
UNIVERSITY OF NORTH TEXAS
HEALTH SCIENCE CENTER AT FORT WORTH
1970

THE EVALUATION OF THE MAXWELL® 16 AND THE

DNA IQ™ CASEWORK SAMPLE KIT FOR THE

EXTRACTION OF DNA FROM

FORENSIC SAMPLES

Tarig Alkhazin, B.S.

APPROVED:

Major Professor

Committee Member

Committee Member

Chair, Department of Cell Biology and Genetics

Dean, Graduate School of Biomedical Sciences

THE EVALUATION OF THE MAXWELL® 16 AND THE DNA IQ™ CASEWORK SAMPLE KIT FOR THE EXTRACTION OF DNA FROM FORENSIC SAMPLES

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the Graduate School of Biomedical Sciences

University of North Texas Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

By

Tarig Alkhazin, B.S.
Fort Worth, Texas
August 2007

ACKNOWLEDGEMENTS

Arthur Eisenberg, Ph.D.

Joseph Warren, Ph.D.

John Planz, Ph.D.

Dan Dimitrijevich, Ph.D.

Xavier G. Aranda, M.S.

Rhonda K. Roby, MPH, M.S.

Curtis Knox, M.S.

Yasser Daoudi, B.S.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The Maxwell® 16 (Promega Corporation, Madison, WI) is a small, self-contained instrument utilizing Promega's DNA IQ™ chemistry for the automated extraction of DNA from 16 biological samples simultaneously. Currently, the Maxwell® 16 is used in conjunction with the DNA IQ™ Reference Sample kit for the automated extraction of DNA from forensic and paternity reference samples. Promega Corporation is currently in the development of the DNA IQ™ Casework Sample kit with the intent of using the Maxwell® 16 instrument in the extraction of DNA from forensic evidentiary samples. Modifications have been made to the Maxwell® 16 to allow the elution of DNA in a smaller volume (Low-Elution Volume (LEV) configuration) that when used in conjunction with the DNA IQ™ Casework Sample kit would optimize DNA yield from forensic casework samples. However, the limited quantity and the low quality of forensic casework samples are significant challenges facing most automated DNA extraction systems. An evaluation study was conducted to test the performance of the Maxwell® 16 instrument along with the DNA IQ™ Casework Sample kit for processing forensic evidentiary samples. Mock evidentiary items used for the evaluation consisted of blood, semen, tissue, and touch samples that are routinely

encountered in forensic casework. Prior to loading on the Maxwell® 16 instrument samples were first digested using the Tissue and Hair Extraction kit (Promega Corporation) designed for optimum DNA recovery.

The extraction of DNA from sexual assault samples typically requires the separation of the sperm DNA deposited by the assailant from the vaginal epithelial cell DNA from the victim. Promega Corporation has developed the Differex™ System which utilizes a manual phase separation technique to obtain both a sperm fraction and an epithelial cell fraction. After separation and digestion, samples were then added to the DNA IQ™ Casework Sample kit cartridges for automated DNA purification using the Maxwell® 16. For comparison, DNA was also obtained from replicate samples processed with the Differex™ System following the standard organic extraction method. The evaluation of the Differex™ system with mock sexual assault samples was conducted in order to determine if this differential extraction process can be used in conjunction with the Maxwell® 16 to improve case processing efficiency.

To evaluate the performance of the Maxwell® 16 and the DNA IQ™ Casework Sample kit, replicate samples were prepared and processed using UNTHSC's standard organic extraction methodology. The DNA obtained by both methodologies was quantified using the Applied Biosystems (AB) Quantifiler Human DNA Quantification kit (Foster City, CA) and the AB 7500 Real-Time PCR system and then amplified using the PowerPlex® 16 kit (Promega Corporation). The amplified DNA was analyzed using the AB 3130*xl* Genetic

Analyzer and the resulting STR electropherograms were analyzed to obtain profiles using GeneMapper® ID *v*3.2 (Applied Biosystems). In addition to assessing the quantity and the quality of the DNA obtained, blank cartridges were simultaneous processed with the mock forensic samples to demonstrate whether the Maxwell® 16 could introduce cross-contamination between samples.

The overall performance and the cost-effectiveness are the ultimate criteria to help determine the utility of the Maxwell® 16 in the processing of forensic evidentiary casework samples.

CHAPTER II

BACKGROUND

Forensic evidentiary samples often contain limited amounts of biological material that may be exposed to environmental conditions that cause degradation. The efficient recovery of amplifiable DNA from a wide variety of challenging samples is essential for the utilization of any automated or manual DNA extraction procedure in forensic casework analysis. This study was designed to evaluate the use of the DNA IQ™ Casework Sample kit in conjunction with the Maxwell® 16 for the automated DNA purification from forensic samples.

Prior studies using on the DNA IQ™ system manually demonstrated that Promega's paramagnetic beads provide a reliable means of extracting DNA from typical forensic casework samples (1). The DNA IQ™ resin combines the DNA binding ability of a silica-based methodology with the automated handling of paramagnetic particles (PMP). Promega Corporation has incorporated a chaotropic agent, guandinium salt, to denature membrane proteins and enzymes to lyse cells and inhibit nucleases. The guanidinium salt also coats the DNA IQ™ resin to facilitate rapid and high affinity binding of the DNA (2)(3). This strong affinity has the potential to make the DNA IQ™ resin a very efficient methodology for the recovery of DNA from evidentiary samples with limited

amounts of biological material. However, the resin can become saturated in the presence of large amounts of DNA. Once saturated, the resin will no longer bind excess amounts of DNA as demonstrated by the blue "Database Applications" box in Figure-1.1. The DNA recovered with the DNA IQ™ system is comparable to that of the standard organic extraction methodology (1). In fact, the DNA obtained from challenging samples with DNA IQ™ often produced better quality STR profiles as compared to DNA extracted using organic extraction (4).

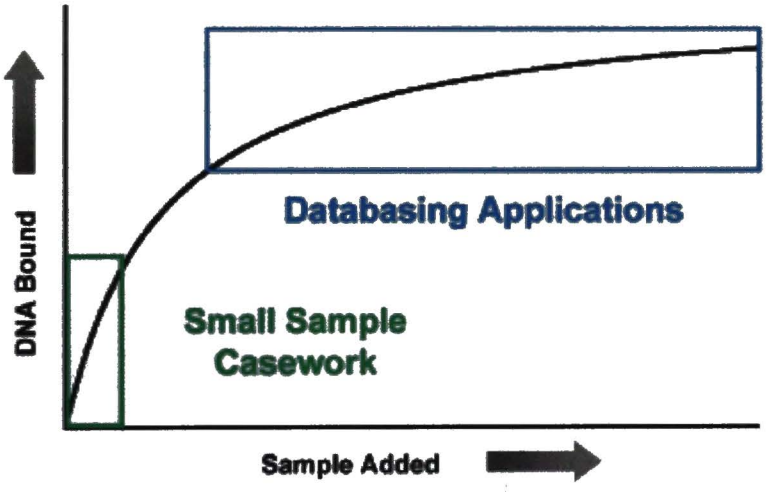

Figure-1.1: The DNA IQ™ resin binding affinity

The DNA IQ™ Casework Sample Kit is used with the Maxwell® 16 instrument (Figure 1.2) was specifically designed to optimize the extraction of DNA from forensic samples (1). The Casework Sample Kit consists of compartmentalized cartridges that are pre-filled with the DNA IQ™ chemicals: Lysis Buffer, DNA IQ ™ resin, and Wash Buffer (Figure-1.3). At the opposite

end of the cartridge, the elution tube contains the Elution Buffer which is firmly positioned on a heating plate platform (Figure-1.4). Using mobile magnetic rods, the Maxwell®16 captures and linearly transports the DNA-bound resin through the purification reagents in the cartridge. The DNA is released from the resin by heating to 60° C. The DNA-free resin is then re-captured by the magnetic rods. The operational modes, forensic or research protocol, are pre-programmed into the instrument's firmware for the simultaneous DNA purification of 16 samples in approximately 30 minutes. The movement of the DNA IQ™ resin through pre-filled cartridges avoids the problems of clogging or contamination due to fixed or disposable tips which is periodically seen with conventional liquid-handling robotic systems.

Figure-1.2: Maxwell® 16 Instrument

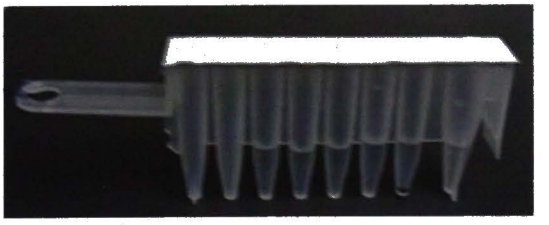

Figure-1.3: Casework cartridge

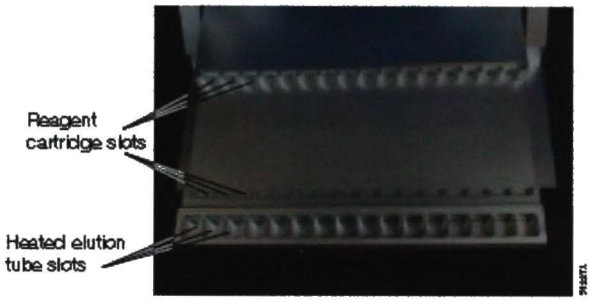

Figure-1.4: Maxwell® 16 Platform

The DNA IQ™ Casework Sample Kit can be used to process blood stains, semen stains, hair, cigarette butts, tissue, and trace samples (5). The protocol used for processing different sample types includes a pre-processing step which requires variations in the reaction volume or in the amount of DTT and Proteinase K. These variations are designed to increase the efficiency of cell digestion within different types of samples. After digestion all samples are processed on the Maxwell® 16 with a single standard protocol for all sample types. However, the amount of Elution Buffer may vary based upon the expected DNA yield (5). The Differex™ System differential extraction kit can be used to manually separate sperm from the epithelial cell fraction in sexual assault samples (6). DNA from both fractions may be purified using a robotic platform (7) (8).

An added advantage of the DNA IQ™ Casework Kit for processing forensic samples is that the DNA IQ™ resin will only bind high quality DNA greater than 80 base pairs. The DNA IQ™ resin will not efficiently bind highly degraded DNA samples which are often found in challenged forensic samples.

The presence of degraded DNA can alter the efficiency of the amplification reaction since smaller fragments of DNA may be preferentially amplified (9). The DNA IQ™ system will also remove PCR inhibitors, such as heme, that is common in DNA extracted from blood. In addition, The DNA IQ™ system does not bind inhibitors such as indigo dyes which are used in denim jeans (10). The removal of PCR inhibitors by DNA IQ™ increases the efficiency of the DNA analysis process by providing more accurate DNA quantification results using quantitative Real-Time PCR (11). The purification of DNA above 80 base pairs which is free of inhibitors can result in a more efficient amplification and the generation of STR profiles without allele or locus drop-out (10). Moreover, the DNA IQ™ resin is compatible with capillary electrophoresis and has no affect on STR profiles (9).

Automation can make DNA analysis a more efficient process, allowing the processing of a larger number of casework samples. Each year, the number of requests for DNA analysis exceeds the processing capacity of forensic laboratories. In 2002, the Census of Publicly Funded Forensic Crime Laboratories reported 61,000 new requests for DNA analysis with only 12,000 completed with 49,000 DNA backlogged cases created (12). The report indicated that an estimated "370 additional full-time employees" would be required to achieve a 30-day turnaround on all DNA analysis requests (12). Automation of DNA extraction can lead to significant reductions in backlogged forensic cases by providing analysts with more time for data interpretation and report generation.

The San Diego Police Department, Los Angeles County Sheriff's Department, and the Virginia Division of Forensic Science are examples of some of the laboratories currently utilizing automated extraction instruments for high-throughput database sample processing (13). Recent validation studies using automated DNA extraction systems has helped demonstrate improved instrument versatility and the adaptability for the extraction of DNA from casework samples (14) (15).

Prior to the utilization of the Maxwell® 16 in forensic casework, quality assurance standards require the Maxwell® 16 DNA extraction system undergo developmental validation (16). The goal of the study was to evaluate the performance of the Maxwell® 16 for processing forensic casework samples in comparison to our currently utilized manual organic DNA extraction methods.

CHAPTER III

MATERIALS AND METHODS

Sample Pre-processing:

Promega has developed a DNA IQ™ Casework Sample Kit for the Maxwell® 16. The kit contains the following components as described in Technical Bulletin (Part# TB354): Lysis Buffer, Elution Buffer, and Casework Sample cartridges. This kit was used for the processing of all samples on a solid support such as swabs or fabric. The Technical Bulletin describes a Trace Sample Pre-processing protocol that uses the Tissue and Hair Extraction Kit (Cat. # DC6740) containing: Incubation buffer, Proteinase K, and DTT described in the technical bulletin. This procedure was designed to maximize the DNA recovery from samples containing a small amount of biological material. The protocol is made up of two sub-sections: Trace Samples on Solid Support, and a Semen Stain Protocol (5).

The protocol for trace samples on a solid support was used to pre-process non-sperm samples found on swabs, fabric, or tissue. In a 1.5ml tube containing the sample, 190µl of Incubation buffer and 10µl of 18mg/ml Proteinase K were added. Tubes were briefly vortexed to completely moisten the sample and then incubated at 56° C for 60 minutes. Lysis Buffer (400µl) was added to the digested

sample and the tube was briefly vortexed. The solid support was transferred to a DNA IQ™ spin basket and placed into the 1.5ml sample tube and centrifuged at maximum speed for two minutes at room temperature to release the remaining liquid. The spin basket was discarded and the lysate, approximately 600µl, was transferred to the sample well on the Casework Sample Cartridge to be processed by the Maxwell® 16 (5).

The protocol developed for Semen Stains was used to pre-process samples containing sperm. Sperm samples on either swabs or fabric were placed in a 1.5ml tube. To the 1.5ml tube, 160µl of Incubation buffer, 20µl of 18mg/ml Proteinase K, and 20µl of 1M DTT was added. The tube was briefly vortexed and then incubated overnight at 70° C. Lysis Buffer (400µl) was added to the digested sperm sample and the tube was briefly vortexed. The swab or fabric cutting was removed and transferred into a DNA IQ™ spin basket and placed back into the 1.5ml sample tube and centrifuged at maximum speed for two minutes at room temperature to recover the remaining liquid. The spin basket was discarded and the recovered lysate, approximately 600µl, was transferred into the sample well on the Casework Sample Cartridge and processed by the Maxwell® 16 (5).

Sample Loading and Purification:

The seal on each Casework Sample Cartridge was carefully removed and approximately 600µl of the lysate was added to well #1 that contains 250µl of Lysis Buffer. A Low Elution Volume (LEV) plunger was loosely placed on well

#8. For each sample, an Elution Tube was filled with 40µl Elution Buffer and placed with the tube open in the appropriate location specified on the Casework Sample Cartridge with the tube open. The Maxwell® 16 instrument was turned on and it automatically ran through its diagnostics. The instrument was then set at the "LEV" operational mode. The prepared Casework cartridges were loaded onto the base-rack of the Maxwell® 16 and the "RUN" command was executed. The Maxwell® 16 initiated the run when the door was closed as prompted by the message on the Liquid Crystal Display (LCD) screen. After the run was completed the elution tubes containing the extracted DNA were capped, stored at 4°C, and the instrument was turned off (17).

Organic Extraction:

For comparison, replicates of the samples processed using the Maxwell® 16 were also processed using UNTHSC's forensic lab organic extraction method. UNTHSC has a protocol for non-sperm samples (Appendix A); a protocol for sperm samples (Appendix B); a protocol for hair samples (Appendix C); and a protocol for bone samples (Appendix D). After completing the appropriate protocol, the DNA extracts were stored at 4° C.

Quantification:

The AB Quantifiler® Human DNA Quantification Kit was used to determine the quantity of DNA recovered from each sample. The quantification

was performed as described in their user manual. Quantification standards were prepared by diluting the 200ng/µl stock solution to: 50, 16.7, 5.56, 1.85, 0.62, 0.21, 0.068 and 0.023ng/µl in TE^{-4} Buffer (10mMTris-Cl pH 8.0, 0.1mM Na_2EDTA). An amplification Master Mix was prepared by combining 10.5µl of Primer Mix and 12.5µl of Quantifiler PCR Reaction Mix for each sample. For each sample or standard, 23µl of the amplification Master Mix was added to an AB 96-well Optical Reaction Plate. To the appropriate well 2µl of either a standard or DNA sample extracts was added. The optical plate was then covered with a clear AB Optical Adhesive Cover which was then firmly fixed using a plastic applicator. The plate was then placed into the 96-well sample block of an AB 7500 Real-Time PCR instrument. The SDS software used by the Real-Time PCR instrument was programmed for a 25µl reaction with the following cycle parameters: 10 min hold at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. After the amplification process was completed (in approximately 105 minutes), data analysis was performed by the SDS software to generate a standard curve data using the quantification standards. The quantification of the DNA samples was determined by the SDS software in comparison with the quantification standards (11).

Amplification:

PCR amplification was performed using the PowerPlex® 16 System on an AB GeneAmp® 9600 Thermal Cycler. DNA was amplified in a total reaction

volume of 25µl in MicroAmp® plates or in 0.2 ml MicroAmp® reaction tubes. Each reaction included 2.5µl of Gold STAR 10X Buffer, 2.5µl of 10X primer pair mix, 0.8µl of (5u/µl) AmpliTaq Gold® DNA Polymerase, and DNA template (up to 1ng of sample) in 19.2µl of nuclease-free water. The DNA was amplified following the cycling parameters recommended by Promega Corporation: a hot start at 95°C for 11 minutes followed by a hold at 96°C for 1 minute; for the first 10 cycles, a denaturation at 94°C for 30 seconds, primer annealing at 60°C for 30 seconds, and an extension at 70°C for 45 seconds. For an additional 22 cycles, a denaturation at 90°C for 30 seconds, a primer annealing at 60°C for 30 seconds, and an extension at 70°C for 45 seconds. Following the cycling reaction there was a 60°C extension hold for 30 minutes, followed by a final hold at 4°C to preserve the amplified DNA (18).

Electrophoresis and Analysis:

The Promega PowerPlex® 16 System was designed to co-amplify sixteen Short Tandem Repeat (STR) loci that include: Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. The fluorescently labeled amplified products were analyzed using a 3130*xl* AB Genetic Analyzer. To prepare the samples for analysis, 9.5µl of Hi-Di Formamide was mixed with 0.5µl of Internal Lane Standard (ILS) which contains DNA fragments ranging from 80 to 500 base pairs in length labeled with a CXR fluorescent dye. To a MicroAmp® Optical 96-

well reaction plate, 1.0µl of PCR product or PowerPlex® 16 allelic ladder was added to 10µl of the Formamide-ILS mix. The samples were denatured at 95° C for 3 minutes and quick chilled in an ice-water bath for 3 minutes (18). The AB 3130*xl* Genetic analyzer auto loads 16 samples simultaneously into each of the capillaries. The fluorescently labeled PCR products are loaded into the AB 3130*xl* Genetic analyzer electrophoresed through AB POP-4 polymer and detected using a 9.9mW laser and a CCD camera.

The 3130*xl* Genetic Analyzer generated STR electropherograms using GeneMapper ID *v*3.2 software. DNA fragments were sized in relationship to the ILS size fragments, and alleles were assigned to each peak in a sample by comparison to an allelic ladder specific for each of the STR loci. Only peaks that above the 100 Relative Fluorescence Unit (RFU) threshold were considered true peaks and given an allele designation.

Sample Preparation to Assess DNA Extraction Yields:

White blood cells (WBC), keratinocytes, and sperm cells were diluted in Isoton® III (Beckman Coulter, Miami, FL) to uniformly suspend the cells. Cell counts were obtained microscopically using a hemocytometer. Varying amounts of WBC (6,500 cells/µl), keratinocytes (16,500 cells/µl), and sperm cells (40,000 cells/µl) were spotted on Dacron swabs and on 1.5cm^2 denim jeans cuttings. The denim jeans were washed using a detergent and then thoroughly rinsed in water prior to the application of samples. The WBC's were spotted at: 32,500; 13,000,

6,500; 3,250; and 650 cells. The sperm were spotted at 200,000; 80,000, 40,000; 20,000; and 4,000 cells. The keratinocyte cells were spotted at 82,500; 33,000; 16,500; 8,250; 1,650 cells. For each of the 5 different counts of the WBC, sperm, and keratinocytes, twelve swabs and twelve jeans cuttings were prepared. This equated to a total of 60 swabs and 60 jean swatches for WBC, sperm and keratinocytes.

Six sets of the swabs and six sets of the jeans cuttings containing the WBCs, sperm cells, and keratinocytes were processed using the Maxwell® 16, while the other six sets of swabs and six sets of cuttings were processed using the organic DNA extraction method (5) (Appendices A and B). The DNA from both the Maxwell® 16 and the organic extraction procedure were eluted/re-suspended in a final volume of 40µl. The DNA from all replicates was quantified using the AB Quantifiler™ kit on the AB 7500 Real-Time PCR System. The total amount of DNA recovered was calculated for each cell type, and the percent recovered was calculated by dividing the amount recovered by the theoretical amount of DNA that was contained within the samples (calculated by multiplying the number of cells by 0.007ng DNA per diploid cell (blood and keratinocyte), or 0.0035ng DNA per haploid cell (sperm).

Sample Preparation to Assess Reproducibility and Precision:

In order to assess the reproducibility and precision of the Maxwell® 16 and the DNA IQ™ Casework Sample Kit, blood from two donors was collected

and diluted in Isoton® III to uniformly suspend cells in order to minimize pipetting errors. For donor 1, a total of 5,600 WBCs were spotted on eight swabs and 3,250 WBCs were spotted on an additional eight swabs. For donor 2, a total of 4,600 WBCs were spotted on eight swabs and 2,300 WBCs were spotted on an additional eight swabs. Half of the blood swab replicates were then processed using the Maxwell® 16 (5). The remaining half was processed using UNTHSC's current DNA extraction method (Appendix A).

The recovered DNA was quantified and standard deviations (SD) for both the Maxwell® 16 and organic extraction DNA yield results were calculated. The coefficient of variation was computed in order to compare the variation in DNA yield between the Maxwell® 16 and the organic extraction method. DNA obtained using the Maxwell® 16 and the organic extraction procedure was amplified using the PowerPlex 16® system, analyzed on the AB 3130*xl* Genetic Analyzer, and the electrophoretic data was interpreted using GeneMapper® ID *v*3.2.

Sample Preparation to Assess Cross-contamination:

Semen (1.0ml) was suspended in 5.0ml Phosphate Buffer Solution (1 X PBS) and a sperm cell count was estimated microscopically using a hemocytometer. Twenty-four swabs were spotted with 256,000 sperm cells, while an additional twenty-four swabs were spotted with 25,600 sperm cells. All 48 semen samples were processed using the Maxwell® 16 (5). A total of forty-eight

blank samples, consisting of DNA-free lysis buffer (400µl) which had been added to the Casework Sample cartridges, were processed along with the forty-eight semen samples in a total of six runs.

For each of the six runs, the 16 cartridges containing the sample lysates or the DNA-free lysis buffer for blanks, were arranged on the Maxwell® 16 base rack as seen in Figure-3.1 below. To refer to a sample or a blank, the run number (1 through 6) and cartridge position (1 through16) is used in the form (run # . cartridge position). In order to detect the presence of DNA, the elution buffer for the forty-eight blank cartridges was quantified using AB 7500 Real-Time PCR system and then amplified using the PowerPlex 16® system. Post-PCR analysis was conducted in order to confirm the presence of PCR product and the occurrence of contamination.

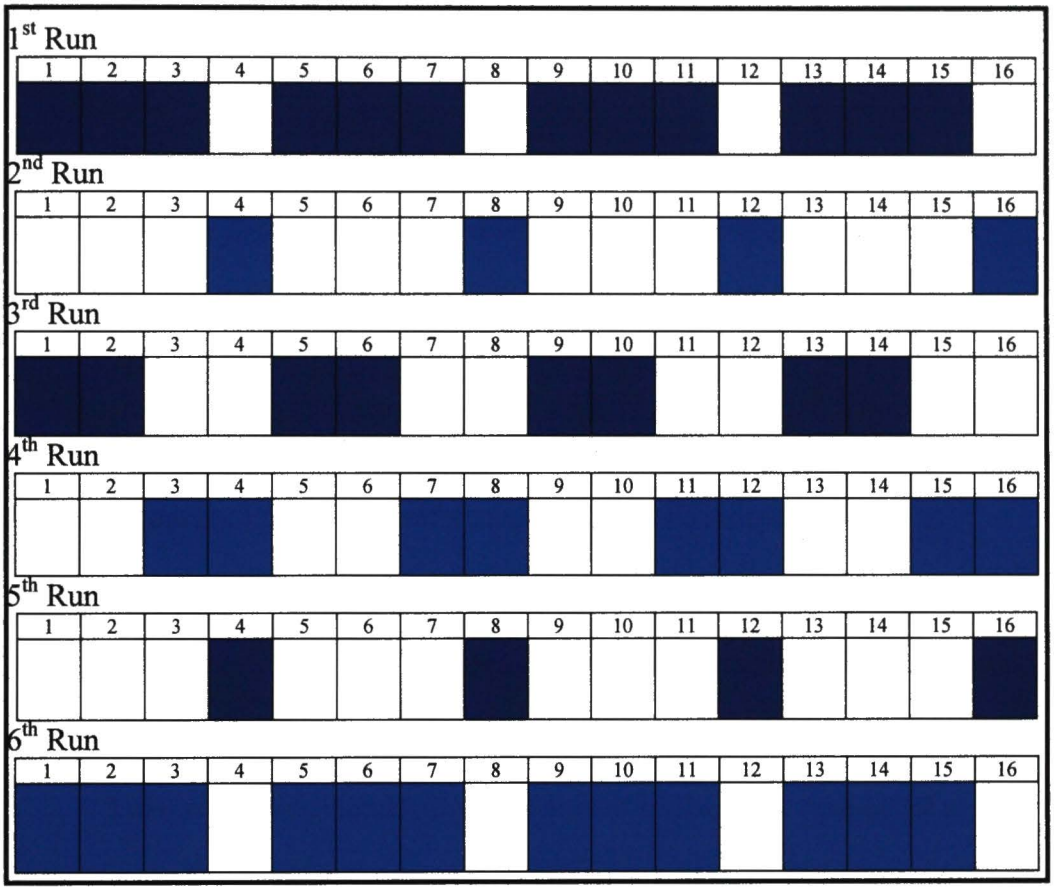

Figure-3.1: The different arrangements of cartridges on the Maxwell® 16 base rack. Cartridges in a run are numbered 1 through 16, and the order of the runs is indicated by the run numbers 1 through 6.
Legend: 256,000 sperm cell sample (dark blue); 25,600 sperm cell sample (light blue), and blanks (white) shown by cartridge numbers 1 through 16

Sample Preparation to Assess Concordance:

Sexual Assault Samples:

To simulate sexual assault evidence, nine plain vaginal swabs were collected from a female donor who had abstained from sexual intercourse for more than one week. Three vaginal swabs were spotted with 25,600 sperm cells, another three vaginal swabs were spotted with 128,000 sperm cells, and an additional three swabs were spotted with 256,000 sperm cells. The male and female donors each provided a reference buccal swabs samples in order to determine their profile. In addition to the simulated sexual assault swabs, three post-coital vaginal swabs were obtained from an anonymous couple following intercourse. These two individuals also provided reference buccal swab samples.

Each of the nine simulated sexual assault swabs, three post-coital swabs, and reference buccal swabs were each dissected in half using a sterile blade. Both halves of the mock sexual assault samples and the post-coital samples were processed with the Promega Differex™ System. The Differex™ System was designed to separate sperm cells and epithelial cells from sexual assault evidence by phase separation and differential centrifugation. Sexual assault swabs were incubated in 400µl of Digestion Buffer (Promega incorporates a yellow dye to simplify the tracking of the aqueous phase) containing 276µg/ml Proteinase K at 37° C for 2 hours. Following incubation, the Digestion Buffer and the swab were transferred into a DNA IQ™ spin-basket and placed in 1.5ml microcentrifuge tube which contained 100µl of a clear, non-aqueous Separation Solution. The tube

was centrifuged at 13,200 rpm for 2 minutes. The aqueous phase containing the epithelial cell DNA in the Digestion Buffer remains on the top layer while the sperm heads are pelleted through the non-aqueous Separation Solution found at the bottom of the 1.5ml tube. The top layer with the epithelial cell DNA was transferred to another 1.5 ml tube for further purification. A total of 500µl of nuclease-free water was carefully added to the top of the non-aqueous Separation Solution layer for 30 seconds at room temperature. The nuclease-free water wash was used to remove any traces of epithelial DNA. The nuclease-free water was removed and the wash step was repeated two additional times (6) (8).

The aqueous layer containing the epithelial DNA and the non-aqueous Separation Solution layer containing the sperm heads were then further purified using the Maxwell™ instrument and the DNA IQ™ Casework Sample Kit. The other half of each sample processed with Differex™ System (both aqueous and non-aqueous Separation Solution layer) was then further purified using the organic DNA extraction method. DNA from reference buccal swabs was extracted using the organic extraction method as well (Appndixes A and B).

Lifted-fingerprint and Touch Samples:

Two fingerprints for each of six different donors were obtained by directly pressing their fingers on the adhesive side of cellophane tape. After the fingerprints were obtained, the non-adhesive surface was cleaned using 70%

alcohol. Each of the 12 fingerprints was cut in half so that each fingerprint could be processed with both the Maxwell® 16 and organic extraction methods.

In order to collect "Touch DNA" samples, common household and office items were swabbed with a Dacron swab pre-moistened with 70% alcohol. For each item, two Dacron swabs were held together and were used to collect any cells that were on the surface. "Touch DNA" was attempted from: a steering wheel; the mouth area of a soda can; the ear piece of an office phone; and a computer keyboard. In addition, a 25-year-old cigarette butt was collected and preserved as a "Touch DNA" sample.

DNA from half of each fingerprint, one of the two "Touch DNA" swabs, and half of the cigarette butt filter paper was extracted using the Maxwell® 16 instrument and the DNA IQ™ Casework Sample Kit. The other half of each these samples was processed following the organic DNA extraction protocol (Appendix A).

Hair and Bone Samples:

Twelve hair samples were obtained from a male donor, and an additional twelve hairs from a female donor. For each hair sample, a 3.0 cm long piece from the root end of the hair was cut and rinsed using Terg-A-Zyme™. Each hair sample was then ground in a Micro-tissue grinder following UNTHSC's hair isolation procedure (Appendix C).

Replicates from two different bone samples (B-3286 and B-3473, approximately 2.0g each) that had been previously analyzed at the UNTHSC Center for Human Identification, were powdered, and then decalcified in 0.5M EDTA for 16 hours. The bone powder was then washed three times using DNase-free water. The bone powder was then digested in 500µl of incubation buffer. 1ml of the DNA IQ™ Casework Sample Kit Lysis buffer was then added to the digested bone samples. DNA was extracted from half of the hair and bone samples using the Maxwell® 16, and the other half using the organic extraction method.

Degraded Liver Tissue Samples:

DNA extraction was attempted from degraded liver tissue obtained from an exhumed body. Duplicate sets of degraded liver tissue (each set had 5 tissue samples, with each sample weighing approximately 35-40mg) were prepared. One set of 5 samples was extracted with the Maxwell® 16 instrument in conjunction with the DNA IQ™ Casework Sample Kit, while the other set was processed using the organic extraction method (Appendix A).

CHAPTER IV

RESULTS

DNA Recovery:

Regardless of the type of cell processed, or the number of cells contained within a sample, the organic extraction method consistently recovered more DNA than the Maxwell® 16. The DNA recovery efficiency for the organic method ranged between 11% (WBC) and 94% (Keratinocytes), while the Maxwell® 16 recovery was as low as 3% (WBC) and did not exceed 20% (Keratinocytes).

The percentage of DNA recovered using the Maxwell® 16 was not linear, with a maximum of 14% (3.2ng) from samples containing 3,200 WBC and then declined with higher WBC counts to approximately 7% (Table-4.1). The percentage of DNA recovered using the organic extraction method had a maximum of 92% from samples containing 3,200 WBC. The percent recovery also declined at higher cell counts to less than 60% with the organic extraction method (Table-4.2). Prior to this study our laboratory evaluated an earlier version of the Casework Sample Kit used in conjunction with the Maxwell® 16. The DNA yields from comparable numbers of cells were significantly greater in our earlier study. With 3,200 WBC cells the earlier study recovered approximately 5.0ng as compared to this study 3.2ng, and with 6,500 WBC we previously

recovered 10.4ng of DNA as opposed to this study 3.18ng. In the earlier study, DNA was extracted from liquid blood samples that had been diluted with Isoton® III. In this study an equivalent number of cells were spotted and dried on Dacron swabs. The difference in yields could be due to the inefficient release of DNA from the Dacron swab prior to processing on the Maxwell® 16.

WBC Count	Theoretical DNA Yield (ng)*	Maxwell® 16 DNA Yields (ng) WBC Swab Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	F		
650	4.6	-	-	0.1	0.2	0.2	0.2	0.17	3.74
3,250	22.8	4.3	7.0	2.4	2.0	1.6	1.7	3.20	14.08
6,500	45.5	3.6	2.1	5.0	5.0	2.1	1.3	3.18	6.99
13,000	91	10.36	14.56	7.16	7.12	7.04	4.48	8.45	9.29
32,500	227.5	11.92	14.28	21.7	24.7	11.6	13.0	16.23	7.14

Table-4.1: Maxwell® 16 DNA yields and % Recovery from WBC swabs

WBC Count	Theoretical DNA Yield (ng)*	Organic DNA Yields (ng) WBC Swab Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	F		
650	4.6	0.42	0.68	0.68	0.3	0.7	0.4	0.55	11.98
3,250	22.8	21.3	24.6	21.1	21.0	18	19.9	20.97	92.19
6,500	45.5	16.9	27.2	27.2	13.0	28.8	17.8	21.82	47.96
13,000	91	72.8	80.4	74.8	61.2	56.8	50.8	66.13	72.67
32,500	227.5	150.8	150.8	98.0	104.8	140.4	97.6	123.73	54.39

Table-4.2: Organic extraction DNA yields and % Recovery from WBC swabs

The percentage of DNA recovered using the Maxwell® 16 with sperm cells dried on Dacron swabs had a maximum of 18.5% from samples containing 20,000 sperm cells and declined at higher sperm cell counts (Table-4.3). The percentage of DNA recovered using the organic extraction method remained relatively constant ranging between 65% and 73% (Table-4.4).

Sperm cell Count	Theoretical DNA Yield (ng)*	Maxwell® 16 DNA Yields (ng) Sperm Swab Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	F		
4,000	14	1.5	1.7	0.98	1.7	1.1	1.3	1.4	10.0
20,000	70	11.4	14.3	11.6	15.9	13.6	10.6	12.9	18.5
40,000	140	12.7	21.1	24.0	20.6	12.9	12.3	17.3	12.3
80,000	280	38.8	34.1	27.6	25.4	22.1	24.0	28.7	10.2
200,000	700	132	65.6	86.8	102	146	104	106.4	15.2

Table-4.3: Maxwell® 16 DNA yields and % Recovery from sperm cells swabs

Sperm cell Count	Theoretical DNA Yield (ng)*	Organic Extraction DNA Yields (ng) Sperm Swab Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	F		
4,000	14	11.8	5.9	10.4	13.0	10.4	9.8	10.2	73.1
20,000	70	54.4	58.8	57.2	52.4	52.8	41.6	52.9	75.5
40,000	140	94.8	-	95.2	106	89	90.8	95.2	67.9
80,000	280	205	192	172	150	166	210	182.9	65.3
200,000	700	460	481	471	466	416	424	453.1	64.7

Table-4.4: Organic extraction DNA yields and % Recovery from sperm cell swabs

The percentage of DNA recovered using the Maxwell® 16 with keratinocytes, had a maximum of 19.9% from samples containing 1,650 cells and declined at higher keratinocyte counts to a low of 4.7% (Table-4.5). The percentage of DNA recovered using the organic extraction method was significantly higher and remained relatively constant averaging over 80% at 16,500 cells and above (Table-4.6).

Keratin. Cell Count	Theoretical DNA Yield (ng)*	Maxwell® 16 DNA Yields (ng) Keratinocyte Swab Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	F		
1,650	**11**	2.2	3.9	2.6	1.6	1.8	1.7	2.3	19.9
8,250	**58**	7.3	2.2	4.5	13.5	12.0	6.16	7.6	13.1
16,500	**115**	5.04	7.6	17.4	11.6	3.6	6.6	8.6	7.5
33,000	**232**	12.6	9.8	5.6	12.4	15.8	13.8	11.6	5.0
82,500	**579**	26.0	18.7	39.4	35.4	21.8	22.6	27.3	4.7

Table-4.5: Maxwell® 16 DNA yields and % Recovery from keratinocyte swabs

Keratin. Cell Count	Theoretical DNA Yield (ng)*	Organic Extraction DNA Yields (ng) Keratinocyte Swab Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	G		
1,650	**11**	3.2	2.9	4.09	4.5	4.9	3.5	3.8	33.5
8,250	**58**	46.8	27.2	36.3	32.3	44.6	41.5	38.1	65.6
16,500	**115**	-	112	109	110	107	108	109	94.7
33,000	**232**	222	199	168	180	178	181	188	81.3
82,500	**579**	510	492	610	541	606	472	538	92.9

Table-4.6: Organic extraction DNA yields and % Recovery from keratinocyte swabs

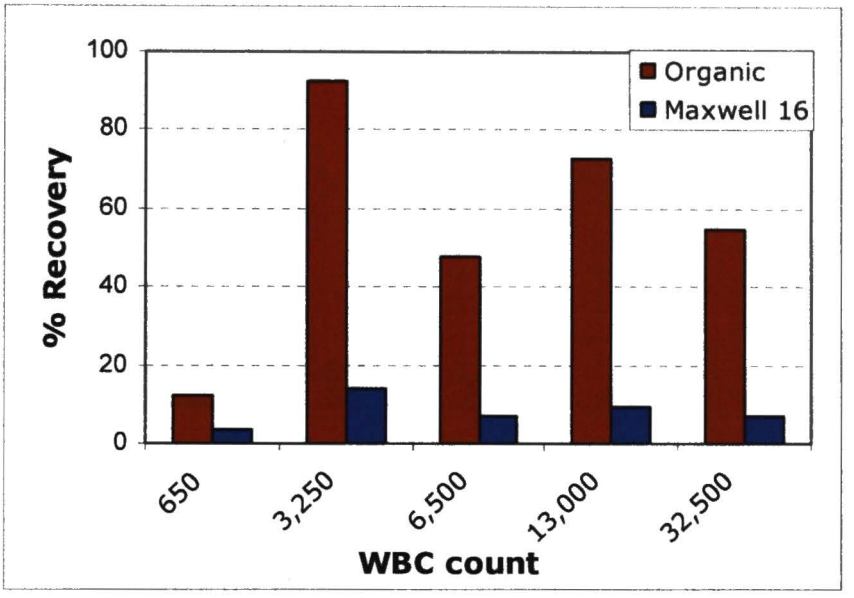

Figure-4.1.: Bar graph of Maxwell® 16 and organic extraction % Recovery from WBC swabs

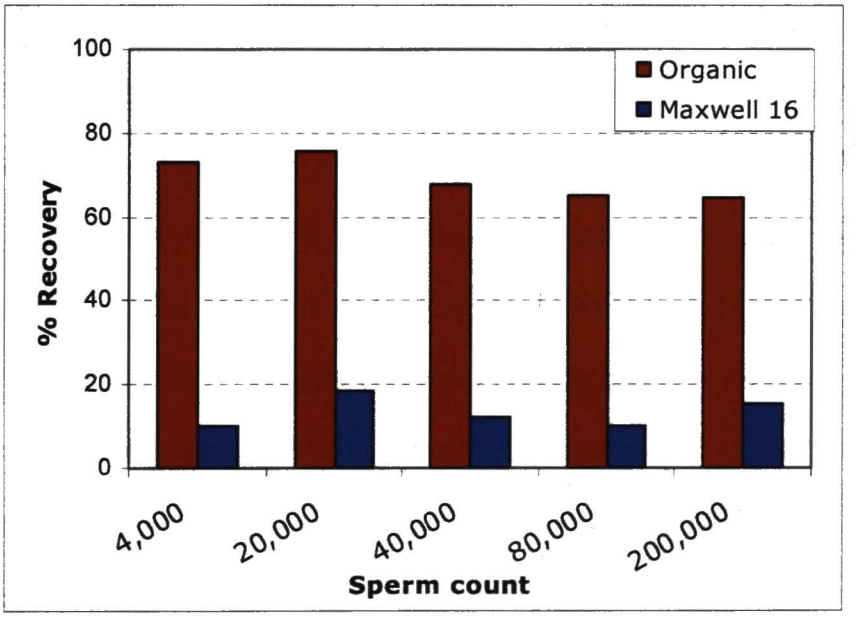

Figure-4.2: Bar graph of Maxwell® 16 and organic extraction % Recovery from sperm cell swabs

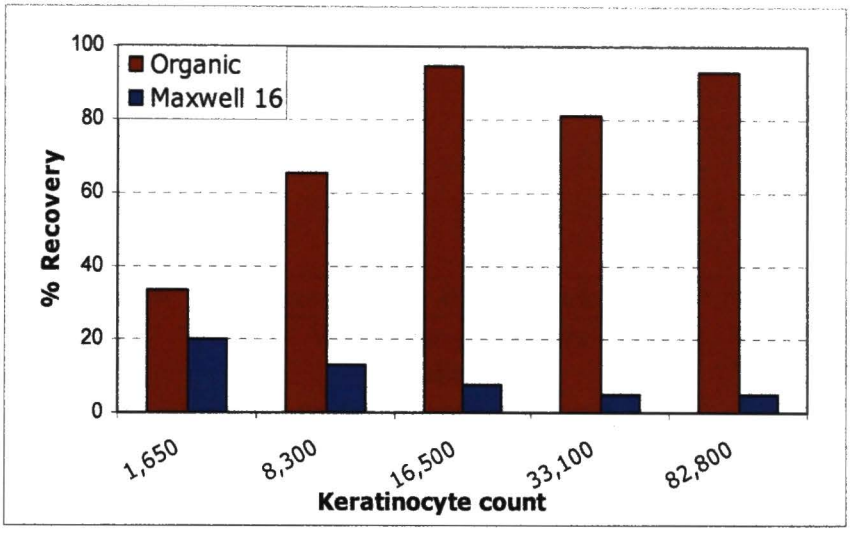

Figure-4.3: Bar graph of Maxwell® 16 and organic extraction % Recovery from keratinocytes swabs

DNA was extracted from varying amounts of blood, sperm, and keratinocycte cells that had been spotted and dried on 1.5cm² denim jeans cuttings. The percentage of DNA recovered using the Maxwell® 16 had a minimum of 0.91% from 200,000 sperm cells (6.36ng of DNA recovered) to a maximum of 25% from 650 WBC (1.13ng of DNA recovered). The DNA obtained from all the denim jeans samples processed with the organic extraction method could not be quantified using Real-Time PCR. The percentage of DNA recovered with the Maxwell® 16 DNA was highest at 25% from 650 WBCs and then declined as the number of WBCs increased to a minimum of 3.68% (Table-4.7). Using sperm cells, the DNA recovery from denim was the lowest amongst all samples used in the study with a high of 4% with 4,000 sperm cells and a minimum of 0.91% with 200,000 sperm cells (Table-4.8). The extraction of DNA from keratinocytes on denim

had a maximum of 16.9% with 1,650 keratinocytes and at a minimum of 4.03% with 82,800 keratinocyte cells (Table-4.9).

WBC Count	Theoretical DNA Yield (ng)*	Maxwell® 16 DNA Yields (ng) WBC Jeans Cuttings Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	G		
650	**4.6**	0.5	1.5	0.5	1.7	1.3	1.2	1.13	25.00
3,250	**22.8**	4.33	2.03	1.7	5.6	1.06	2.5	2.88	12.67
6,500	**45.5**	-	2.7	2.7	6.7	2.6	6.8	4.32	9.50
13,000	**91**	11.4	9.8	-	10.5	6.6	7.2	9.12	10.02
32,500	**227.5**	7.7	9.9	6.8	4.4	10.3	10.9	8.35	3.68

Table-4.7: Maxwell® 16 DNA yield and % Recovery using WBC on denim jeans

Sperm Cell Count	Theoretical DNA Yield (ng)*	Maxwell® 16 DNA Yields (ng) Sperm Jeans Cuttings Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	G		
4,000	**14**	0.3	0.5	0.6	0.1	0.7	1.4	0.57	4.07
20,000	**70**	1.5	2.4	0.9	0.3	3.8	1.9	1.80	2.56
40,000	**140**	2.5	1.8	1.6	-	4.5	1.2	2.33	1.66
80,000	**280**	6.6	6.0	3.2	3.9	3.9	3.2	4.48	1.60
200,000	**700**	0.8	7.7	0.2	9.04	-	14.1	6.36	0.91

Table-4.8: Maxwell® 16 DNA yield and % Recovery using sperm cells on denim jeans

Keratin. Cell Count	Theoretical DNA Yield (ng)*	Maxwell® 16 DNA Yields (ng) Keratinocyte Jeans Cuttings Replicates						Mean DNA Yield (ng)	% Recovery †
		A	B	C	D	E	F		
1,650	**11**	2.7	2.4	1.2	2.5	1.4	1.6	1.95	16.90
8,250	**58**	11.6	9.2	11.8	8.6	6.6	9.2	9.49	16.34
16,500	**115**	10.1	16.5	12.2	11.3	11.5	10.6	12.03	10.41
33,000	**232**	15.2	11.5	-	12.9	9.2	5.7	10.90	4.71
82,500	**579**	20.0	29.1	18.7	20.1	30.8	21.6	23.4	4.03

Table-4.9: Maxwell® 16 DNA yield and Recovery using keratinocytes on denim jeans

* Based on 0.007ng DNA per diploid cells and 0.0035ng DNA per haploid cells

† Calculated as %Recovery = $\frac{\text{mean DNA yield X 100}}{\text{expected DNA yield}}$

- No Detectable DNA

Reproducibility:

The average quantity of DNA extracted from 6,500 WBCs from donor 1 using the Maxwell® 16 (5.4ng) was three times less than the organic method (16.57ng). DNA yields obtained using the Maxwell® 16 had a smaller standard deviation (2.32) than the organic method (4.55). The percent coefficient of variation (%CV) was greater using the Maxwell® 16 (43%) than for the organic extraction (27%) method. Using 3,250 WBCs, the average DNA yield obtained with the Maxwell® 16 (2.22ng) was at least four times less than that of the organic method (10.32ng). Standard deviations decreased overall but the Maxwell® 16 standard deviation (1.08) remained lower than that of the organic method (2.52). The %CV using the Maxwell® 16 (49%) remained greater than that of organic extraction (24%). Results are summarized in Table-4.10.

Donor 1				
	6,500 WBCs		**3,250 WBCs**	
	DNA Yield		**DNA Yield**	
Replicate #	**Maxwell®16**	**Organic**	**Maxwell®16**	**Organic**
1	7.14	23.96	1.29	7.44
2	4.11	20.72	1.54	7.04
3	8.04	15.08	0.46	11.40
4	7.74	18.68	3.28	15.04
5	6.42	10.32	3.25	9.84
6	1.85	11.40	1.81	11.60
7	5.13	16.44	3.11	10.32
8	2.77	15.96	3.00	9.84
Mean	5.40	16.57	2.22	10.32
SD	2.32	4.55	1.08	2.52
%CV	43	27	49	24

Table-4.10: SD and CV for Donor 1 blood DNA yields obtained using the Maxwell® 16 and organic extraction

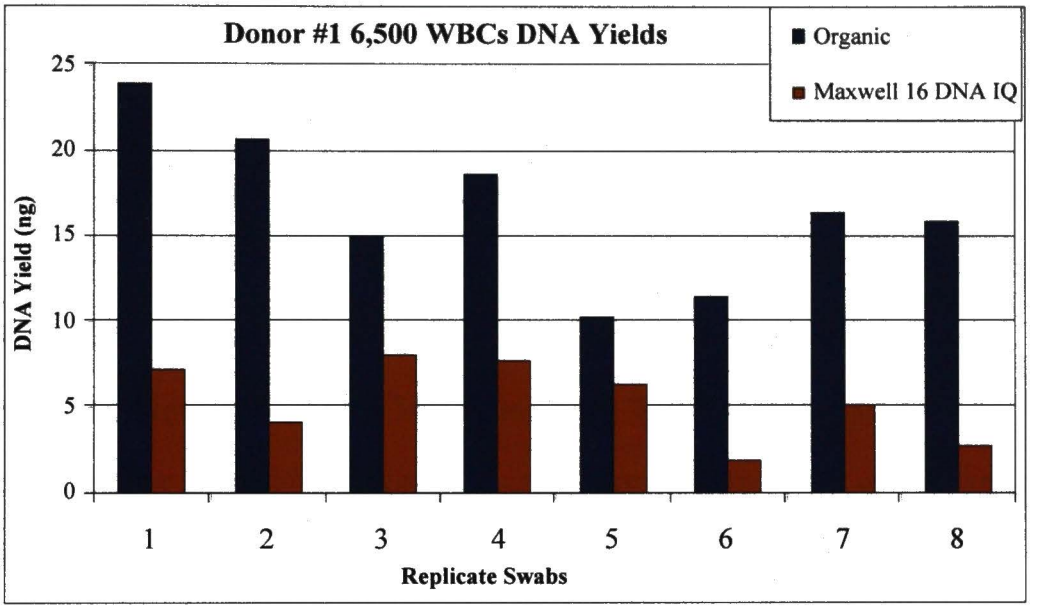

Figure-4.4

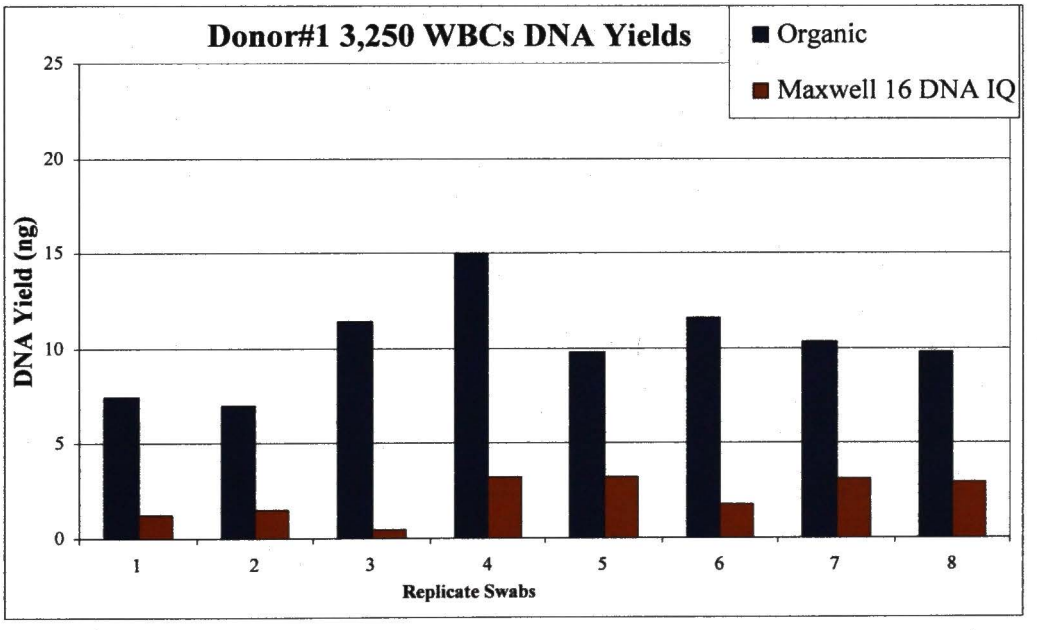

Figure-4.5

Bar graphs illustrating the variability of DNA yields obtained using the Maxwell® 16 and organic extraction from Donor 1 6,500 WBCs (Figure-4.4), and Donor 1 3,250 WBCs (Figure-4.5)

The average quantity of DNA extracted from 4,600 WBC from donor 2 using the Maxwell® 16 (2.47ng) was at least three times less than the organic method (8.5ng). The Maxwell® 16 again had a smaller standard deviation (0.82) than the organic method (1.15). Using 2,300 WBCs from donor 2, the average DNA yield obtained with the Maxwell® 16 (1.46ng) was three times less than that of the organic method (4.53ng). Using 2,300 WBCs, a smaller SD was calculated for DNA yields obtained using the Maxwell® 16 (0.48), while a larger SD was calculated for the organic method (1.68). Results are summarized in Table-4.11.

Donor 2				
Replicate #	4,600 WBCs DNA Yield		2,300 WBCs DNA Yield	
	Maxwell®16	Organic	Maxwell®16	Organic
1	1.42	9.36	1.12	5.12
2	2.63	6.28	2.18	5.40
3	3.91	9.00	1.72	6.48
4	2.08	8.28	1.48	0.77
5	1.98	8.80	0.57	4.04
6	1.73	9.28	1.39	5.00
7	3.10	9.68	1.82	4.88
8	2.90	10.97	1.42	4.56
Mean	2.47	8.50	1.46	4.96
SD	0.82	1.15	0.48	0.77
%CV	33	14	33	15

Table-4.11: SD and CV for Donor 2 blood DNA yields obtained using the Maxwell® 16 and organic extraction

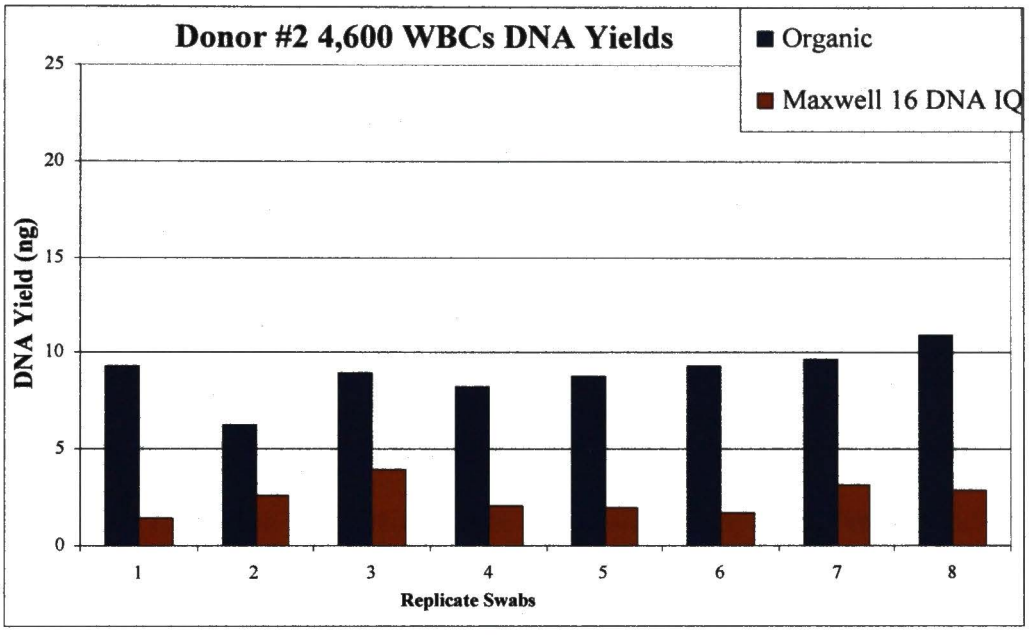

Figure-4.6

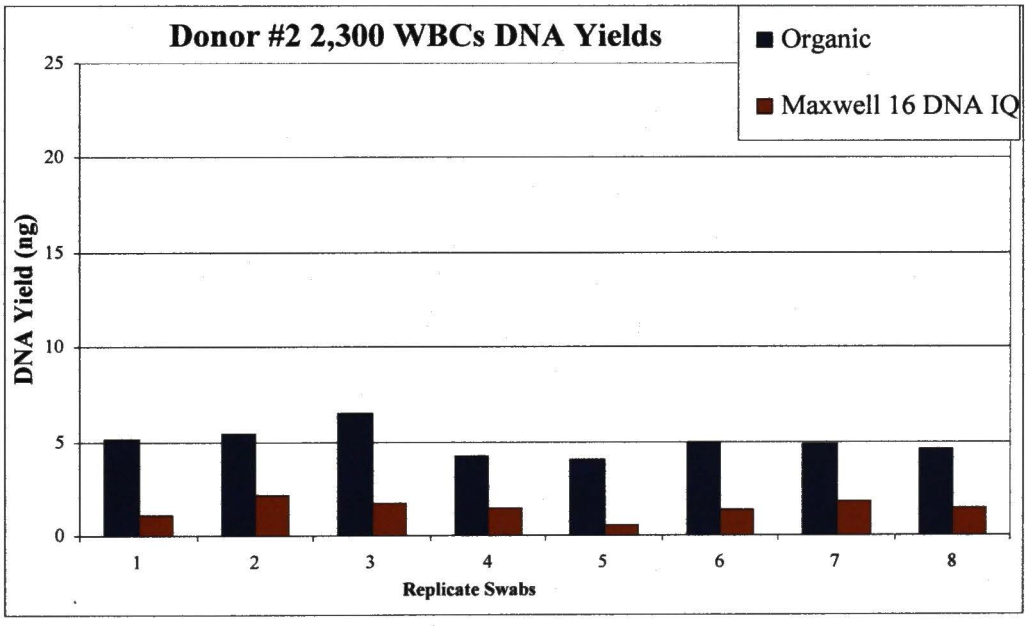

Figure-4.7

Bar graphs illustrating the variability of DNA yields obtained using the Maxwell® 16 and organic extraction from Donor 2 4,600 WBCs (Figure-4.6), and Donor 2 2,300 WBCs (Figure-4.7)

The DNA extracted from the blood swabs using the Maxwell® 16 had an average cycle threshold (Ct) value for the internal positive control (IPC) of 27.1, whereas the Ct threshold for DNA extracted from the replicate swabs with the organic extraction was 29.3. Post-PCR analysis of the STR profiles obtained using the Maxwell® 16 and the organic extraction method reflected the differences indicated by the different Ct values. For example, the DNA extracted by the organic extraction method from samples 2 and 3 containing 5,600 WBC from donor 1, resulted in a partial profile due to allelic dropout at Penta E and/or Penta D. The same samples exhibited a decline in the RFU values as allele the sizes increased from 80 to 500 base pairs giving the characteristic "ski-slope" pattern in electropherograms (Figure-4.8 and 4.9). All other samples (1, 4, 5, 6, 7, and 8) from donor 1, and all samples (1-8) from donor 2 produced full profiles (Figure-4.10 and 4.11). DNA obtained using the Maxwell® 16 resulted in balanced peaks and full profiles from the blood swabs prepared from both donor 1 and donor 2.

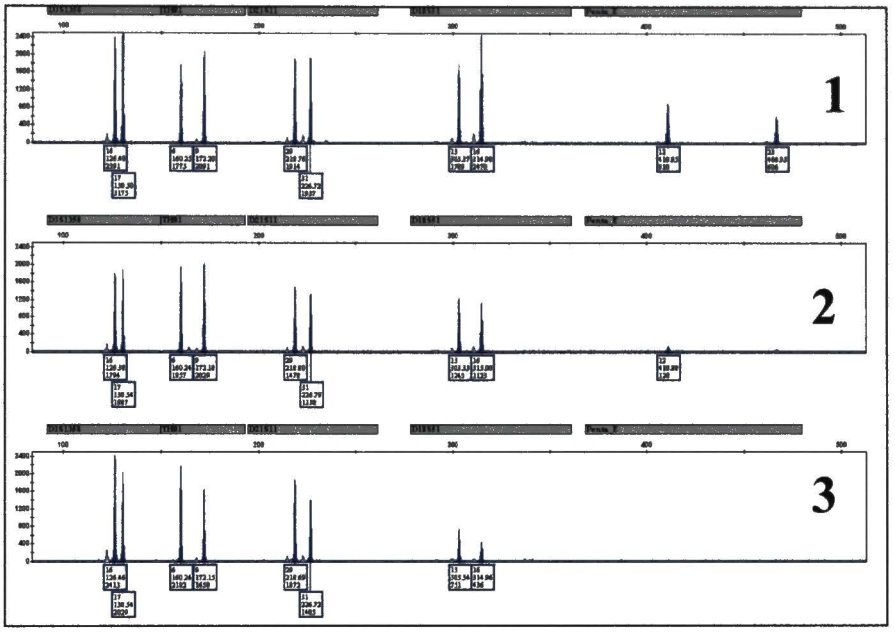

Figure-4.8

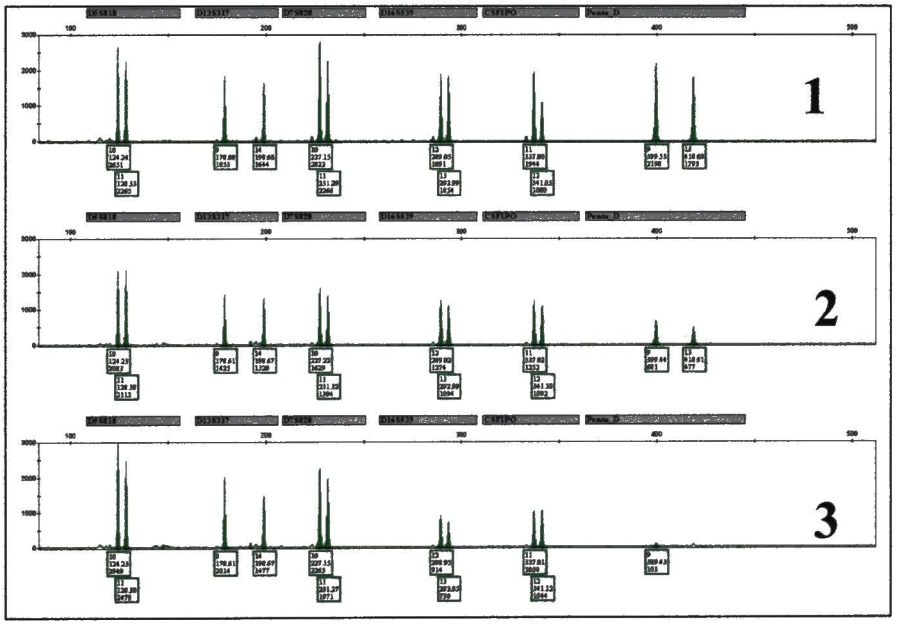

Figure-4.9

STR electropherograms of DNA from Donor 1 6,500 WBCs obtained using organic extraction and showing "ski-slope" pattern or allelic drop-out in samples 2 and 3 blue dye (Figure-4.8) and in samples 2 and 3 green dye (Figure-4.9).

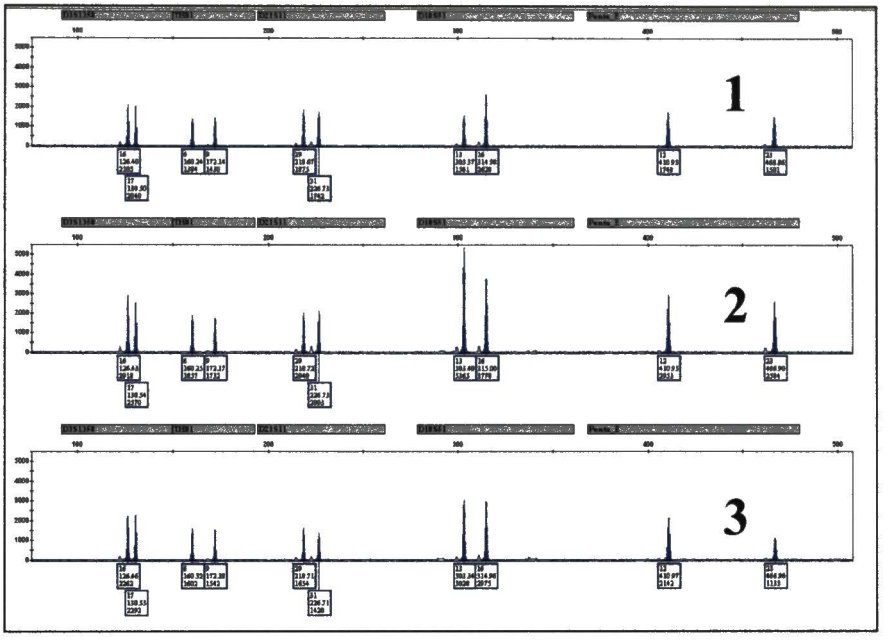

Figure-4.10

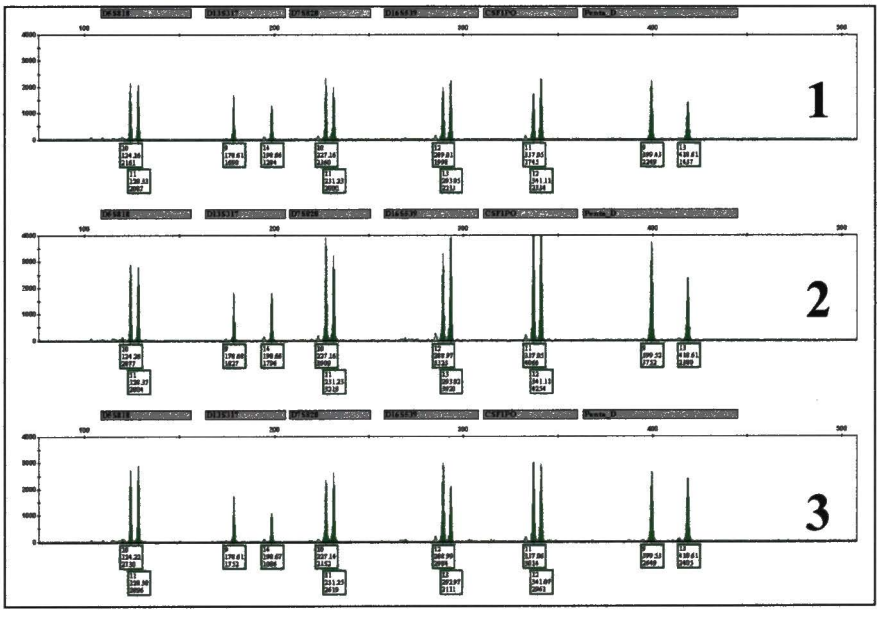

Figure-4.11

STR electropherograms of DNA from Donor 1 6,500 WBCs equivalent blood obtained using the Maxwell® 16 and showing full profiles in representative samples 1, 2, and 3 FL blue dye (Figure-4.10) and in samples 1, 2, and 3 JOE green dye (Figure-4.11).

Cross-contamination:

No cross-contaminating DNA was detected in any of the 48 blanks using quantitative Real-Time PCR. Post-PCR analysis, however, revealed that six of the 48 blanks had detectable STR peaks indicating possible contamination. One blank was traced back to the 1st run (1.12), two to the 4th run (4.2 and 4.10), and three to the 5th run (5.6, 5.10, 5.13). The STR peaks had RFU values ranging from 102 to as much as 319. Only three of the six blanks (4.10, 5.6, 5.10, and 5.13) had what appeared to be a combination of both off-ladder and true alleles, while only off-ladder peaks were observed in the remaining three blanks (1.12, 4.2, and 4.10). None of the true alleles could be attributed to DNA from the sperm samples used in the study. The exceptions were an allele 15 (D3S1358), allele 10 (D7S828), allele 11 (CSF1P0), and an allele 14 (vWA) in blank 5.6; allele 11 (TPOX), and X (AMEL) in blanks 5.10, and 5.13 respectively. The results are summarized in Table 4.12 below.

	Allele Labels (RFU)						Ref. DNA Profile
	Blank Location (Run . Position on Maxwell® 16)						
LOCI	1.12	4.2	4.10	5.6	5.10	5.13	
D3S1358	-	OL(134)	-	15(170), 18(102)	-	OL(214)	14,15
TH01	-	OL(137)	-	-	-	-	6,7
D21S11	-	-	-	-	-	-	29,31
D18S51	-	-	-	13.2(289)	-	14.2 (247)	12,13
Penta E	-	-	-	-	OL (159)	OL(102)	15,17
D5S818	OL(123)	-	-	11(116)	-	OL(185)	12
D13S317	-	-	-	-	-	-	12,13
D7S828	-	-	-	10(147)	-	-	10
D16S539	-	-	8(207)	9(155)	-	-	12,13
CSF1P0	-	-	-	11(107)	OL(112)	OL(171), OL(117)	11,12
Penta D	-	-	-	-	-	-	9,14
vWA	-	-	-	14(102)	-	-	14,16
D8S1179	-	-	-	-	-	-	11,12
TPOX	-	-	OL(319)	8(117)	11(110)	-	10,11
FGA	-	-	-	-	-	-	21,28
AMEL	-	-	-	-	-	X(178)	X,Y

Table-4.12: A table of the STR profiles for the six blanks showing true (red) and off-ladder alleles (black), the peak RFUs, and their respective loci. The six blanks (1.12, 4.2, 4.10, 5.6, 5.10, and 5.13) had signs of possible contamination after processing by the Maxwell® 16

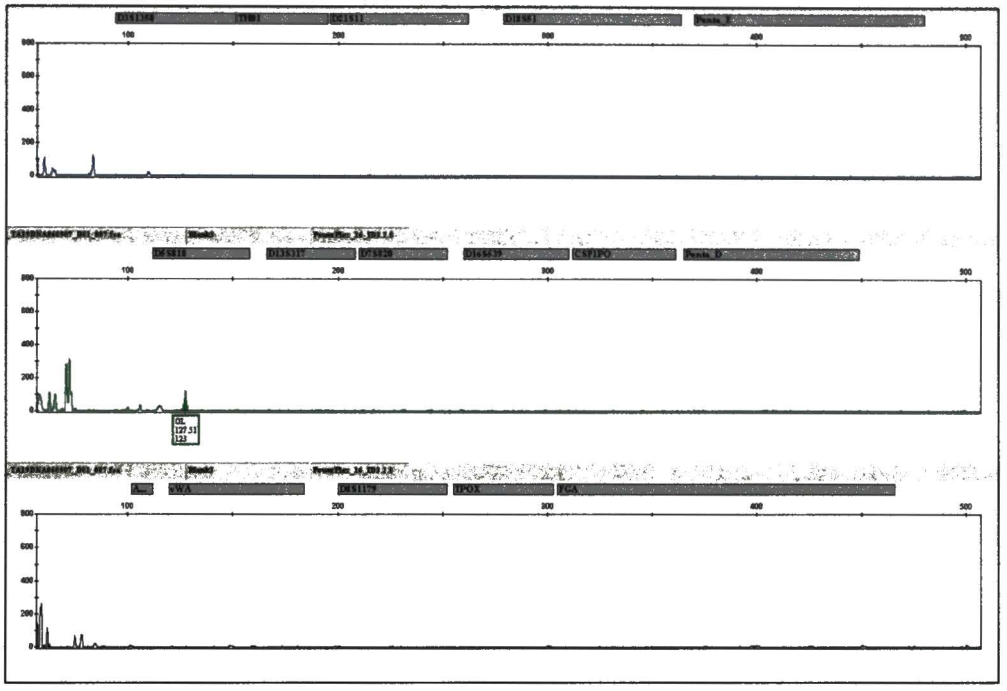

Figure-4.12

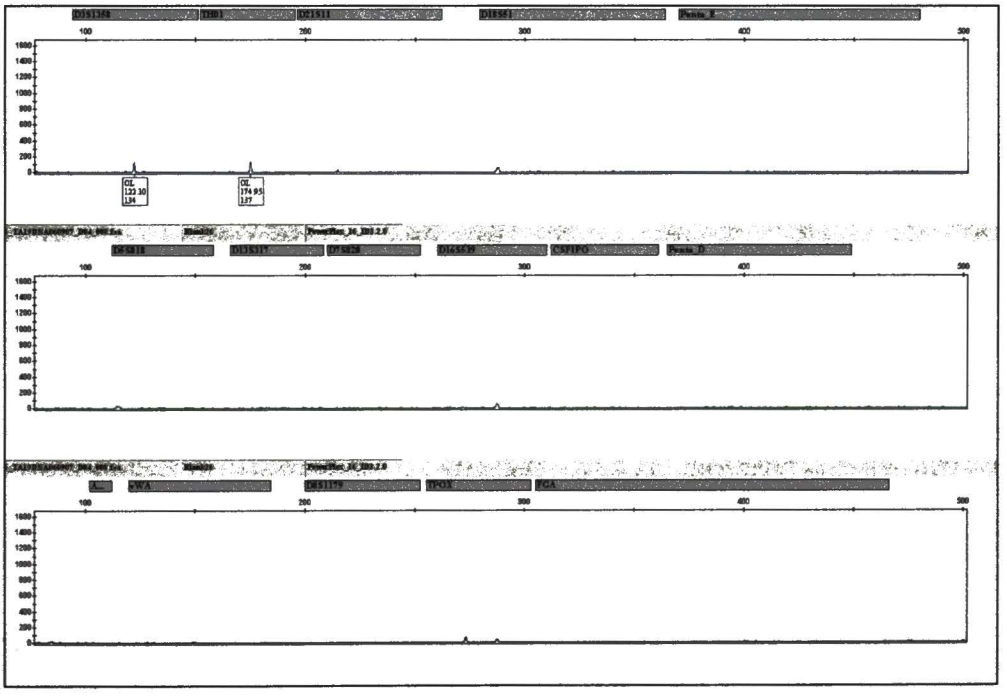

Figure-4.13

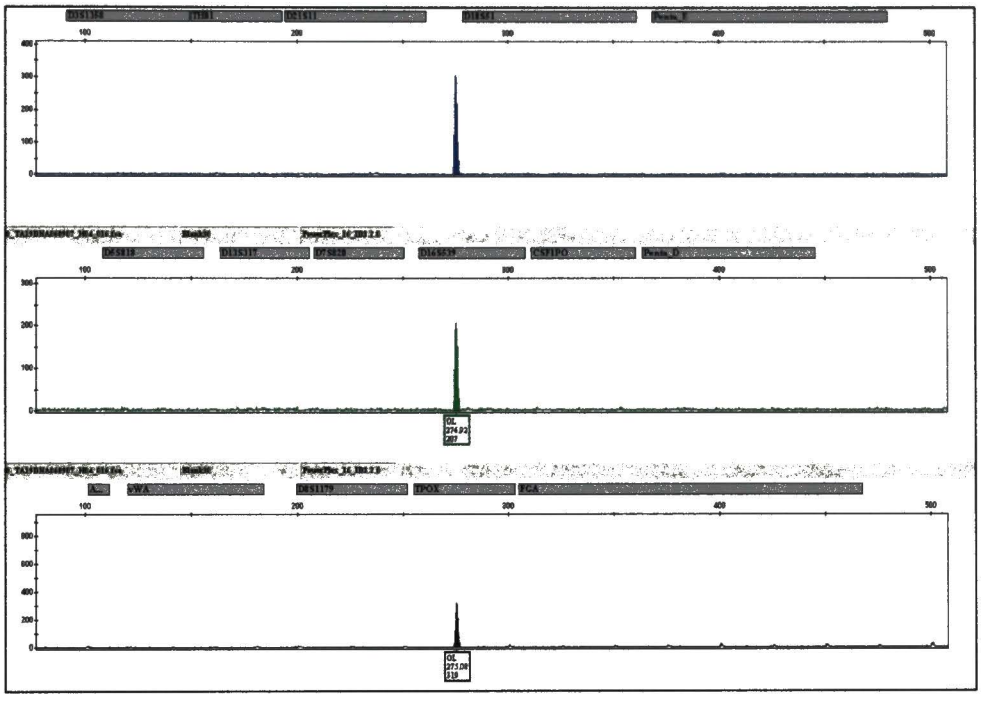

Figure-4.14

STR electropherograms of blank 1.12 (Figure-4.12), blank 4.2 (Figure-4.13), and blank 4.10 (Figure-4.14). Erroneous peaks or spikes labeled as off-ladder peaks are observed in the three blanks

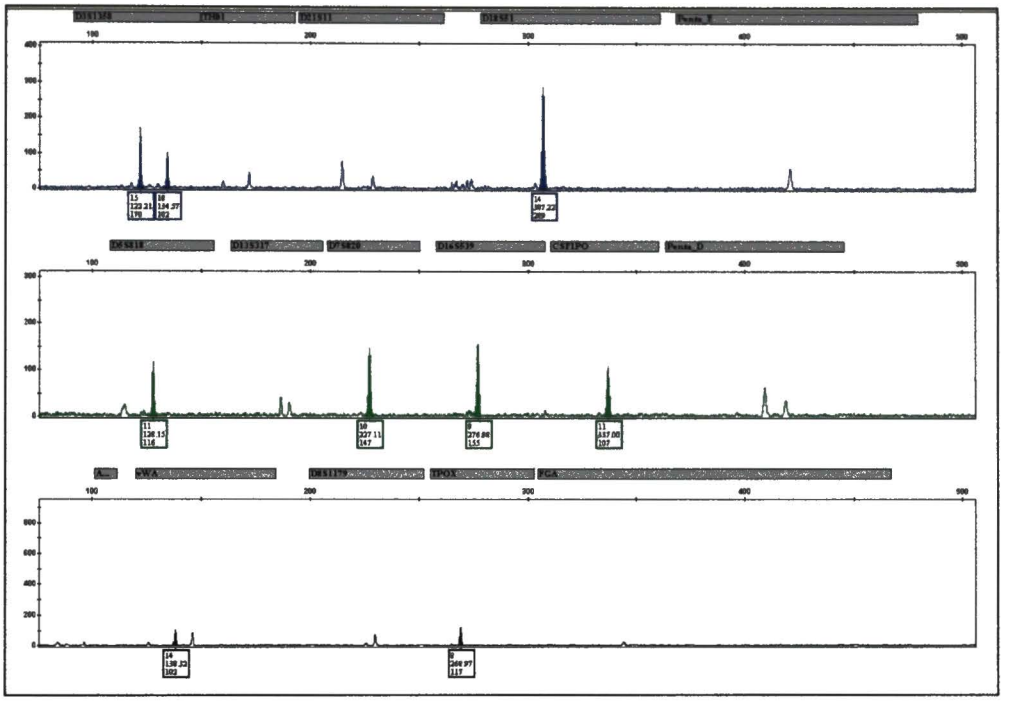

Figure-4.15

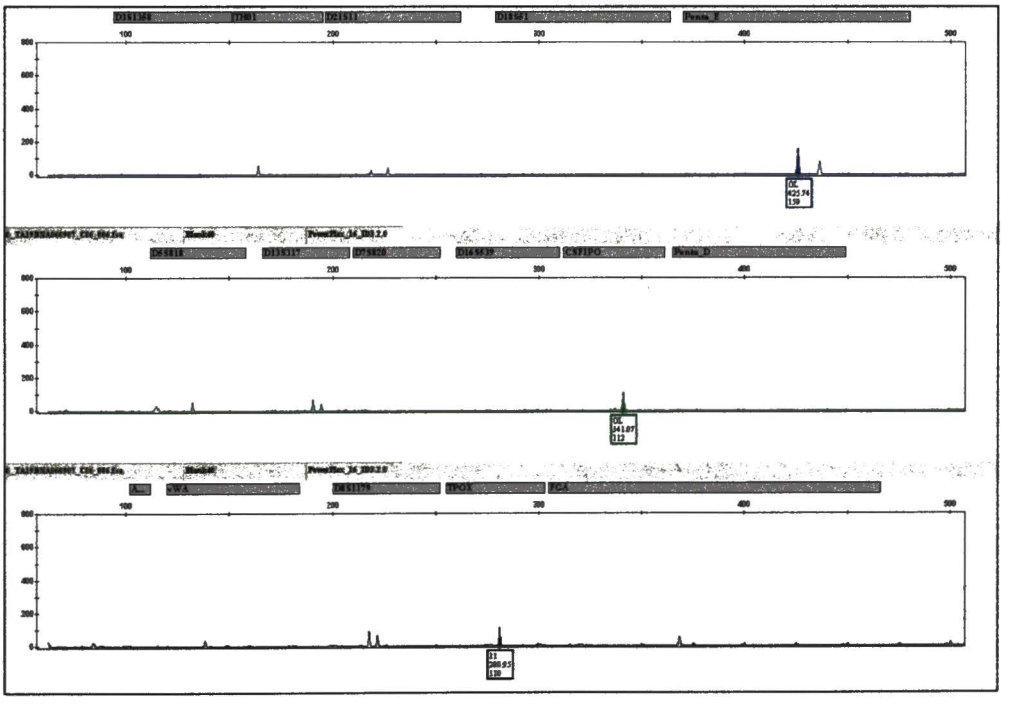

Figure-4.16

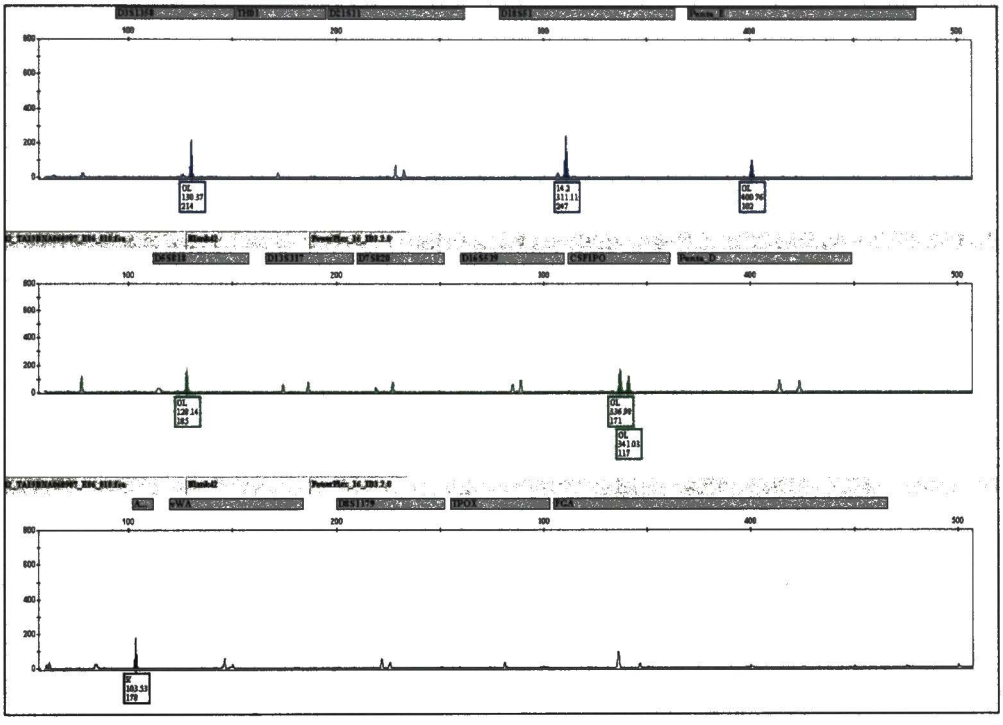

Figure-4.17

STR electropherograms of blank 5.6 (Figure-4.15), blank 5.10 (Figure-4.16), and blank 5.13 (Figure-4.17). A combination of true and off-ladder alleles observed in the three blanks.

Concordance:

Sexual Assault:

Both the Maxwell® 16 and the organic extraction method produced DNA from the epithelial and sperm fractions. The average DNA yield from 25,600 (A), 128,000 (B), and 256,000 sperm cells (C) was 14.5ng, 129ng, and 370ng respectively using the organic extraction method. Using the Maxwell® 16, the average DNA yield from 25,600 (A), 128,000 (B), and 256,000 sperm cells (C) was 7.5ng, 6.6ng, and 10.7ng respectively.

Epithelial Fraction	DNA Yield (ng)	
	Maxwell® 16	Organic
1A	265.5	>2000
2A	291.4	>2000
3A	450.9	>2000
1B	139.4	>2000
2B	192.6	>2000
3B	124.7	>2000
1C	387.2	>2000
2C	280.2	>2000
3C	465.3	>2000
PC-1	88.8	>2000
PC-2	109.2	34.76
PC-3	134.4	>2000

Table-4.13

	DNA Yield (ng)	
	Maxwell® 16	Organic
1A	8.4	15.8
2A	6.6	16.2
3A	7.4	11.6
1B	9.3	185.6
2B	8.3	60.0
3B	2.2	141.2
1C	20.4	210.4
2C	9.3	471.6
3C	19.2	428.0
PC-1	2.7	51.2
PC-2	1.4	163.6
PC-3	28.2	42.4

Table-4.14

Tables of DNA yields obtained from epithelial fraction (Table-4.13) and sperm fraction (Table-4.14) using the Maxwell® 16 and organic extraction. Post-coital (PC) samples DNA yields are included in the tables.

The DNA extracted from all samples using either the Maxwell® 16 or the organic extraction method produced full STR profiles. The profiles obtained with DNA extracted with the Maxwell® 16 matched the respective reference samples profiles and were concordant with the profiles generated with DNA extracted using the organic extraction method. Three epithelial fraction samples (1C, 2C, and 3C) processed by the Maxwell® 16 Samples produced a mixed profile with alleles attributed to DNA from both the male and female donors. Samples 1C, 2C, and 3C exhibited a mixture in all but four loci. The major-minor contributors were easily determined with ratios of at least 3:1. Results are summarized in Table-4.15.

	Epithelial Fraction								
LOCI	**1A**	**2A**	**3A**	**1B**	**2B**	**3B**	**1C***	**2C***	**3C***
D3S1358	+	+	+	+	+	+	+	+	+
TH01	+	+	+	+	+	+	+	+	+
D21S11	+	+	+	+	+	+	+	+	+
D18S51	+	+	+	+	+	+	+	+	+
Penta E	+	+	+	+	+	+	+	+	+
D5S818	+	+	+	+	+	+	+	+	+
D13S317	+	+	+	+	+	+	+	+	+
D7S828	+	+	+	+	+	+	+	+	+
D16S539	+	+	+	+	+	+	+	+	+
CSF1PO	+	+	+	+	+	+	+	+	+
Penta D	+	+	+	+	+	+	+	+	+
vWA	+	+	+	+	+	+	+	+	+
D8S1179	+	+	+	+	+	+	+	+	+
TPOX	+	+	+	+	+	+	+	+	+
FGA	+	+	+	+	+	+	+	+	+
AMEL	+	+	+	+	+	+	+	+	+

Table-4.15: Table of epithelial samples STR profiles resulting from Maxwell® 16 processing and illustrating overall concordance. (Legend found in footnote at the end of the chapter)

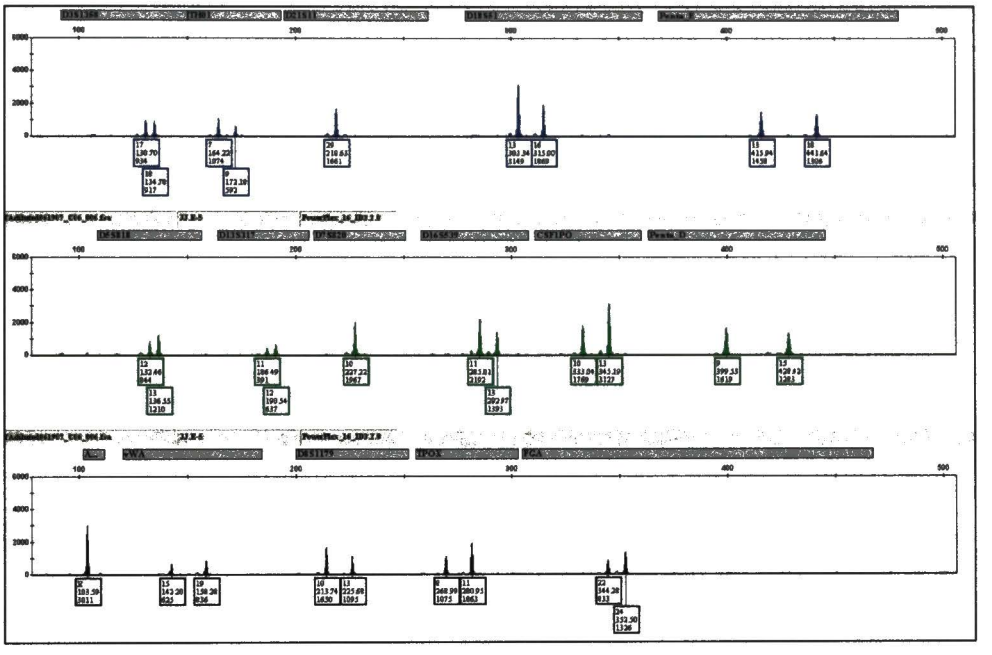

Figure-4.18: STR electropherogram of a representative epithelial fraction sample resulted from DNA obtained using the Maxwell® 16

DNA extracted from five sperm fraction samples (2A, 3A, 1B, 3B, and 3C) using the Maxwell® 16 resulted in a profile showing a mixture. However, it was possible to separate the profiles into a major and a minor contributor were there was at least in a 2:1 ratio; the major contributor lowest RFU was 1605 and the minor contributor highest RFU was 506. The results from the Concordance study are summarized in Table-4.16. One post-coital epithelial fraction sample (PC-1) resulted in a mixture profile with more than three alleles at the loci TH01, D5S818, and D16S539 when processed with the Maxwell® 16. The three post-coital sperm fraction samples processed using the Maxwell® 16 resulted in a mixture profile at one locus for PC-1, three loci for PC-2, and ten loci for PC-3. A

major and a minor contributor could be determined for the mixture profiles based on significant differences between the lowest RFU values for the major contributor (1,117) and the minor contributors highest RFU (469). None of the STR profiles from the sperm or epithelial fractions processed using the Maxwell® 16 had any drop-out. A reliable comparison could be made between all post coital epithelial or sperm fractions and their respective reference samples. The STR profiles generated from the DNA obtained from both the epithelial and sperm fraction matched the profiles generated from the DNA obtained from the known reference samples. Concordance results for post-coital samples are summarized in Table-4.17.

LOCI	Sperm Fraction								
	1A	2A*	3A*	1B*	2B	3B*	1C	2C	3C*
D3S1358	+	+	+	+	+	+	+	+	+
TH01	+	+	+	+	+	+	+	+	+
D21S11	+	+	+	+	+	+	+	+	+
D18S51	+	+	+	+	+	+	+	+	+
Penta E	+	+	+	+	+	+	+	+	+
D5S818	+	+	+	+	+	+	+	+	+
D13S317	+	+	+	+	+	+	+	+	+
D7S828	+	+	+	+	+	+	+	+	+
D16S539	+	+	+	+	+	+	+	+	+
CSF1PO	+	+	+	+	+	+	+	+	+
Penta D	+	+	+	+	+	+	+	+	+
vWA	+	+	+	+	+	+	+	+	+
D8S1179	+	+	+	+	+	+	+	+	+
TPOX	+	+	+	+	+	+	+	+	+
FGA	+	+	+	+	+	+	+	+	+
AMEL	+	+	+	+	+	+	+	+	+

Table-4.16: Table of sperm fraction samples STR profiles resulting from Maxwell® 16 processing and illustrating overall concordance.(Legend found in footnote at the end of the chapter)

	Epithelial Fraction			Sperm Fraction		
	1*	2	3	1*	2*	3*
D3S1358	(+)	(+)	(+)	(+)	(+)	(+)
TH01	(+)	(+)	(+)	(+)	(+)	(+)
D21S11	(+)	(+)	(+)	(+)	(+)	(+)
D18S51	(+)	(+)	(+)	(+)	(+)	(+)
Penta E	(+)	(+)	(+)	(+)	(+)	(+)
D5S818	(+)	(+)	(+)	(+)	(+)	(+)
D13S317	(+)	(+)	(+)	(+)	(+)	(+)
D7S828	(+)	(+)	(+)	(+)	(+)	(+)
D16S539	(+)	(+)	(+)	(+)	(+)	(+)
CSF1PO	(+)	(+)	(+)	(+)	(+)	(+)
Penta D	(+)	(+)	(+)	(+)	(+)	(+)
vWA	(+)	(+)	(+)	(+)	(+)	(+)
D8S1179	(+)	(+)	(+)	(+)	(+)	(+)
TPOX	(+)	(+)	(+)	(+)	(+)	(+)
FGA	(+)	(+)	(+)	(+)	(+)	(+)
AMEL	(+)	(+)	(+)	(+)	(+)	(+)

Table-4.17: Table of epithelial and sperm fractions STR profiles from post-coital samples illustrating overall concordance (Legend found in footnote at the end of the chapter)

Lifted-fingerprints and Touch Samples:

The Maxwell® 16 yielded DNA that was detectable by Real-Time PCR from only seven of the twelve fingerprints lifted using scotch tape. The DNA yields ranged from 0.01 to 0.1ng. All touch samples that were processed by the Maxwell® 16 yielded detectable quantities of DNA; from as low as 0.03ng (phone) to as high as 1.08ng (soda can). The organic extraction method, however,

obtained detectable DNA from eleven out of twelve lifted fingerprints with DNA yields ranging from 0.2 to 2.6ng. As for the touch samples, the organic extraction method obtained a DNA yield ranging from 0.2ng (window) to 17.23ng (soda can). The biggest difference in DNA yields was observed in the phone swab; the organic extraction method obtained 80 times more DNA than the Maxwell® 16. Results are summarized in Table-4.18 below.

Fingerprint Samples	DNA Yield (ng)	
	Maxwell® 16	Organic
1A	0	0.68
1B	0.05	0
2A	0	0.09
2B	0.01	0.24
3A	0.03	0.48
3B	0	0.23
4A	0.10	0.19
4B	0.06	0.49
5A	0	2.70
5B	0.00	0.77
6A	0.02	0.68
6B	0.09	0.70
Soda can	1.09	17.32
Keyboard	0.91	5.24
Phone	0.03	2.41
Steering wheel	0.14	3.57
Cig. Butt	0.16	0.30

Table-4.18: Table of lifted-fingerprint and touch sample DNA yields obtained using the Maxwell® 16 and the organic extraction method

Post-PCR analysis of the DNA obtained using the Maxwell® 16 from two lifted fingerprints (4A and 6A) resulted in partial profiles. No profiles were obtained from the other ten fragments. The partial profiles generated from the lifted fingerprints (4A and 6A) were consistent with the profiles from the reference sample obtained from the know contributor. However, DNA from one lifted fingerprint (1B) generated a partial profile that was inconsistent with the reference sample and was attributed to contamination from the individual performing extraction procedure. DNA from four touch samples (soda can, keyboard, phone, and steering wheel) resulted in mixture partial profiles that were consistent with their known contributor. The DNA extracted from the cigarette butt sample generated a mixture profile. However, the source of the DNA could not be determined since no known profiles were available for the individual(s) who had smoked the cigarette. Concordance results for these studies are summarized in Table-4.19.

LOCI	STR Results				
	Soda Can	Keyboard	Phone	Steering wheel	Cigarette
D3S1358	+	+	+	+	+
TH01	+	+	+	+	+
D21S11	+	+	+	+	+
D18S51	+	+	+	+	+
Penta E	NR	NR	NR	NR	+
D5S818	+	+	+	+	+
D13S317	+	+	DO	+	+
D7S828	+	+	DO	+	+
D16S539	+	+	+	+	+
CSF1PO	+	+	+	+	+
Penta D	NR	NR	NR	NR	+
vWA	+	+	+	+	+
D8S1179	+	+	+	+	+
TPOX	+	+	+	+	+
FGA	+	+	DO	+	NR
AMEL	+	+	+	+	+

Table-4.19: STR results of touch samples resulting from DNA obtained using the Maxwell® 16 (Legend found in footnote at the end of the chapter)

Post-PCR analysis of DNA obtained using the organic extraction method resulted in a mixture profile from only one lifted fingerprint sample (5A) with no results at the FGA locus. Only DNA obtained from the soda can produced a partial profile. At 6 of the 16 loci detected, the STR profiles were consistent with the reference sample (Figure-4.19 and 4.20). The other loci exhibited allelic drop-out. The remaining seven touch samples, although yielding significant quantities of DNA, produced no STR results.

LOCI	STR Results	
	Finger print 5A	Soda Can
D3S1358	+	DO
TH01	+	+
D21S11	+	+
D18S51	+	DO
Penta E	+	DO
D5S818	+	+
D13S317	+	DO
D7S828	+	+
D16S539	+	DO
CSF1PO	+	DO
Penta D	+	DO
vWA	+	+
D8S1179	+	DO
TPOX	+	DO
FGA	NR	+
AMEL	+	DO

Table-4.20: STR results of lifted-fingerprint and touch samples resulting from DNA obtained using the organic extraction method. (Legend found in footnote at the end of the chapter)

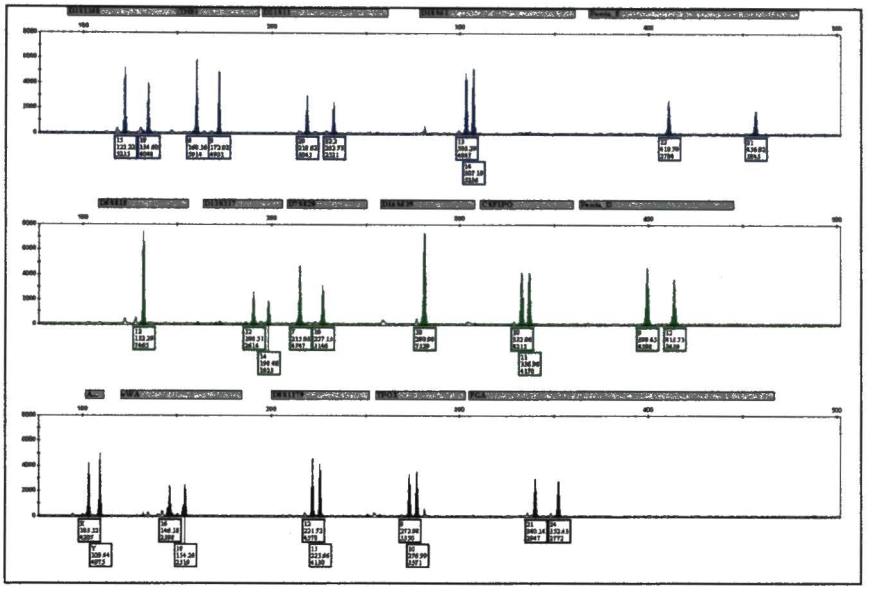

Figure-4.19: STR electropherogram of soda can sample processed by the Maxwell® 16

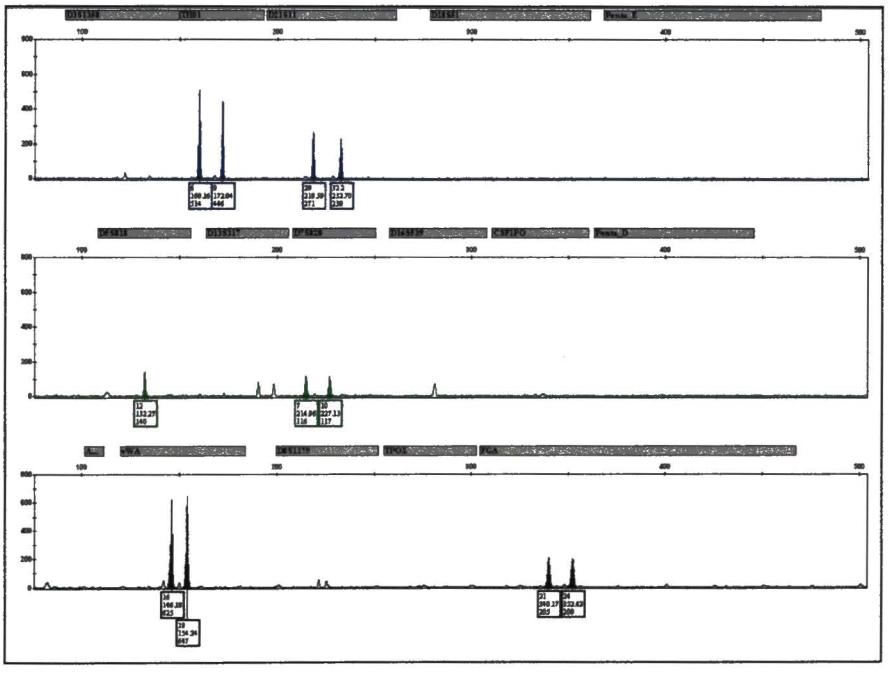

Figure-4.20: STR electropherogram of soda can sample processed by the organic extraction method

Hair and Bone Samples:

Overall, the quantity of DNA extracted from the male donor's hair samples exceeded that of the female with both extraction methods. All hair samples resulted in a significant DNA yield except for two samples (2 and 4) from the female donor. These samples which had been extracted using the organic method resulted in a DNA yield of 0.1ng. The DNA yield results from the hair samples are summarized in Tables-4.21 and 4.22.

Hair Samples (male donor)	DNA Yield (ng)	
	Maxwell® 16	Organic
1	113.2	112.0
2	23.8	158.0
3	24.2	12.2
4	36.8	149.6
5	38.0	125.2
6	28.6	304.0

Table-4.21: Male Hair samples DNA yield

Hair Samples (female donor)	DNA Yield (ng)	
	Maxwell® 16	Organic
1	6.4	27.2
2	11.0	0.2
3	6.9	0.4
4	3.3	0.1
5	6.2	35.4
6	31.8	34.7

Table-4.22: Female Hair samples DNA yield

The STR profiles from the DNA extracted from all of the hair samples processed using the Maxwell® 16 matched the donors profile at all loci. The STR profiles generated from the hair samples are summarized in Table-4.23.

LOCI	Male Hair Donor						Female Hair Donor					
	1	2	3	4	5	6	1	2	3	4	5	6
D3S1358	+	+	+	+	+	+	+	+	+	+	+	+
TH01	+	+	+	+	+	+	+	+	+	+	+	+
D21S11	+	+	+	+	+	+	+	+	+	+	+	+
D18S51	+	+	+	+	+	+	+	+	+	+	+	+
Penta E	+	+	+	+	+	+	+	+	+	+	+	+
D5S818	+	+	+	+	+	+	+	+	+	+	+	+
D13S317	+	+	+	+	+	+	+	+	+	+	+	+
D7S828	+	+	+	+	+	+	+	+	+	+	+	+
D16S539	+	+	+	+	+	+	+	+	+	+	+	+
CSF1PO	+	+	+	+	+	+	+	+	+	+	+	+
Penta D	+	+	+	+	+	+	+	+	+	+	+	+
vWA	+	+	+	+	+	+	+	+	+	+	+	+
D8S1179	+	+	+	+	+	+	+	+	+	+	+	+
TPOX	+	+	+	+	+	+	+	+	+	+	+	+
FGA	+	+	+	+	+	+	+	+	+	+	+	+
AMEL	+	+	+	+	+	+	+	+	+	+	+	+

Table-4.23: STR results for male and female hair samples resulting from DNA obtained using the Maxwell® 16. All samples matched their reference samples and their organic extraction replicates across all loci. (Legend found in footnote at the end of the chapter)

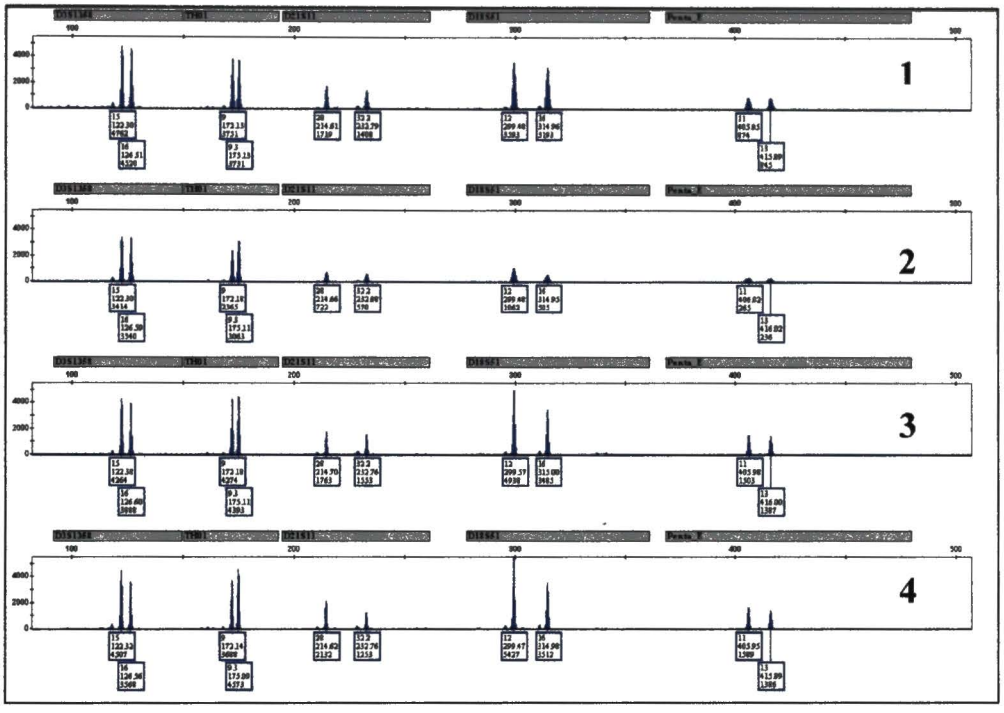

Figure-4.21

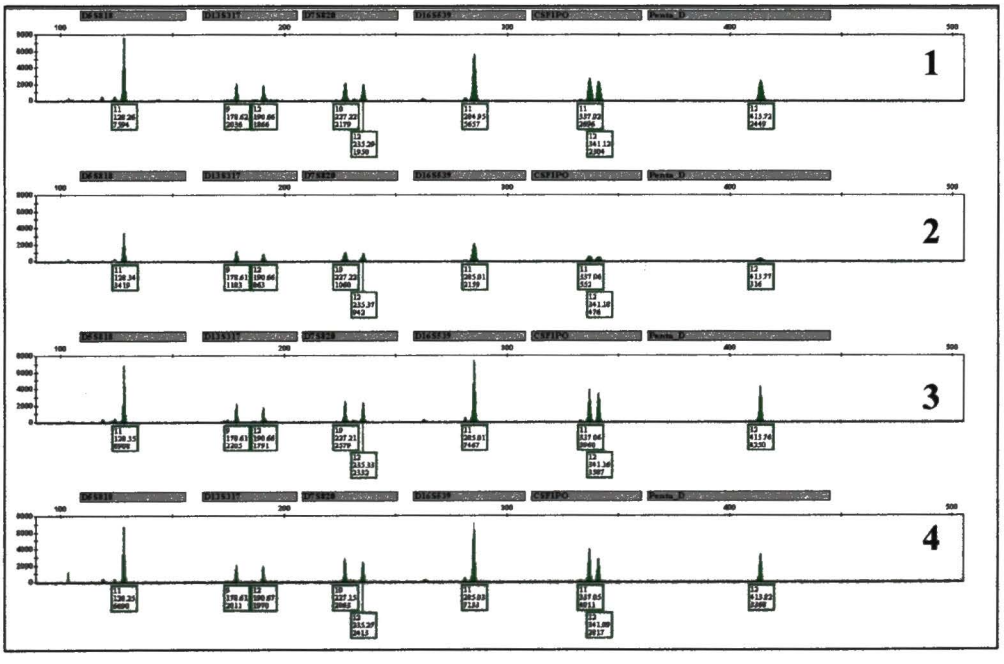

Figure-4.22

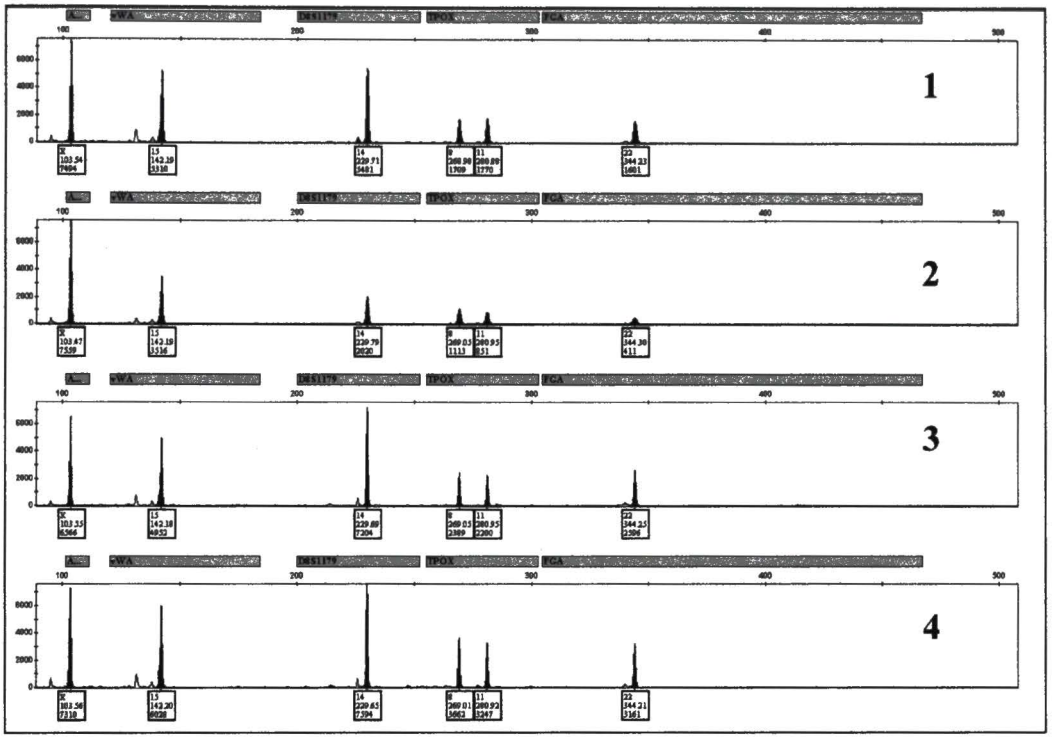

Figure-4.23

STR electropherograms showing the FL blue due (Figure-4.21), the JOE green dye (Figure-4.22), and the TMR yellow dye (Figure-4.23) of four representative female hair samples processed using the Maxwell® 16. No drop-out observed and full profiles were obtained

All male hair samples, except for sample #6, processed using the organic extraction method resulted in STR profiles that matched the reference sample at all loci. Sample #6 resulted in a mismatch at D7S828. Two of the six female hair samples (#2 and #4) produced no results for at least 12 loci. The same two samples also had a DNA concentration of less than 0.006ng/μl and, therefore, less than 200pg total DNA was available for the PCR reaction. Concordance results are summarized in Table-4.24.

	Male Hair Donor						Female Hair Donor					
LOCI	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
D3S1358	+	+	+	+	+	+	+	NR	+	NR	+	+
TH01	+	+	+	+	+	+	+	NR	+	NR	+	+
D21S11	+	+	+	+	+	+	+	NR	+	NR	+	+
D18S51	+	+	+	+	+	+	+	NR	+	+	+	+
Penta E	+	+	+	+	+	+	+	NR	+	NR	+	+
D5S818	+	+	+	+	+	+	+	NR	+	NR	+	+
D13S317	+	+	+	+	+	+	+	NR	+	NR	+	+
D7S828	+	+	+	+	+	+	+	NR	+	+	+	+
D16S539	+	+	+	+	+	+	+	NR	+	NR	+	+
CSF1PO	+	+	+	+	+	+	+	NR	+	NR	+	+
Penta D	+	+	+	+	+	+	+	NR	+	+	+	+
vWA	+	+	+	+	+	+	+	NR	+	NR	+	+
D8S1179	+	+	+	+	+	+	+	NR	+	NR	+	+
TPOX	+	+	+	+	+	+	+	NR	+	NR	+	+
FGA	+	+	+	+	+	+	+	NR	+	NR	+	+
AMEL	+	+	+	+	+	+	+	NR	+	+	+	+

Table-4.24: STR results for male and female hair samples resulting from DNA obtained using the organic extraction method. Partial profile and no results were obtained for female hair samples 2 and 4 respectively. (Legend at footnote in end of the chapter)

No detectable or amplifiable DNA was obtained from either of the two bone samples provided for this study. Previously when these same samples were processed using the organic extraction method, a full profile was obtained from bone sample B-3286 and a partial profile was obtained from bone sample B-3473 using the Profiler and COfiler amplification system (Applied Biosystem, Foster City, CA).

Degraded Liver Tissue Samples:

Of the six degraded liver samples, five samples processed with the Maxwell® 16 had no detectable quantities of DNA and one liver sample yielded a very low quantity of DNA (0.12ng). Using the organic extraction method, three liver samples yielded a detectable quantity of DNA that averaged 0.06ng. The quantification results from the DNA extracted from the liver samples may have been erroneous as a result of PCR inhibitors inherent in the degraded liver. The IPC Ct values observed for the Maxwell® 16 averaged 27.6, while that of organic extraction were around 32 (Table-4.25).

	Maxwell® 16			Organic		
Liver Sample	Input mass (g)	DNA Yield (ng)	IPC Ct	Input mass (g)	DNA Yield (ng)	IPC Ct
1	36.6	NR	27.7	37	0.036	31.6
2	35.5	0.12	27.6	40.2	0.103	31.1
3	34.9	NR	27.7	39.4	NR	29.8
4	42.2	NR	27.8	34.3	NR	29.4
5	41.5	NR	27.9	35	0.052	35.4
6	39.2	NR	27.9	34.8	NR	34.2

Table-4.25: DNA yields and IPC Ct values for liver samples processed using the Maxwell® 16 and the organic extraction method

Post-PCR analysis of the DNA obtained from the liver samples processed using either the Maxwell® 16 or the organic extraction method did not demonstrate the presence of any amplified DNA products. STR profiles were not observed even in those samples that had detectable DNA with Real-Time PCR quantification (sample #2 extracted using the Maxwell® 16 and samples 1, 2, and 5 processed using the organic extraction method).

- [+] alleles matching their reference samples and/or the organic extraction replicates
- [-] alleles not matching their reference samples or the organic extraction replicates
- [DO]Allelic drop-out
- [NR] No Result

CHAPTER V

DISCUSSION

Although the DNA IQ™ Casework Sample Kit chemistry was designed for forensic samples, the organic extraction method was consistently more efficient at recovering DNA from every cell type (WBC, sperm cell, and keratinocyte). There are several possible explanations for the low DNA yields observed with the Maxwell® 16. First, it was noticed that the DNA IQ™ resin was not efficiently captured and transferred by the magnetic rod and plunger. As a result, not enough resin was available for capturing the DNA in the lysate, and portions of the DNA-bound resin were not re-captured during the wash steps as seen in Figure-5.1. Loss of DNA IQ™ resin during the processing could significantly the low DNA recovery. Second, the Lysis Buffer was found to have a basic pH of ~8.5; a significantly higher pH than the manufacturer specifications of pH ~5. While the Lysis Buffer components are proprietary, such a significant increase in pH is likely to have an adverse effect on ion, DNA, and resin charge. Such an adverse effect may hinder the DNA binding capability of the resin and result in a decrease DNA recovery. The previous version of the DNA IQ™ Casework Sample Kit provided significantly greater yields. The greater DNA yield could have been the result of extracting DNA from liquid blood samples as

opposed to the blood samples dried on Dacron swabs. The blood swabs used in this current study potential could have entrapped DNA in the Dacron fibers as the white blood cells were lysed. If the DNA was not efficiently eluted off the swab, the DNA recovery would be greatly reduced [19]. The inefficient recovery of DNA and the low DNA yields observed with the Maxwell® 16 and the DNA IQ™ Casework Sample Kit is strongly disadvantageous in processing forensic casework samples which may initially contain limited amounts of DNA. However, the DNA obtained by the Maxwell® 16 from touch samples resulted in better quality STR profiles than the organic extraction method. Although, a greater DNA yield was obtained using the organic extraction method, the DNA obtained in several samples could not be reliably amplified. Unlike the organic extraction, full and partial profiles were obtained using the Maxwell® 16 which also confirmed previous studies using earlier versions of the DNA IQ™ Casework Sample Kit.

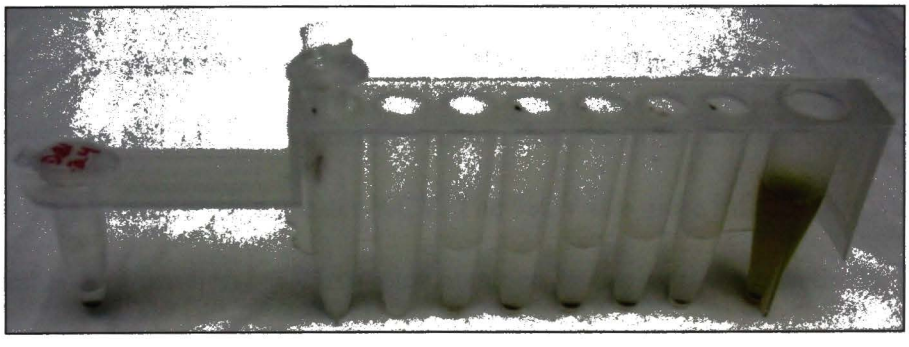

Figure-5.1: Inefficient resin capture observed in Casework Cartridge

The organic extraction method did not successfully extract amplifiable DNA from denim jeans. The dye co-precipitated with DNA and prevented detection using Real-Time PCR. The indigo dyes can both inhibit PCR amplification as well as quench the fluorescence. Similarly, liver tissue is a source of many PCR inhibitors such as collagen, heme, bilirubin, and bile salts which are co-extracted when using the organic method. The DNA IQ™ system used by the Maxwell® 16 was better at removing PCR inhibitors, including the indigo dye. The effectiveness of the Maxwell® 16 in the removal of PCR inhibitors was evident in the complete STR profiles obtained from denim jeans samples and the consistent IPC Ct values observed in liver samples. Blood stains on denim jeans or other fabrics are frequently encountered in forensic casework. For these types of samples which may contain significant amounts of PCR inhibitors, the Maxwell® 16 would be the preferred method for DNA extraction.

The automation provided by the Maxwell® 16 could minimize the human error associated with manual pipetting and help eliminate potential sample switching and contamination. However, the DNA IQ™ chemistry may have also introduced the inconsistency observed in DNA yields. The DNA yields with the Maxwell® 16 had a lower standard deviation than the organic extraction method, while, the Maxwell® 16 a greater %CV. The %CV is a more informative way of expressing the variation in DNA yields obtained by the extraction methods. In this case, the %CV indicated that the Maxwell® 16 was not as consistent as an analyst performing the manual organic extraction procedure.

The incorrect pH of the Lysis Buffer in the Casework Sample Kit could explain the low and inconsistent DNA yields. The Casework Sample Kit contains an excess of resin in relation to the amount of DNA recoverable from most forensic samples. Below the saturation point, the amount of DNA binding to the resin varies with the amount of DNA released from the cells. Another reason behind the variable DNA yields obtained by the Maxwell® 16 could be attributed to the inefficient capture of the resin and its inconsistent carryover during the wash steps. While the Maxwell® 16 more efficiently captured the DNA-bound resin in some sample cartridges, other cartridges had evidence of variable amounts of DNA-bound resin that was not efficiently captured and resulted in lower DNA yields from replicate samples.

A total of six blanks showed signs of potential contamination, but only the three blanks in run #5 displayed six peaks that would be considered true alleles. This was the only indication of potential contamination. The six true alleles could have originated from the semen samples, but the actual cause of the contamination cannot be determined with certainty. The contamination seen in blanks 5.6, 5.10, and 5.13 are not likely to have originated from previous runs since the intra-run as blanks 4.6, 4.10, and 4.13 do not display any contamination. Contamination in blanks 5.6, 5.10, and 5.13 originating from adjacent samples or from an external source is a more likely explanation. The off-ladder, erroneous peaks and spikes observed in run #1 and run #4 are not consistent with contamination and are of little concern, but still need to be further investigated.

Previous studies with the Differex™ system reported minor carry-over between the sperm and epithelial fractions. This carry-over was evident in the majority of sexual assault samples processed in their study (8). A potential explanation fro the carry-over could be the partial lysis of epithelial cells. The intact vaginal epithelial cells could then pellet with the sperm cells. It was also noticed that after removing the top Digestion Buffer layer, a gel-like inter-phase remained after the three wash steps. The inter-phase was suspected to contain cell debris that could trap epithelial cell DNA. In this study, the efficient separation of the two fractions was possible and single-source profiles were obtained with the use of the Differex™ system and the Maxwell® 16.

All hair samples were viewed microscopically to check for the presence of a root. However, some female hair samples (#2 and #4) may have had fewer cells at the root that resulted in low amounts of DNA. The lower yield of DNA obtained with the organic method could explain the insufficient amount of amplifiable DNA. The hair samples processed by the Maxwell® 16 may have had larger roots with enough of cells that resulted in sufficient quantities of amplifiable DNA. Very small amounts of DNA were obtained from the degraded liver samples. An equivalent amount of intact liver tissue would normally yield an abundance of DNA. The liver tissue used in this study appeared highly decomposed and the DNA was probably degraded to a point where very little if any amplifiable DNA could have been recovered by either extraction method. The

Maxwell® 16 was less efficient extracting DNA and obtained DNA from only one sample compared to three samples of six using the organic method.

No DNA was obtained from either bone sample using the Maxwell® 16. The most likely explanation is that the Tissue and Hair extraction Kit used to pre-process samples for the Maxwell® 16 does not efficiently extract DNA from bone samples as reported by previous studies [20]. The organic method previously obtained a sufficient amount of DNA to generate STR profiles. In the absence of a more efficient method for pre processing bone samples the Maxwell® 16 should not be used to extract DNA from bone samples. Promega Corporation has developed a modified bone protocol for the Maxwell® 16. The "Bone Extraction Protocol to be used with the DNA IQ ™ System" includes a bone incubation buffer and 1M DTT specifically designed to more efficiently extract DNA from pulverized bone in comparison to the Tissue and Hair Extraction kit. Future testing of the Maxwell® 16 using the Casework Sample kit may consider incorporating this new bone protocol.

CHAPTER VI

CONCLUSION

At this time the results from this study would indicate that the Maxwell® 16 cannot replace the use of organic extraction method for the extraction of DNA from critical forensic evidentiary samples. Based on the results obtained in this study, the organic extraction procedure provides significantly greater yields of DNA from evidentiary samples containing limited amounts of DNA. However, for samples with sufficient amounts of DNA, that may contain significant amounts of PCR inhibitors, the Maxwell® 16 may be preferential over the organic extraction method. With samples that contain an abundance of DNA, it may be more time efficient and cost effective to extract the DNA using the Maxwell® 16. If the resin retention and capture are improved, and the correct pH for the Lysis Buffer is utilized, the Maxwell® 16 along with the DNA IQ™ Casework Sample kit may have the potential to be utilized by forensic laboratories. With samples that contain a significant amount of inhibitors the Maxwell® 16 could be used in conjunction with the organic extraction procedure to improve the overall quality of the STR profiles generated by forensic analysts. The Maxwell® 16 can be beneficial in low to medium throughput laboratories if

the DNA recovery is improved by the manufacturer without a loss in the current quality of DNA being purified.

APPENDIX A

Organic DNA Extraction: Blood, Tissues and Stains (non-sperm)

Purpose: Extraction of DNA from blood stains, saliva stains/swabs, tissue, and liquid whole blood using Proteinase K digestion and protein/lipid extraction using organic solvents. This procedure can also be used as a general extraction protocol for other body fluids or stains that do not contain sperm.

A. Sample Preparation

1. If processing a dried stain: dissect the swab or fabric into thirds on a clean cutting surface.
2. Add sample to an appropriately labeled, sterile 1.5 ml screw top microcentrifuge tube.
3. If processing liquid blood: transfer 30-100 µl of liquid blood, depending on analyst's evaluation of sample quality, to an appropriately labeled, sterile 1.5 ml screw top microcentrifuge tube.

B. Protein Digestion

1. Add 300-600 ml of Stain Extraction Buffer (Working Solution) and 5 ml of 20 mg/ml Proteinase K to each sample tube. The volume of SEB added must be sufficient to allow the sample medium to move freely when tube is agitated.
2. Briefly vortex and pulse spin the samples to force the sample into the extraction solution.
3. Incubate samples at 56 °C (± 1°C) for a minimum of 2 hours (24 hour maximum).

NOTE: One hour is usually sufficient for reference samples and at least six to twenty four hours may be needed for evidentiary samples.

Phenol-Chloroform-IAA Extraction

This step removes residual proteins and lipids from the DNA extracts. Prior to use, visually inspect the PCIA solution for clarity. Any reagent with a red hue should not be used. NOTE: PCIA is stored with a buffer barrier as a supernatant. Make sure that you pipette the reagent from below the aqueous layer.

1. After digestion, remove evidence sample (stained material only), place in spin basket and centrifuge at 10,000-14,000 rpm for 4 minutes.

Note: This step is not necessary for liquid blood or tissue samples that should be fully digested.

2. Add 300 ml of buffered **phenol/chloroform/isoamyl alcohol (PCIA,** 25:24:1) to each tube.
3. Vortex tubes for 30 seconds to attain a milky emulsion, then centrifuge for 3 minutes at 14000 rpm.

4. Carefully remove the aqueous phase (supernatant) from each tube and transfer it to a new sterile tube. **Avoid drawing any of the interface into the pipette tip!**

5. The DNA can now be concentrated by one of the following two accepted methods.

C. Ethanol Precipitation

1. Add 1.0 mL of cold absolute alcohol to the aqueous layer in the new sample tube, vortex briefly and let incubate at -20°C or less for 30 minutes.
2. Centrifuge for 20 minutes at 10,000-14,000 rpm and decant alcohol when finished.
3. Add 1.0 ml of 70% ethanol and centrifuge at 10,000-14,000 rpm for 10 minutes. Carefully pipette off 70% ethanol. Evaporate off remaining alcohol.
4. Add 50 – 100 µl of sterile H_2O and let DNA resolubilize at 56°C (± 1°C) for two hours.
5. Record volume of DNA extract on worksheet and store DNA extract at 2-8°C. For long-term storage DNA should be stored at –20°C or less.

APPENDIX B

Organic DNA Extraction from Semen

A. Sperm Digest

* Add 450µl *SEB* stock to each sample and to the blank control.
* Add 10µl *Proteinase K* (20mg/ml) and 20µl DTT (1.0M) to each sample. Mix by stirring with the pipette tip.
* Incubate at 56°C (±1°C) for 120 minutes or overnight.

B. PHENOL/CHLOROFORM EXTRACTION

THIS STEP REMOVES RESIDUAL PROTEINS AND LIPIDS FROM THE DNA EXTRACTS. PRIOR TO USE, VISUALLY INSPECT THE PCIA SOLUTION FOR CLARITY. ANY REAGENT WITH A RED HUE SHOULD NOT BE USED.

***NOTE:* PCIA IS STORED WITH A BUFFER BARRIER AS A SUPERNATANT. MAKE SURE THAT YOU PIPET THE REAGENT FROM BELOW THE AQUEOUS LAYER.**

Extraction procedure:

* Add an equal volume (~ 500µl) of buffered phenol-chloroform to each of the microcentrifuge tubes and vortex vigorously for 15 seconds.
* Centrifuge the tubes at 13,000×g for five minutes.
* Transfer the aqueous layer (top layer) into a fresh 1.5ml microcentrifuge tube.

C. Ethanol Precipitation

1. Add 1.0 ml of cold absolute ethanol to each sample tube, vortex for 5 seconds and place in a freezer at -20°C or less for 30 minutes.
2. Centrifuge at >10,000 RPM for 20 minutes and decant off alcohol.
3. Add 1.0 ml of 70% ethanol, microcentrifuge at >10,000 RPM for 10 minutes, decant and pipette off 70% ethanol.
4. Incubate at 56 °C (±1°C) for 5 minutes or longer with tube caps open to evaporate ethanol.
5. Add 60 µl of TE-4 buffer, cap tube and vortex briefly, then incubate at 56°C (±1°C) for 30 min to 2 hours to re-solubilize DNA.
6. Record volume of DNA extract on Sample table (Page 1) and store DNA extract at 4°C. For long-term storage DNA should be stored at –20°C or less.

APPENDIX C

Organic DNA Extraction From Hair

PREPARATION OF GRINDERS

Micro Tissue Grinders are used to grind hairs for DNA extraction. The grinders consist of matched sets of mortars and pestles, and should be used as such. To facilitate working with the grinders, the grinders may be placed in a 1.5 ml microcentrifuge tube that has had the cap removed. Prior to beginning hair extraction the grinders should be cleaned using the following protocol:

1. Carefully rinse the grinders with sterile H_2O. Using cotton tip applicators and warm 5% (w/v) **Terg-a-zyme™** detergent scrub the pestles and the inside of the mortars.

2. Rinse the grinders with sterile H_2O and add approximately 200 ml of **1N H_2SO_4**. Place the pestles in the mortars and briefly simulate grinding. Allow the mortars to soak in **1N H_2SO_4** for a minimum of 20 minutes.

3. Rinse the grinders in sterile H_2O. Remove the pestles and pulse spin the mortars in a microcentrifuge to collect the remaining water. Remove the remaining water from the mortars using a sterile pipette.

Place the grinders in a rack and place in the UV crosslinker for a minimum of 15 minutes

Grinding and DNA extraction of hair samples

1. A **reagent blank** should be prepared for each grinder used. Prepare the blank by placing 200 ml of **Stain Extraction Buffer (SEB)** working solution into the grinder and briefly simulate grinding. Transfer the liquid into a sterile 1.5 ml microcentrifuge tube and set aside until step 6.
2. To the same grinder add 200 ml of **SEB** and add the hair fragment to be processed.
3. Move the pestle up and down to force the hair into the bottom of the mortar. Grind until fragments are no longer visible.
4. Remove the pestle from the mortar. If fluid is adhering to the pestle head, gently pass it along the inner lip of the mortar until the liquid flows to the bottom of the mortar.
5. Transfer the homogenized liquid to a sterile 1.5 ml microcentrifuge tube.
6. Add 5 ml of 600U/ml of **Proteinase K** to each tube (samples and reagent blanks).
7. Vortex on low speed briefly and pulse spin in a microcentrifuge. Place tubes in a 56°C (± 1°C) heat block for a minimum of 2 hours and a maximum of 24 hours.

PHENOL-CHLOROFORM-IA EXTRACTION.

This step removes residual proteins and lipids from the DNA extracts. Prior to use visually inspect the PCIA solution for clarity. Any reagent with a red hue should not be

used. NOTE: PCIA is stored with a buffer barrier as a supernatant. Make sure that you pipette the reagent from below the aqueous layer.

6. Add 200 ml of buffered **phenol/chloroform/isoamyl alcohol (PCIA,** 25:24:1) to each tube.
7. Vortex tubes for 30 seconds, then centrifuge for 3 minutes at 14000 rpm.

MICROCON™ 100 CONCENTRATION

1. Assemble and label a **Microcon™ 100** microconcentrator for each sample. Prepare the concentrator by adding 200 ml of sterile **H_2O** to the filter side of each concentrator.
2. Carefully remove the aqueous phase (supernatant) from each tube and transfer it to the appropriate concentrator. **Avoid drawing any of the interface into the pipette tip!**
3. Cap each Microcon YM-100 unit containing sample and place into microcentrifuge.
4. Centrifuge the Microcon YM-100 units for 15-20 minutes (or as required to minimize retained volume) at 500 x g. Discard flow-through solution.
5. Add 400µl of sterile **H_2O** to each Microcon YM-100 unit.
6. Centrifuge units for 15-20 minutes (or as required to minimize retained volume) at 500 x g.
7. Discard the filtrate cups and add 20-60µl of **hot** (80-90°C) sterile **H_2O** to the filter side of each concentrator and place a retentate tube on top of each concentrator.
8. Briefly vortex the concentrator with the retentate tube facing up and then invert the microconcentrator and centrifuge at 1000 x g for 3 minutes.
9. Discard the concentrators and place caps on the retentate tubes.
10. Record volume of DNA extract on Sample worksheet and store DNA extract at 2 – 8°C. For long-term storage DNA should be stored at –20°C or less.

Reference:

1. **B. Budowle, J. Smith, T. Moretti, J. DiZinno. 2000**
   DNA Typing Protocols: MolecularBiology and Forensic Analysis, 3:13- 45
2. **FBI Laboratory, DNA Unit II. Rev 4. 1999**
   Mitochondrial DNA Sequencing Protocol, 3.1

APPENDIX D

Organic DNA Extraction of Bones and Teeth

A. Grinding of Teeth and Bones with SPEX 6750 Freezer Mill

1. Prepare a reagent blank by swabbing the inside of the cylinder of the SPEX 6750 Freezer Mill (Part No. 6751) with a sterile, dry swab. A reagent blank is prepared for each cylinder in which a sample is extracted utilizing the SPEX 6750 Freezer Mill. Cut off the tip of the swab and place in a sterile 1.5 ml microcentrifuge tube.
2. Clean the outer surface of the bone/tooth using a Dremel tool with an emery disk to remove the outer surface of possible contaminants. The emery disk should only be used for one sample and discarded. Clean bone with ethanol.
3. Cut a section of the bone approximately 1-2 cm x 1-2 cm using a Dremel tool with a separating disk. The separating disk should be used for only one sample and then discarded. An entire cleaned tooth may be used.
4. Assemble the sample cylinder by inserting one end plug into the cylinder. End plugs are inserted concave side in. Place the impactor in the sample cylinder along with the bone/tooth section. Insert the second end plug.
5. Keeping the top of the SPEX 6750 Freezer Mill open, fill reservoir with liquid nitrogen (approximately 5 liters). After an initial chill down period, additional liquid nitrogen should be added to the reservoir to the fill line prior to processing sample.
6. Close the lid of the Freezer Mill and wait for sample cylinder to cool (about 4-5 minutes).
7. Set the grinding time to about 5 minutes and adjust impact frequency to the highest setting at which the impactor noise give a steady and uniform rattle (typically about 15). Refer to the user's manual for additional programming information if needed.
8. When grinding cycle is complete, open lid and remove sample cylinder with the Extractor and Cylinder Opener.
9. Visually inspect the samples and if sample is not pulverized sufficiently, reinsert sample cylinder for additional grinding.
10. Once grinding is complete, remove sample cylinder from the Freezer Mill and allow warming to room temperature or proceeding with sample removal while cylinder is still cold.
11. Place cylinder into the Clamp Frame Assembly and holding the cylinder with the extractor place the other end of cylinder in its place and tighten clamp knob.

12. Swing the clamp section into its horizontal position and place the cylinder section of the extractor in the appropriate position. Turn knob clockwise until end plug is removed.

13. Remove the impactor and empty the pulverized sample onto a new piece of weigh paper. Carefully pour a portion of the pulverized sample into a sterile 1.5 ml microcentrifuge tube for DNA extraction. Store remaining sample at 2-8°C or at -20°C or less for long term storage.

B. Sample Preparation

* Use sufficient bone/tooth powder to fill a sterile screw top microcentrifuge tube no more than half way.
* Include the reagent blank swab(s) collected in the previous step for processing from this point
* Use extra care to avoid contaminating the evidence itself by handling only one item at a time and re-sealing before handling another.

B1. Decalcification

1. Carefully pour a portion of the pulverized sample into a sterile screw top tube. Fill the tube to approximately one fourth to one half way to the top of the tube. Place RB swab into a separate extraction tube.
2. Add 0.6 to 1.0 ml 0.5 M EDTA pH 8.0 to each tube and close tube tightly.
3. Vortex vigorously to suspend bone powder.
4. Incubate tubes with agitation up to 24 hours at room temperature (16 hours is optimal).
5. Centrifuge tubes one minute at 7000 x g to pellet bone powder.
6. Remove supernatant.
7. Wash RB swab and bone powder by adding 1 ml dH_20 to each tube. Close lid, vortex briefly. Centrifuge tubes one minute at 7000 x g to pellet bone powder. Remove supernatant.
8. Repeat water wash step one more time for a total of two washes.

REFERENCES

1. Caltagirone A, Houde J, Lapointe M, Arsenault S, Jolicoeur C. Validation of the PROMEGA DNA IQ™™ system for casework samples. *promega.* Quebec, Canada:;61.

2. Smith CE, York CK, inventors; Promega Corporation, assignee. Methods of Isolating Biological Target Materials Using Silica Magnetic Particles. WI, USA patent 6027945. 1997, 2000.

3. Ronquillo N. *Validation of The BioMek® 2000 Laboratory Automated Workstation and The DNA IQ™ System For Forensic Casework.* [Master of Science]. Fort Worth, TX: University of North Texas Health Science Center; 2005.

4. Jian Ye, B. S., Anquan Ji MS, Parra E, et al. A simple and efficient method for extracting DNA from old and burned bone. *J Forensic Sci.* 2004;49.

5. DNA IQ™ casework sample kit for Maxwell® 16. USA: Promega Corporation; 2007;TB354. Available from: www.promega.com.

6. Differex™ System. USA: Promega Corporation; 2006;TBD020. Available from: www.promega.com.

7. Greenspoon S, Ban J. Robotic extraction of mock sexual assault samples using the biomek® 2000 and the DNA IQ™ system. *Profiles in DNA* [serial online]. 2002;5:2/12/2007. Available from: www.promega.com.

8. Flanagan L, Mandrekar P, Olson R, Tereba A. A new approach to differential extraction. .

9. Tereba A, Mandrekar P, Flanagan L. An introduction to the DNA IQ system: The smart way to purify DNA. .

10. Bassetti J. An introduction to PCR inhibitors. *Profiles in DNA* [serial online]. 2007;10:June 14, 2007. Available from: http://www.promega.com/profiles/1001/ProfilesInDNA_1001_09.pdf.

11. Richard ML, Frappier RH, Newman JC. Developmental validation of a real-time quantitative PCR assay for automated quantification of human DNA. *J Forensic Sci.* 2003;48:1041-1046.

12. Peterson JL, Hickman MJ. Census of publicly funded forensic crime laboratories, 2002. U.S. Department of Justice; 2005;207205 Accessed 2/9/2007.

13. Crispin SJ. *Validation and Optimization of Automated Instrumentation for Mitochondrial DNA Database Sample Processing.* [Master of Science]. Fort Worth, TX: University of North Texas Health Science Center; 2004.

14. Greenspoon S, Ban J, Sykes K, et al. Application of the BioMeK 2000 laboratory automation workstation and the DNA IQ system to the extraction of forensic casework samples. *J Forensic Sci.* 2004;49.

15. Shawn A. Montepetit, Fitch, Ian, O'Donnel PT. A simple automated instrument for DNA extraction in forensic casework. *J Forensic Sci.* 2005;50.

16. Butler JM. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers.* second ed. London, UK: Elsevier Academic Press; 2005.

17. Maxwell(R) 16 instrument operating manual. Madison, WI: Promega Corporation; 2007;TM274.

18. PowerPlex(R) 16 system. USA: Promega Corporation; 2007;TMD012. Available from: www.promega.com.

19. Voorhees J, Ferrance J, Landers J. Enhanced elution of sperm from cotton swabs via enzymatic digestion for rape kit analysis. *J Forensic Sci.* 2006;51:574-579.

20. Mandrekar P, Flanagan L, Tereba A. Forensic extraction and isolation of DNA from hair, tissue and bone. *Profiles in DNA.* 2002:11-13.