicating that the DNase in the blank sample was completely inactivated prior to the addition of the DNA extract. As the activity of DNase is directly dependent on the presence of Mg^{2+} and Ca^{2+} divalent ions, removal of these divalent ion activators by the addition of EDTA was selected as the DNase inactivation method.

Forensic samples are often exposed to environmental conditions such as extreme temperature shifts, light, heat, and humidity that could potentially lead to degradation of the DNA. The age of the samples may also affect the quality and condition of the cells and DNA. As a result, aged semen stains dating back to 1952–1991, sperm samples subjected to multiple freeze/thaw cycles, and sperm samples subjected to 75°C with 80% humidity were studied to determine whether physically compromised sperm samples would be lysed prior to the addition of DTT, resulting in loss of sperm cell DNA due to destruction by DNase. Samples were subjected to both the conventional and the selective degradation differential digestion processes, and the

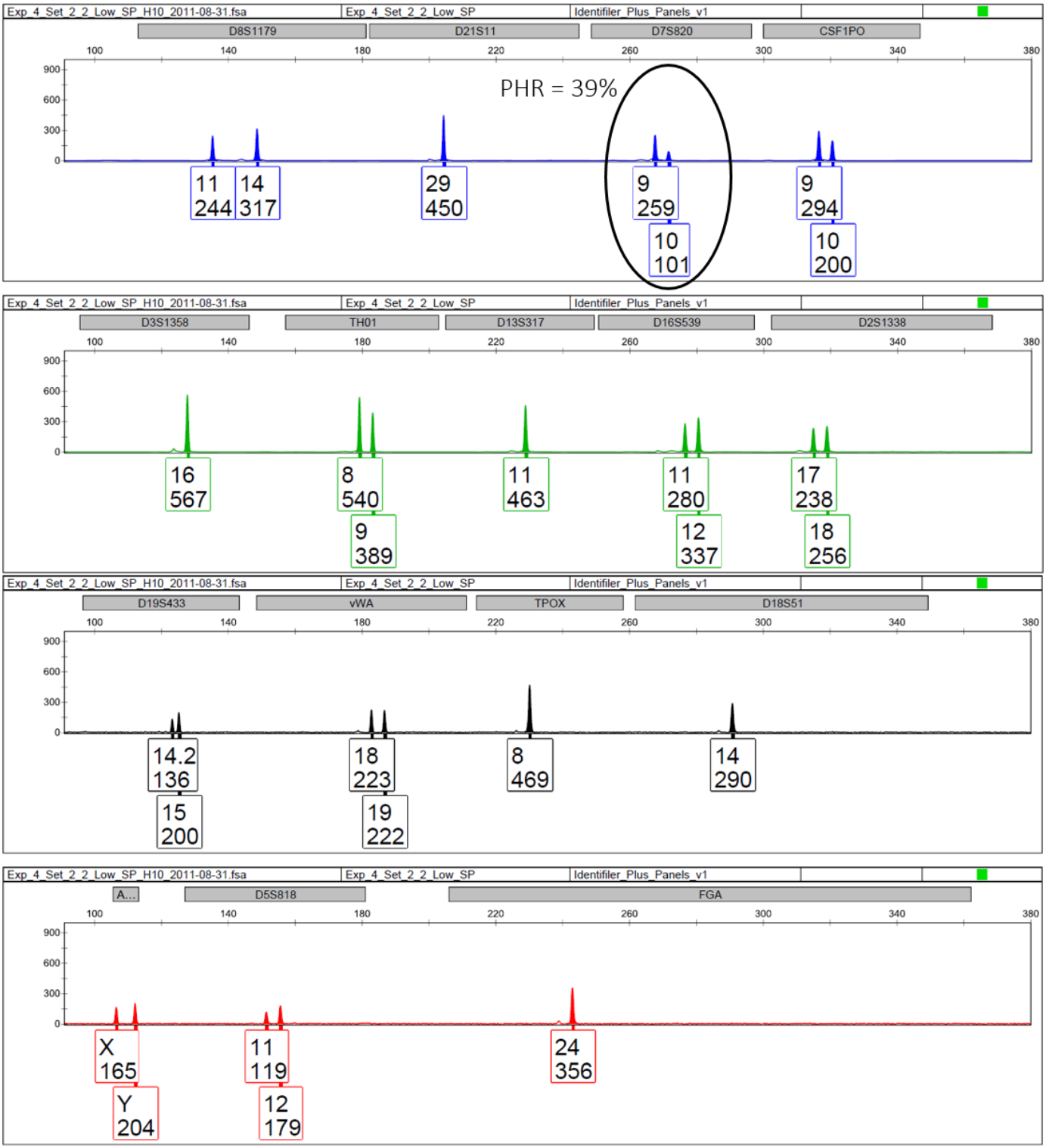

FIG. 2—*Electropherogram of a sample prepared with the selective degradation differential digestion process using the initial reagent concentrations outlined in the Garvin et al. publication: 360 units of DNase, 10 mM $MgCl_2$, and 5 mM $CaCl_2$. Data are scaled to 1000 RFU. The peak heights in this profile are low at about 100–500 RFUs, with significant peak height imbalances at some loci.*

DNA typing results were evaluated to compare the results of each method. No degradation was observed in the samples subjected to multiple freeze/thaw cycles, whereas complete degradation was observed in the samples subjected to heat and humidity. The amount of degradation varied in the aged semen stains depending on storage condition. Stains stored in frozen condition yielded full or close to full DNA profiles, while stains stored at room temperature showed significant signs of degradation with many loci dropping out. However, regardless of whether the samples were degraded, no difference in DNA yield

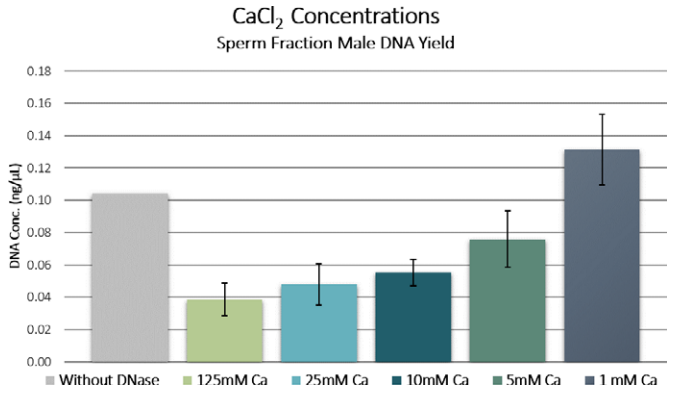

FIG. 3—*Male DNA yield of the sperm cell fraction of samples with varying CaCl2 concentrations. A decrease in CaCl2 concentration resulted in an increase in DNA yield. Error bars represent one standard deviation. The "Without DNase" sample represents the conventional differential digestion process. Sample value is n = 1. 125 mM was the CaCl2 concentration recommended in the Garvin et al. publication.*

or STR DNA typing data was observed in the sample sets (Fig. 7). Compromised or aged samples that resulted in low partial profiles did so regardless of whether the conventional or the selective degradation differential digestion process was used. These results indicated that environmentally compromised and aged sperm samples could withstand DNase treatment and produced similar results to samples processed by the conventional differential digestion process.

The sperm fractions for samples processed with the selective degradation technique resulted in single source DNA profiles most of the time, demonstrating the ability of DNase in eliminating residual nonsperm DNA (Fig. 8). However, DNA mixtures in the sperm fractions resulting from nonsperm DNA carryover were observed in some samples, especially those with low sperm count (Fig. 9). In general, the major sperm donor in these DNA mixtures was distinct from the minor nonsperm donor DNA carryover. These results demonstrate that DNase efficiency is not 100%. DNase efficiency could be increased with higher concentrations of DNase. However, the sperm DNA profiles were much more robust with lower concentrations of DNase. The advantages of having robust DNA profiles that were reflective of the amount of DNA detected and amplified were determined to be more important than achieving clean, single source DNA profiles, especially in samples with low sperm count.

Automation

The optimized selective degradation differential digestion protocol was applied to robotics in order to transition to automation. Automation of the selective degradation differential digestion protocol employed the VERSA 1100 liquid handler (Aurora Biomed) (Fig. 10) and SlicPrep™ 96-well plates (Promega) (23). The VERSA 1100 has 15 deck positions and includes an on-deck heating and shaking unit used for incubation and swab agitation steps and a gripper for moving the plate from one position to another (Fig. 11). A reagent drop system enables fast and easy liquid dispensing without the use of pipette tips. A four-

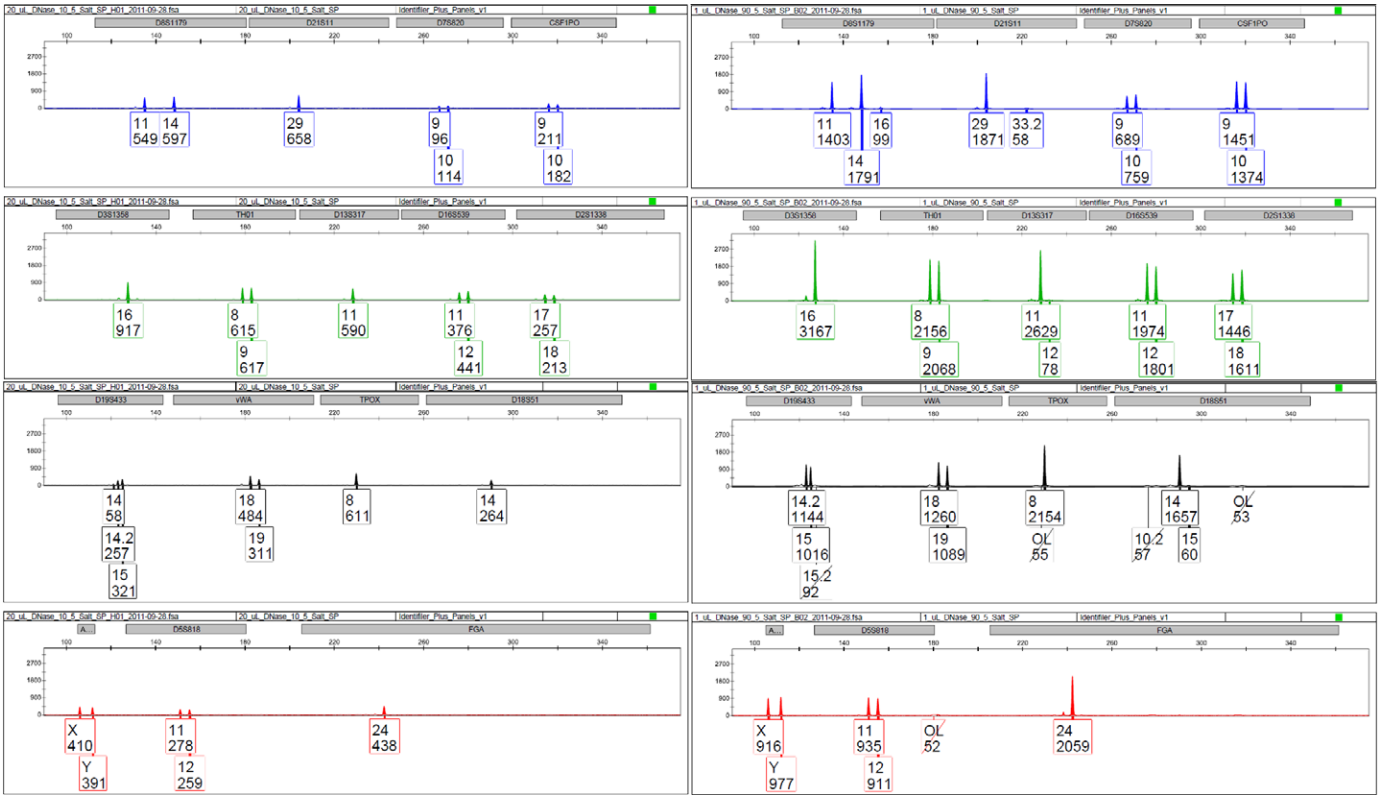

FIG. 4—*Electropherograms of duplicate samples digested with varying amounts of DNase. Data scaled to 3500 RFUS. The profile on the left was prepared with the initial Garvin et al. recommended DNase and ionic concentration, resulting in very low peak heights. The profile on the right was prepared with the optimal DNase and ionic concentration, showing significant improvement in peak height. Trace alleles consistent with carryover from the nonsperm fraction were observed in the profile on the right. This is a result of the DNase not being 100% efficient in removing all residual amounts of nonsperm DNA. See sections below for further discussion.*

FIG 5—*Electropherograms of replicate samples digested with varying amounts of DNase and $MgCl_2$. Data scaled to 2000 RFUs. The first profile was digested with the initial Garvin et al. recommended DNase and ionic concentration, resulting in very low peak heights. The second profile was digested with a decrease in amount of DNase concentration, showing significant improvement in peak height. However, carryover from the nonsperm fraction is present. The third profile was digested with the same amount of decreased DNase concentration, but with an increased amount of $MgCl_2$ concentration. This resulted in a reduced amount of carryover from the nonsperm fraction.*

channel pipettor head is used for liquid handling, preparation of microscope slides, and transfer of the samples from the 96-well plate to individual 2.0 mL tubes for DNA extraction on the EZ1 Advanced XL BioRobot®. Liquid level sensing on the instrument allows for careful removal of the supernatant without disturbing the cell pellets at the bottom of each well on the plate.

The SlicPrep™ 96 device is a 2.2-mL 96-deep-well plate that includes a 96-well spin basket insert and a U-shaped collar. Sample substrates are placed inside of the spin basket which has seven 1 mm perforations at the bottom to allow for liquid flow between the spin basket and 96-well plate during incubation. The U-shaped collar is inserted between the spin basket and 96-well plate during centrifugation steps to create space by raising the spin basket by approximately 1 cm (23).

The automated differential digestion protocol was designed to mimic the manual selective degradation differential digestion process (Fig. 12). Four pauses were incorporated into the protocol to allow for the manual intervention required for two centrifugation steps (6 min at 1450 x *g*), removal of sample tubes and microscope slides, and manual sample agitation. Sample agitation is required for efficient release of biological material into solution because epithelial and sperm cells may adhere quite strongly to various substrates. A manual agitation step where samples were agitated by the analysts using sterile toothpicks was added to the automated process due to the observation that simply shaking the samples on the robot without manual agitation resulted in a significant decrease in DNA recovery. The tight and narrow wells of the SlicPrep™ plate filled with one

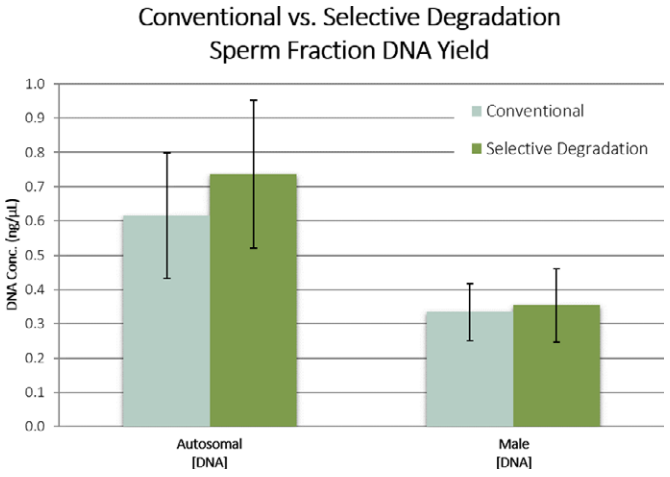

FIG 6—*Mean DNA yield comparing the conventional and selective degradation differential digestion processes. Error bars represent one standard deviation.*

whole swab did not allow sufficient space for effective agitation of the sample.

Automation of the differential digestion process was achieved on the VERSA 1100 without compromising on the quantity or quality of the recovered DNA. The DNA yield and STR DNA typing data of samples processed using selective degradation were comparable to samples processed using the conventional differential digestion. No contamination was detected for samples processed on the VERSA 1100.

Application to Casework

Automation with the use of the 96-well SlicPrep™ plate enabled processing of sexual assault kits in a high throughput and high efficiency manner. Each sexual assault kit was designated one column on the plate, allowing for a maximum of 12 kits to be processed at one time. With one sexual assault kit per column, an analyst can take up to seven samples per kit, using the last empty well as the reagent blank. The availability of seven wells for sampling has potential advantages. This process negates potential laboratory requirements that only the top one to three probative samples be taken forward in fear of having too many samples to manage at one time during the manual differential digestion. In situations where more than seven samples need to be taken for one sexual assault kit, two columns can be used for one case.

Each plate is typically shared between two and three analysts, because having one person manages all 12 sexual assault kits and generating up to 96 aqueous extracts, 96 nonsperm fractions, 96 sperm fractions, 96 predigest slide wells, and 96 postdigest slide wells is overwhelming to say the least and introduces more potential for human error. However, with multiple people sampling into a single 96-well plate with narrow wells, the concern of contamination cannot be dismissed. As a result, a plate mat septa which fits into the wells of the SlicPrep™ plate is used to cover the plate. The septa can easily be cut into strips, so that only one column is exposed while the rest of the plate is covered. This allows the analyst to place samples from one case into a column without contaminating the rest of the plate. In most cases, only half of a swab is sampled because of the narrow wells. However, whole swabs can be sampled, as well as underwear, clothing, and condom cuttings. Samples on the plate are tracked using the Oakland Police Department in-house Laboratory Information Management System (LIMS).

It is important to keep in mind that the spin basket insert can be inserted into the plate in either orientation. Therefore, our protocol incorporates marking the spin basket insert in relation to the plate to avoid inadvertently reinserting the spin basket in the wrong orientation after centrifugation steps, leading to contamination of the entire plate.

Aqueous extracts are prepared by the VERSA 1100, and all aqueous extracts are kept for potential p30 or amylase testing. All sperm and nonsperm fractions are extracted and brought forward to DNA quantitation. Any potential aspermic samples can be caught using this process without the time-consuming and less sensitive presumptive semen testing of every sample. Microscopic slides are available for visual examination for sperm.

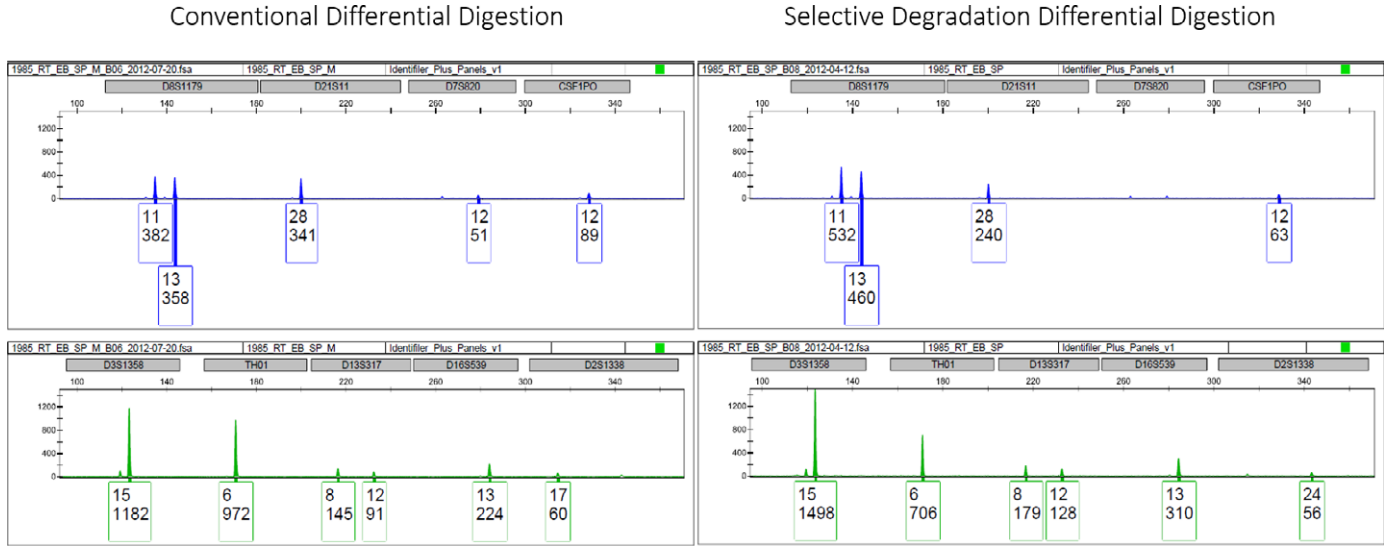

FIG. 7—*Electropherograms of a semen stain stored at room temperature from 1985 processed using the conventional differential digestion and the selective degradation differential digestion processes. Data scaled to 1500 RFU. Degradation is observed for both samples. However, the STR DNA typing results for both samples are very similar, demonstrating that the use of DNase does not have a negative effect on degraded samples.*

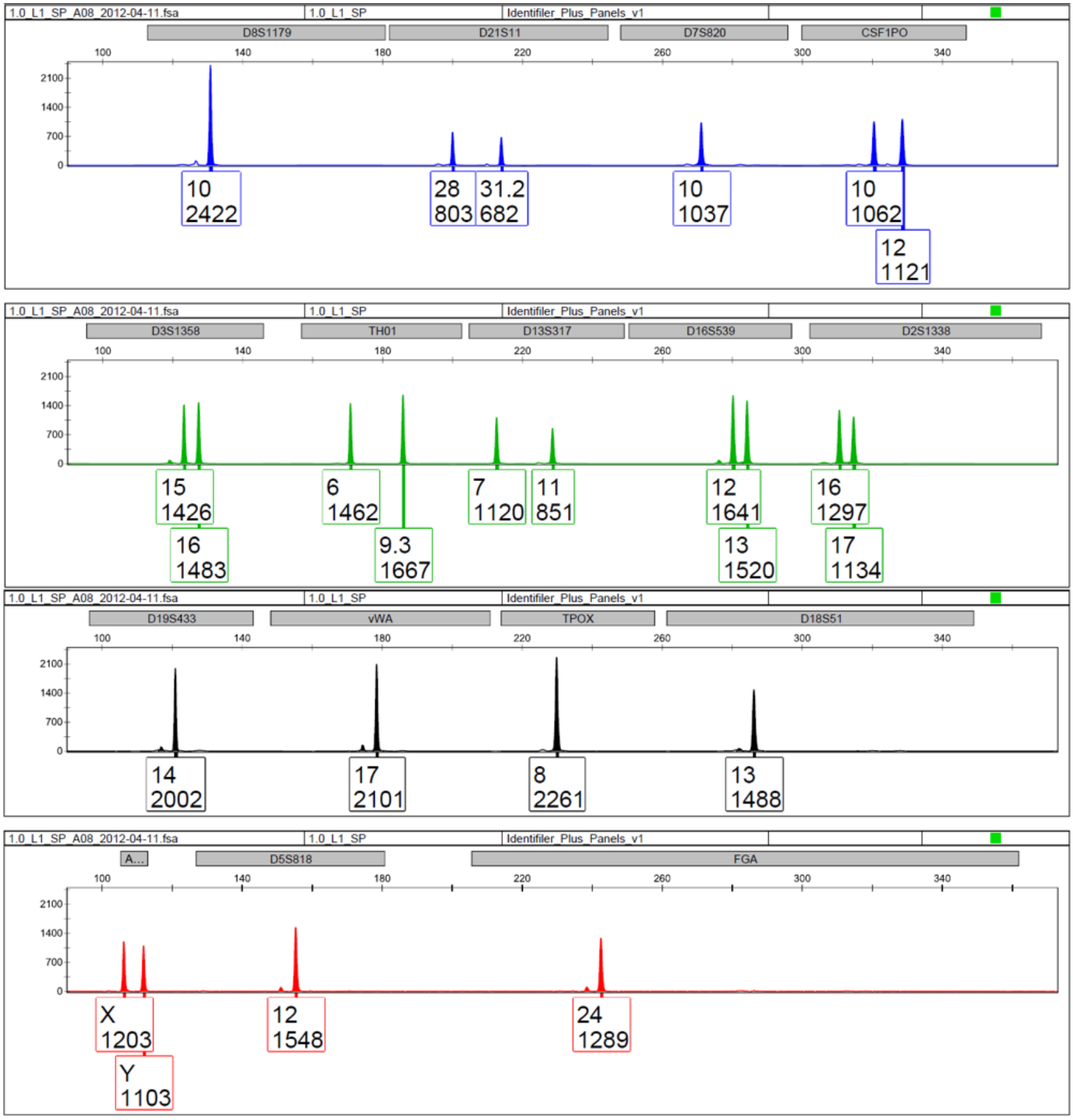

FIG. 8—*Electropherogram of the sperm fraction of a sample with a decent amount of sperm. Based on microscopic examination, the sample contained approximately 170 sperm cells. Based on quantitation data, approximately 1 ng of DNA was amplified for the sperm fraction.*

Casework Evaluation—Throughput and Statistics

Implementation of the automated differential digestion process in the Oakland Police Department Criminalistics Laboratory has significantly increased our throughput of processing sexual assault cases. The push to eliminate our sexual assault kit backlog using the automated differential digestion process was conducted between January and August of 2014. Within this 8-month period, a total of 343 sexual assault kits were processed with eight analysts. During this time, each analyst was also responsible for additional nonsexual assault kit casework.

With multiple assailants commonly reported for our sexual assault cases, the flexibility to take up to seven samples per sexual assault kit, or more if using multiple columns, provides the assurance that all potential assailants are identified. The success rate, defined by the number of cases with sufficient probative DNA for DNA typing (e.g., male DNA detected on female

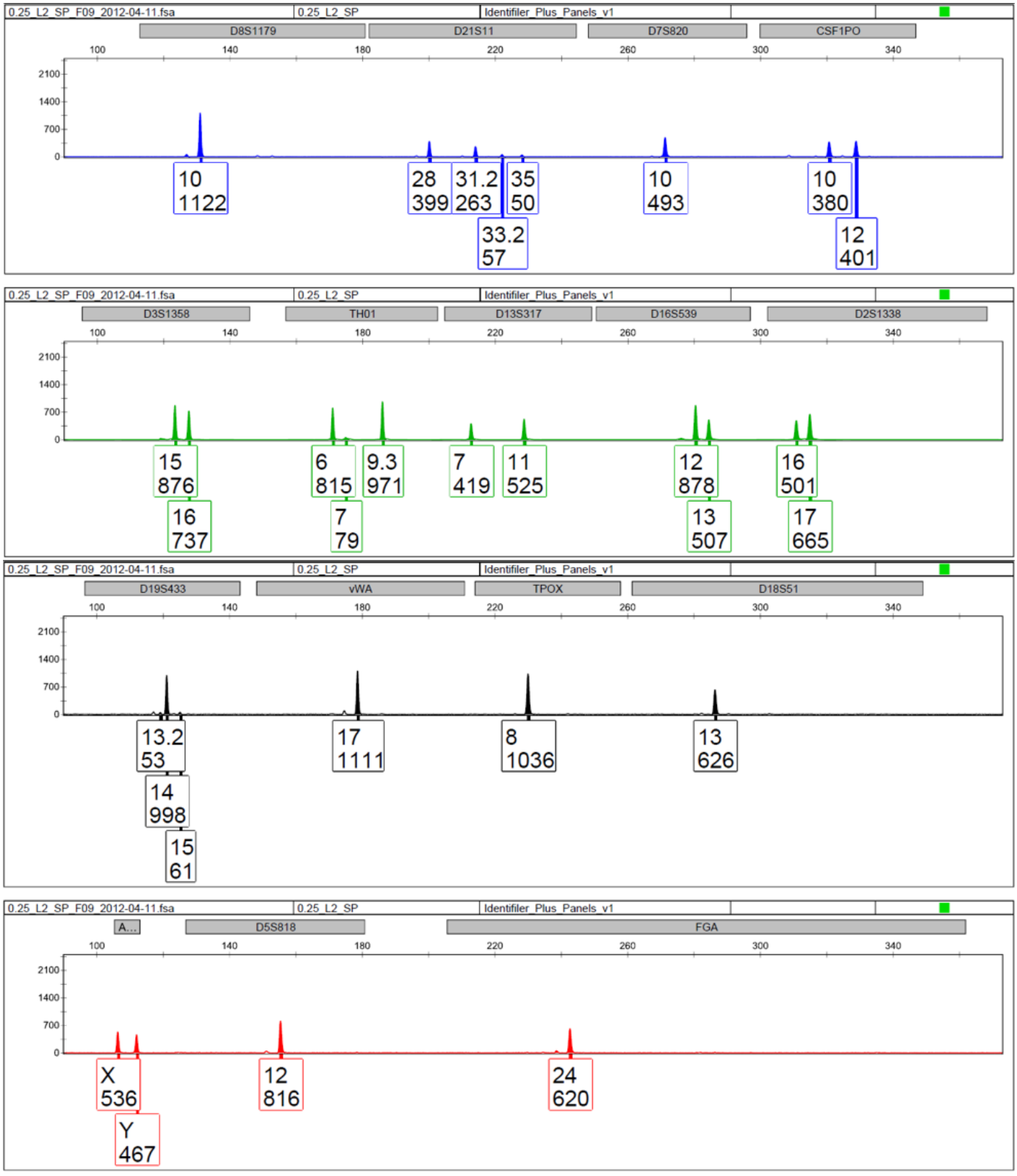

FIG. 9—*Electropherogram of the sperm fraction of a sample with a low amount of sperm. Based on microscopic examination, the sample contained approximately 20 sperm cells. Based on quantitation data, approximately 500 pg of DNA was amplified for the sperm fraction.*

victim swabs), was compared between the automated and the manual conventional differential digestion process. The automated process during the project period had a success rate of 58%, while the conventional process was 52%. The similar success rates demonstrate that the automated process is on par with the conventional process. The slight increase in success rate for

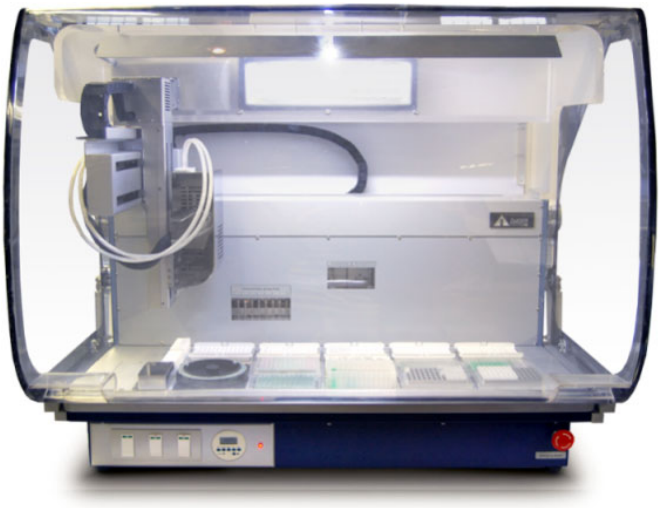

FIG. 10—*VERSA 1100 liquid handler by Aurora Biomed.*

the automated process may be due to more samples being taken for each sexual assault kit as compared to the manual conventional process.

An entire plate of 96 sexual assault evidence samples can be processed in about 8 h using the automated differential digestion process. It would take about six analysts to process the same number of samples in the same amount of time. An additional advantage to the automated process is the significant amount of hands-off time the analyst has. The analyst may use this time to perform other duties, such as setting up the extractions runs or finishing up paperwork. Fig. 13 shows the processing time from when a case is assigned to when it is subjected to quantitation. Although the significant decrease in processing time may be due to additional factors such as improvement of workflow within the laboratory, the impact of utilizing the automated differential digestion process can be gleaned by comparing the processing time for three years before (manual differential digestion) and three years after (automated differential digestion) the VERSA 1100 was implemented.

One drawback to the automated differential digestion process is the quality of the predigest and postdigest slides. As swabs are manually agitated only prior to the postdigest slides, the number of cells observed for the predigest slides does not accurately reflect the number of cells on the swab. As for the postdigest slides, it has been observed that for samples with higher amounts of epithelial cells, such as in vaginal swabs, more cellular debris is observed in the slides, sometimes to a point where it covers the entire slide. This is because the sperm pellet is no longer washed prior to making the postdigest slides, thus the cellular debris remaining in the sample also gets stained. The purpose of microscopic examination is to identify sperm and to estimate the number of cells present in the sample. This might be an issue for the samples where the sperm may not be readily visible due to the amount of cell debris. However, the quality of the slide does not affect the quality of the sample and the downstream processes it is subjected to.

Contemporary Kit Program

After the successful implementation of the automated differential digestion process into the laboratory, the Contemporary Kit Program was devised. The purpose of this program is to ensure that a backlog of sexual assault kits will not occur again within the Oakland Police Department Criminalistics Laboratory. The aim is to ensure every sexual assault kit submitted to the Oakland Police Department will be evaluated, and if appropriate, analyzed contemporaneously. Each week, an analyst is assigned to examine the sexual assault kits that have been submitted to the department within the past week. In this program, almost every sexual assault kit is analyzed, unless specifically requested by the Special Victims Unit to not do so. The reasons for no analysis of a sexual assault kit are typically due to outside jurisdiction (crime did not occur in Oakland) or the investigator has determined that no crime has occurred. The goal of this program is to upload an eligible profile into the Combined DNA Index System (CODIS) within 10–15 business days of starting the analysis and to publish a report to the investigators within 40 business days of starting the analysis.

Currently, the Oakland Police Department has no sexual assault kit backlog and approximately 92% of all submitted

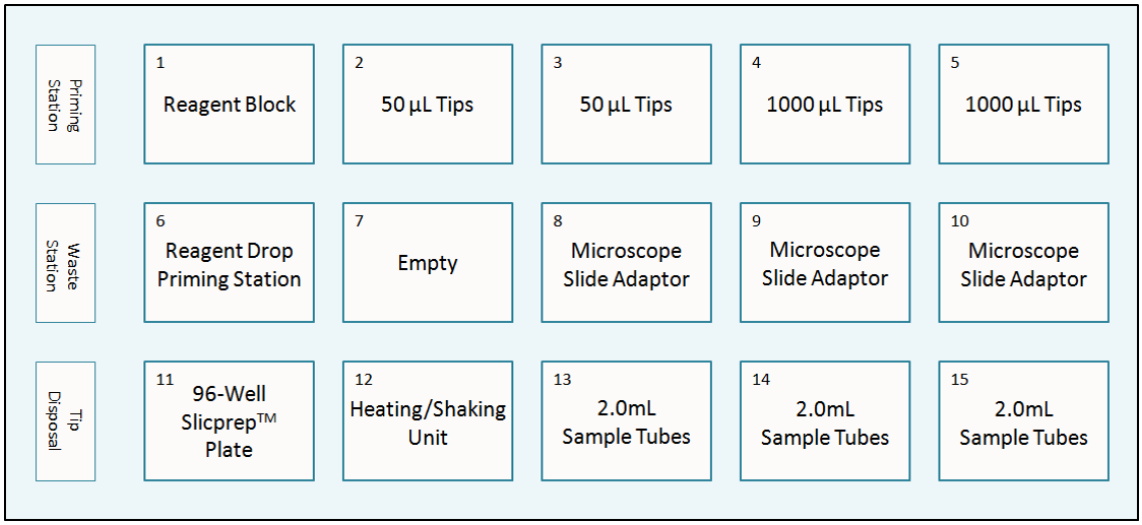

FIG. 11—*VERSA 1100 Deck Layout.*

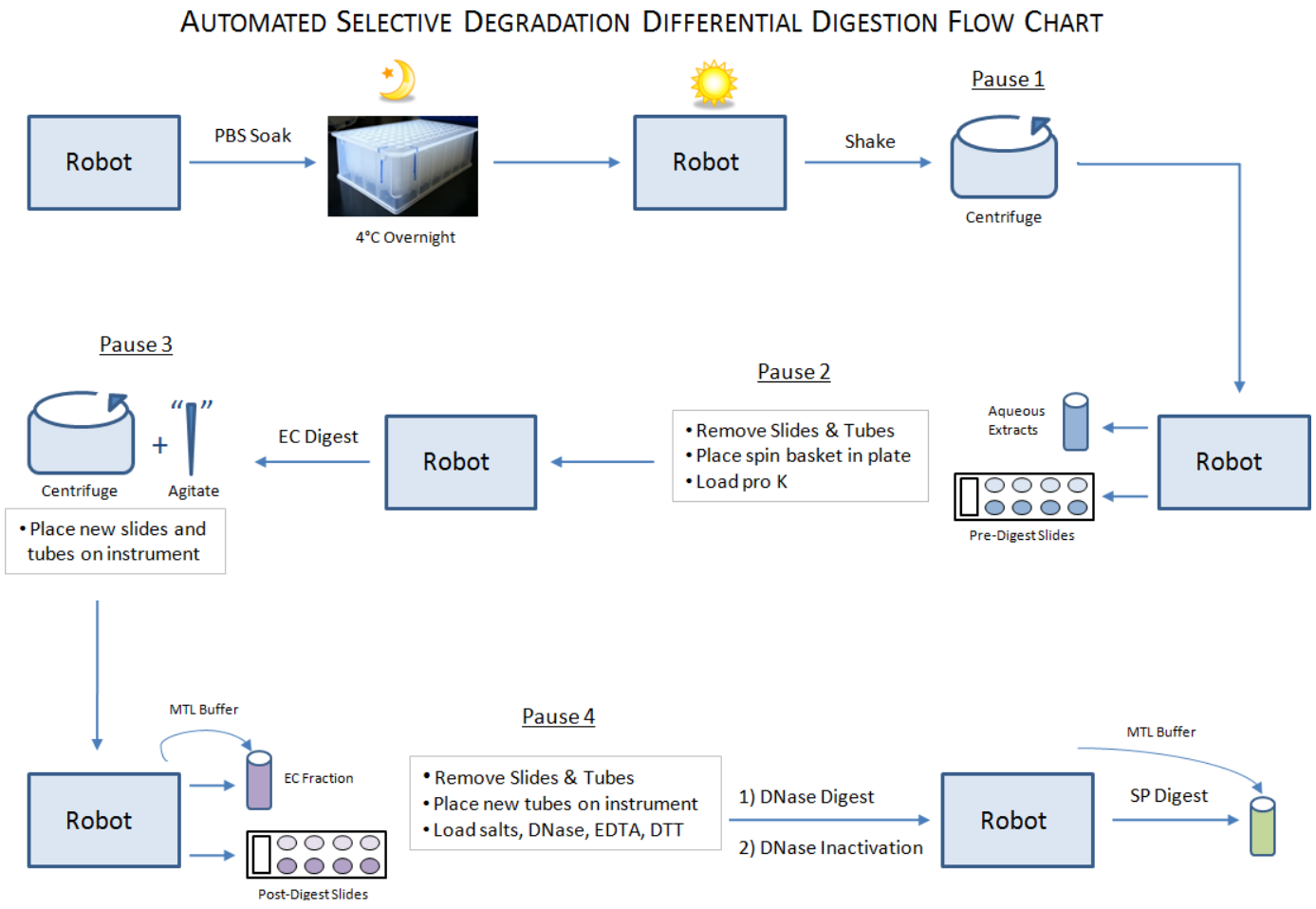

FIG. 12—*Flowchart of the automated differential digestion process.*

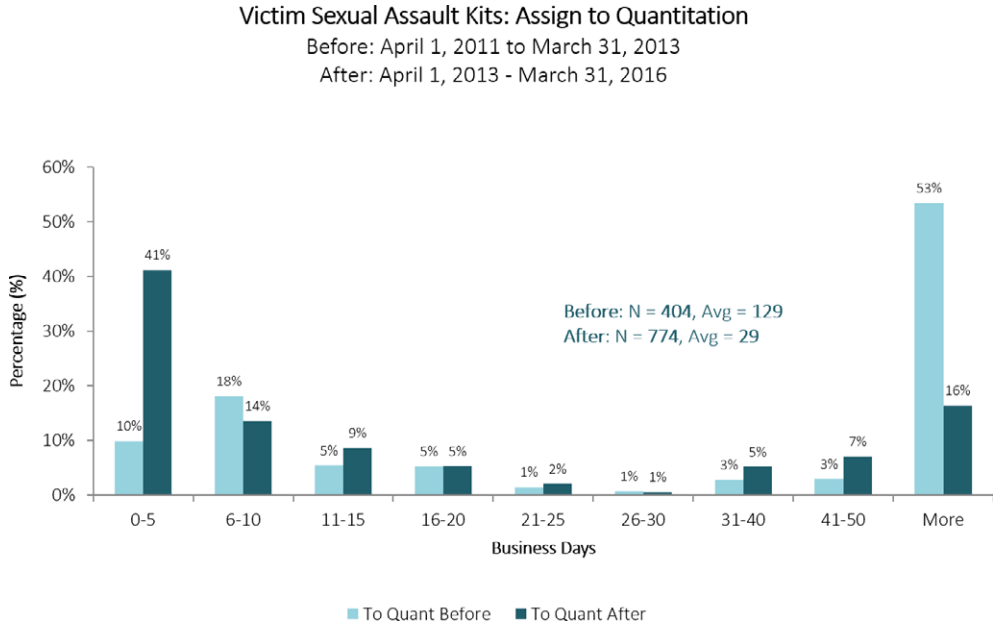

FIG. 13—*Turnaround time of victim sexual assault evidence kits from assign date to quantitation date. The assign date typically reflects the date that an analyst begins sampling the sexual assault evidence kit.*

sexual assault kits move forward to analysis. On average, an eligible profile is uploaded into CODIS in 10 days and a report is published to the investigator in 23 days. To truly capture the quick turnaround time for analysis of sexual assault kits in Oakland, the average time from the date of the crime to the date an eligible profile is uploaded into CODIS is 20 days. This accomplishment and the continued success of the program are due in large part to the automation of the differential digestion process.

Conclusion

Through use of the selective degradation technique, automation of the differential digestion process was achieved without compromising the quantity and quality of the recovered DNA. The conventional differential digestion is a time-consuming manual process; automation of this process reduces analyst bench time thereby increasing the time available for more casework, data interpretation, report writing, and casework review. With the increase in demand for casework per analyst and reduced turnaround time, many laboratories will find that automation of the differential digestion process will aid in the reduction of sexual assault evidence kit backlogs. Using the automated selective degradation differential digestion process, a full plate of 96 sexual assault evidence samples can be processed in approximately 8 h with minimal analyst interaction. Processing the same number of samples by the conventional differential digestion process would take approximately six times as long and would require the full engagement of the analyst. The minimal number of manual manipulation steps of the automated process also reduces the possibility of contamination and human error such as sample switching. Although automation of the differential digestion was achieved using the VERSA 1100, any differential digestion protocol utilizing selective degradation by DNase can be automated with a wide variety of liquid handler instruments. However, some platforms may work better than others depending on specific requirements of the protocol and the functionalities provided by the instrument.

Although the initial evaluation of the use of DNase for selective degradation of residual nonsperm DNA during the differential digestion process led to observations of reduced DNA yield and poor STR DNA typing data, optimization of the process resulted in significant improvements such that the data obtained were comparable to the conventional differential digestion process. The selective degradation technique employing DNase can produce high DNA yield and high-quality STR DNA typing data, with easily interpretable sperm fraction DNA profiles. However, specific parameters associated with the use of DNase must be carefully evaluated to avoid the potential loss of DNA evidence due to the activity of DNase. These parameters include, but are not limited to, the amount of DNase used and the magnesium and calcium divalent cation concentrations. Experiments performed on aged semen stains and samples subjected to heat and humidity and multiple freeze–thaw cycles also demonstrated that the DNase treatment did not have an appreciable negative effect on the DNA yield or quality of STR DNA typing results on environmentally compromised samples.

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