

## Automation of a Differential Digestion Protocol for Processing Sexual Assault Kits Using the VERSA Automated Liquid Handling Workstation

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Separation of sperm cell DNA from epithelial cell DNA in processing sexual assault kits remains a time consuming, manual task contributing to the backlog of evidence processing. Rapid and accurate sample processing is of high importance to ensure DNA evidence is presented properly for potential conviction. An automated differential digestion protocol was developed and adapted for use on the VERSA 1100 automated liquid handling workstation. The automated protocol gave DNA yields of similar concentration to the conventional manual protocol resulting in similar short tandem repeat (STR) typing profiles. The automated protocol demonstrated high levels of reproducibility across a set of sample replicates with no detectable cross contamination. Automation of the differential digestion protocol by the VERSA 1100 automated liquid handling workstation resulted in a six fold decrease in sample processing time. Automation by the VERSA liquid handling system significantly decreased sample processing times and proved to be a capable automated solution for differential digestion of sexual assault kits.

### INTRODUCTION

Since the advent of DNA sequencing and forensic fingerprinting, DNA analysis has become a routine part of criminal investigations, notably in the investigation of sexual assault cases. Forensic science laboratories are faced with an increase in demand for sexual assault evidence analysis, while lacking sufficient analysts to perform the labor-intensive work required for DNA analysis. As a consequence, the large number of requests quickly results in an overwhelming backlog. This high demand has led laboratories to transition to automation for processing cases in a more efficient manner. While many areas of DNA analysis have adopted automation, the differential digestion process remains a time-consuming, manual task.

The current differential digestion process requires multiple wash and centrifugation steps to remove residual epithelial DNA from the sperm fraction. An automated differential digestion protocol was developed using a selective degradation technique, which replaces these labor-intensive steps by using DNase I to digest the remaining epithelial DNA. Studies on the use of DNase on evidence samples demonstrated that the selective degradation technique produced DNA yield and STR DNA typing data that were comparable to the conventional protocol. Sensitivity, reproducibility, and contamination studies were performed on a VERSA 1100 robotic liquid handler to automate the differential digestion process. The automated protocol utilized 96-well plates for high efficiency and

incorporated microscope slide preparations for the confirmation of the presence of sperm.

## METHODS

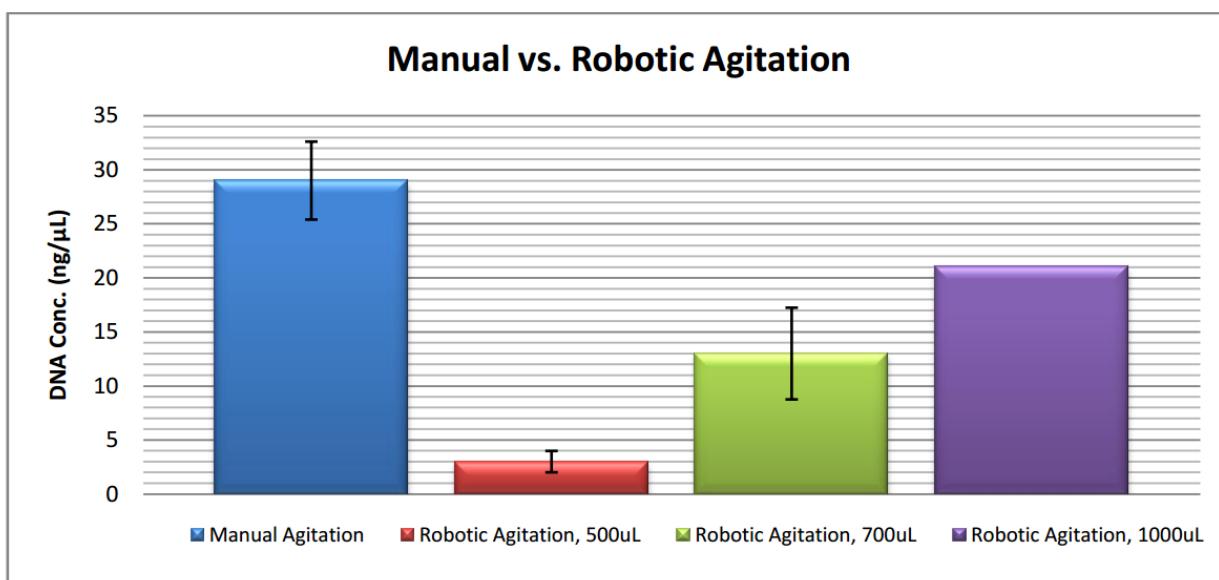
### *Preparation of samples*

Semen samples were obtained from healthy donors and centrifuged to remove seminal fluid. The remaining sperm cells were resuspended in phosphate buffered saline (PBS) at pH 7.4. Vaginal epithelial samples were obtained from swabs, and vaginal cells were resuspended in PBS pH 7.4 before use in any experiments. Mock sexual assault samples were prepared by mixing 15 µL of vaginal cells with the same volume of dilute sperm samples. As the specific concentration of sperm cells was not measured, the exact

concentration is not known; however, epithelial cells were in a large excess to sperm cells to mimic the conditions observed in a sexual assault case.

### *Differential digestion of mock sexual assault samples*

Samples were lysed using 510 µL of Tween-80 buffer solution (20 mM Tris-HCl, 1 mM EDTA and 2% Tween-80). To selectively digest the epithelial cell DNA, the samples were treated with 290 µL Tween-80 buffer, 15 µL units of DNase I (1 U/µL), 90 mM MgCl<sub>2</sub>, and 5 mM CaCl<sub>2</sub>. Samples were digested for 15 minutes at 56°C before terminating the reaction with 20 µL EDTA (0.5 M). The remaining sperm fraction was lysed with 10 µL proteinase K (20 mg/mL) and 20 µL of



**Figure 1.** Average male DNA yield of sperm fractions from samples digested manually and robotically using the VERSA 1100 automated liquid handling workstation. Cotton swabs were soaked in PBS and agitated manually with a toothpick or shaken using the orbital shaker on the VERSA 1100. The 500 µL sample was half a swab, while swabs soaked in 700 µL or 1000 µL were cut into 4 or 5 smaller pieces prior to agitation. Robotically prepared samples were shaken for 30 minutes at 2500 rpm. The 1000 µL sample consisted of a single replicate (n=1).

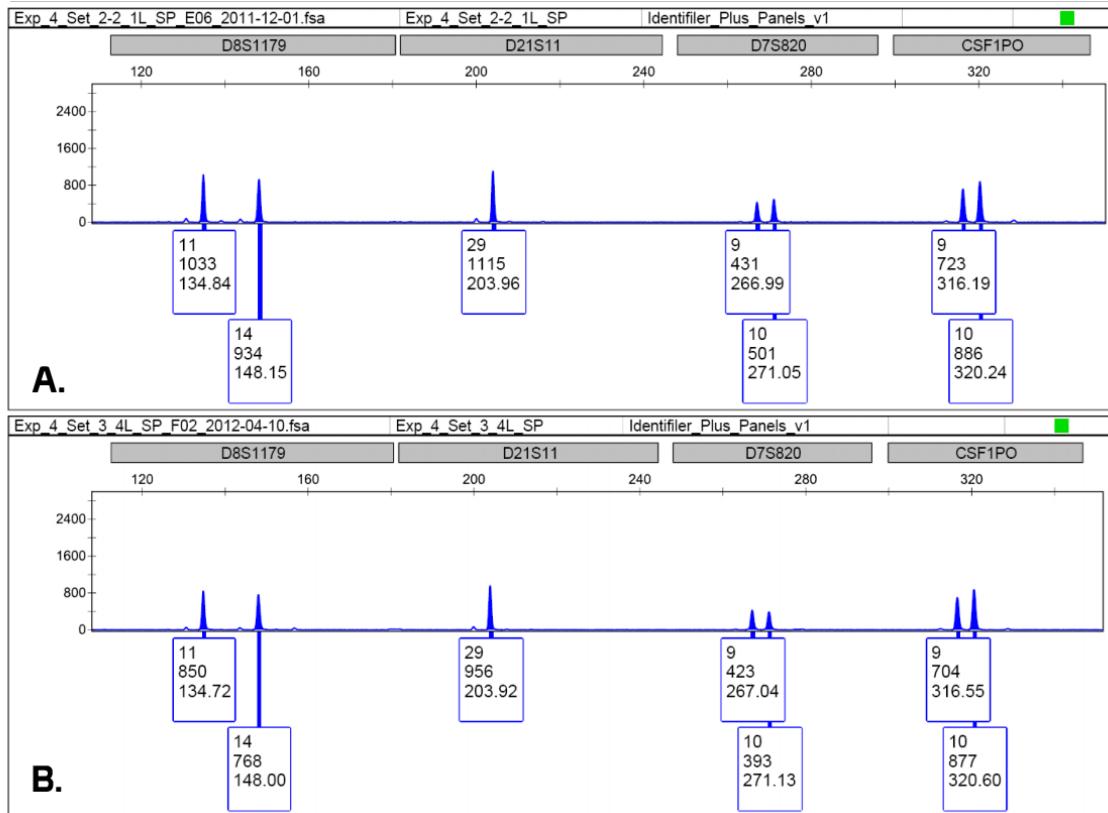
dithiothreitol (DTT)

#### Purification and assessment of DNA

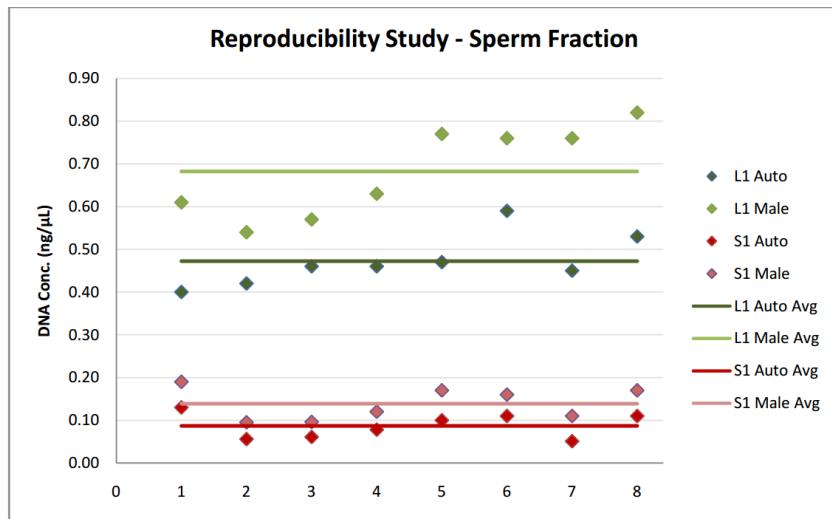
DNA was purified following digestion using an EZ1 Advanced XL BioRobot (Qiagen) and quantified by qPCR using the Plexor HY system (Promega). STR typing data was assessed by amplifying purified DNA using the AmpF/STR Identifiler Plus PCR Amplification Kit (Applied Biosystems) and separated by capillary electrophoresis using an ABI 3130 Genetic Analyzer (Applied Biosystems) with 5 second injections.

#### RESULTS & DISCUSSION

Initial recovery from the robotically processed samples showed a lowered cell recovery from the swab substrate using the shaker on the VERSA as compared to manual agitation with a toothpick (**Figure 1**). Increasing the volume of liquid for soaking the swabs increased the cell recovery to approximately 90% of the manual agitation method. However, this volume exceeded the capacity of the SlicPrep™ plate used for this process. Therefore, the protocol was modified to incorporate a manual agitation step to effectively



**Figure 2:** Electropherogram comparison of a single sample digested using both manual and automated protocols. Samples were digested (A) manually using the conventional differential digestion method, (B) robotically using the selective degradation protocol on the VERSA 1100 automated liquid handling workstation. All data scaled to 3000 RFU.



**Figure 3:** Graph of the reproducibility study. The study consisted of two sets of eight samples (L1 and S1). The graph shows the autosomal DNA concentration and the male DNA concentration. The horizontal line across each set of data represents the average value for that particular set of data.

release cells from the swab. Further optimization of this step may produce a fully automated protocol for releasing cells from the swab substrate. Evaluations of the electropherograms revealed that samples processed by the VERSA automated liquid handling workstation produced STR typing data which was consistent with the conventional method (**Figure 2**).

The ability of the VERSA to avoid sample cross contamination was assessed through strategic placement of blank samples on a 96 well plate. No DNA was detected in the blank samples indicating that the VERSA robotic system produces a contamination free preparation (data not shown).

Sample replicates for samples prepared on the VERSA 1100 liquid handling workstation showed very low variation between replicates (**Figure 3**). The coefficients of variance (CVs) were found to be 13% and 16% for the recovered sperm DNA fractions across two independent experiments;

this is below the expected coefficient of variance for the quantitation system alone (~20%). The VERSA 1100 has a CV of less than 5% at one microliter providing a high level of consistency.

## CONCLUSIONS

Through the use of the selective degradation technique, automation of the differential digestion process was achieved without compromising the quantity or quality of the DNA recovered. Automation of the process resulted in less hands-on time for the analyst and an overall six-fold decrease in sample processing time. Automation allows higher throughput and gives greater time for data analysis, report preparation, and other casework required by forensic scientists. With minimal manual interaction, the VERSA was able to satisfactorily perform the differential digestion method, providing a validated automated protocol for processing sexual assault kits.