

**Comparison of QIAcube® Differential to Manual Differential Extraction When Purified Using the QIAamp® DNA Blood Mini Kit**

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**Abstract**

In a 2008-2012 survey performed by the U.S. Department of Justice, an average of 237,868 victims reported being sexually assaulted each year, which calculates to an occurrence roughly every two minutes.(10) Although only about half of all sexual assaults are reported, a great deal of time and effort goes into processing evidence from these cases due to the potential of samples containing female-male mixtures on which differential extractions must be performed. In forensic casework, a differential extraction is a method that incorporates the combination of phase separation with differential centrifugation to isolate sperm cells from other cell types in order to generate two distinct profiles of the victim and the assailant. Traditionally, differential extractions have been performed manually, requiring an analyst to undergo repeated pipetting and multiple centrifugation steps. Due to the hands-on nature of the approach the quality and consistency of the separations tend to be variable from analyst to analyst. A combination of the number of sexual assault cases reported along with the time required to analyze samples from these cases has caused backlogs to become commonplace among many crime labs across the country. Bringing an automated differential extraction procedure online would benefit analysts by not only reducing the backlog of the laboratory but also by streamlining the workflow of a lengthy process and removing analyst to analyst variability.

This study focused on determining the utility of Qiagen®'s (Hilden, Germany) QIAcube® for differential extraction of samples and compared it to the Qiagen® QIAamp® DNA Blood Mini manual method currently being used by the Washoe County Sheriff's Office (WCSO). The QIAcube®, introduced in 2007, was originally designed to extract nucleic acids and proteins and, therefore, is capable of centrifuging, vortexing,

pipetting, and extracting a supernatant from a pelleted sample.(9) This study evaluated the QIAcube®'s abilities, using a standard protocol, to perform differential separations on up to 12 mock sexual assault samples at a time. Experiments included a buffer study comparing three potential buffers incorporated into the lysis mixtures; a sensitivity study based on a 1:3 semen dilution series, with and without female epithelial cells present; a cross contamination study using mixed female blood and semen; as well as a matrix and mock evidence study consisting of a mixture of female epithelial cells and semen pipetted onto different substrates along with various proficiency test samples. All studies were performed by a graduate student using a combination of four QIAcubes®. For comparison, the sensitivity and reproducibility studies were also performed by an experienced analyst. There was no sign of cross-contamination between samples, even though the tubes remain open all at once in the instrument. Interestingly, the manual method consistently yielded DNA concentrations approximately twice as high as the QIAcube® for the sperm fraction. Extensive troubleshooting was performed to include the use of different reagents and temperatures as well as a variety of protocol variations. In conclusion, the WCSO Crime Lab will not be utilizing the QIAcube® to perform differential extractions unless future modifications of the standard protocols result in higher male yields.

### **Introduction**

The National Center for Injury Prevention and Control released a 2012 survey that indicated nearly 1 in 5 women and 1 in 71 men in the United States reported experiencing rape at some time in their lives.(11) As a result, forensic scientists must handle a significant amount of sexual assault kits and this can result in backlogged evidence

requiring analytical processing. The most pertinent evidence collected in these cases contains both the DNA from the victim and perpetrator. These types of samples require a special technique called differential extraction. Differential extraction is a technique that separates sperm cells from non-sperm cells in order to produce two distinct purified profiles of the victim and perpetrator. The success of differential extraction is based on the disulfide bonds within the plasma membrane of the sperm. The disulfide bonds of the spermatid plasma membrane make the spermatozoa more difficult to lyse than other cell types. All non-sperm cells can be lysed and separated from the untouched sperm cells, followed by sperm lysis, resulting in purification of each cell type.

Differential extraction is one of the most time-consuming techniques performed in a DNA forensic laboratory, mainly because the most common procedures are performed manually, requiring several steps including multiple washes. Although there are many different techniques that can be used for differential extraction, the general steps follow the same principles and result in the same products, namely one purified sperm fraction and one purified epithelial fraction. The first step requires the sample to be incubated in a mixture composed of a buffer, a detergent and Proteinase K. A component of the buffer functions as a chelating agent by sequestering divalent and trivalent metal ions such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .(3) Although the metal ions remain in solution, once bound to this chelating agent their reactivity is substantially diminished. The detergent lyses the cell membranes of the epithelial cells and denatures the histone proteins associated with the DNA in the cells. When the histones are denatured they lose their conformational shape and release the DNA into the solution. Proteinase K rapidly digests nucleases, intended to degrade DNA.(1) Many times the detergent and buffer are combined in the same pre-

mixed digest buffer provided in a particular kit or prepared within the laboratory. A commonly used chelating agent and detergent found in these digest buffers are ethylenediaminetetraacetate acid (EDTA) and sodium dodecyl sulfate (SDS) respectively. After the mixture has finished incubating and the sample substrate has been spun-down to collect all of the liquid remaining in it, the solution is centrifuged to pellet the sperm at the bottom, leaving the epithelial DNA suspended. The supernatant is removed and saved until it is ready to be purified and continue through the DNA workflow. The sperm pellet will be washed a minimum of three times in order to remove any excess epithelial DNA that may have been left behind. The second step in the differential extraction process requires the sperm pellet to be incubated in the same mixture of detergent, buffer, and Proteinase K along with the addition of Dithiothreitol (DTT). DTT is a reducing agent that has a very high conformational propensity to form six-membered rings with the disulfide bonds found in the plasma membrane of sperm.(6) After DTT has acted on the plasma membrane of the sperm the remaining chemicals can act as before in order to release the DNA. As with the epithelial fraction, the sperm fraction will now continue through the DNA workflow by first purifying the DNA.

The results from these manual differential extractions tend to be variable based the validated procedure used in a particular lab as well as on the experience, skill and personal lab practices between individual analysts performing the procedure. Studies have shown that different laboratories fluctuate in the efficiency of differential extraction based on the validated method being used.(12) Variability between labs and analysts is a result from the fact that there is not an established best practice when performing differential DNA extractions. Several automated differential extraction techniques have

been developed within the past several years in an attempt to save time and obtain more consistent results, regardless of the analyst performing the procedure. The Qiagen® QIAcube® is one of the first robotic workstations developed with an option to perform differential extraction. Developed in 2007, the instrument was designed to purify proteins and nucleic acids by centrifuging, vortexing and pipetting reagents for up to twelve samples.(9) Protocols are available for use with the QIAcube® for a number of different procedures, including standard sample extraction differential extraction. A study performed in 2014 showed that the QIAcube® resulted in comparable sensitivity and specificity as various other purification methods designed to extract viral nucleic acids.(4) Yet another study for 2012 compared two manual extraction techniques to the QIAcube® and found that all procedures resulted in similar results, indicating the QIAcube® provides an efficient option while reducing the potential for contamination.(7) The WCSO Crime Lab currently has four QIAcube® instruments used for DNA extraction and sample purification. Two of the instruments have the standard differential wash protocol offered by Qiagen®, where the epithelial fraction is separated from the sperm pellet, the sperm pellet is washed and the sperm lysis buffer, containing DTT, is added. The remaining two instruments contained a different differential wash protocol offered by Qiagen®, where the instrument separates the epithelial fraction and washes the sperm pellet but the sperm lysis buffer is added manually. This standard protocol was designed so an analyst could obtain an un-digested sample from the sperm pellet in order to confirm the presence of sperm through microscopic examination. Efficiency and reliability of the QIAcube® using these procedures was compared with the validated QIAamp® DNA Blood Mini manual procedure currently employed at the WCSO Crime

Lab. The studies focus primarily on comparing the amount of DNA obtained from the QIAamp® DNA Blood Mini Kit manual procedure with the QIAcube® Differential Wash protocol coupled with the QIAcube® purification protocol utilizing the QIAamp® DNA Blood Mini Kit.

### **Materials and Methods**

20 µL of an in-house male semen sample was digested manually by adding a mixture of 500 µL Buffer G2, 15 µL Proteinase K and 20 µL DTT to the sample followed by vortexing vigorously for 10 seconds. The sample was then incubated for approximately 10 min at 70 °C with 900 rpm mixing. 500 µL of Buffer AL was added to the samples and incubated at 56 °C for 10 minutes at 550 rpm following the sperm lysis. After incubation, the sample was again vortexed vigorously for 10 seconds and placed on the Qiagen® QIAcube® for purification eluting in approximately 50 µL of AE Buffer. The DNA extract obtained from the QIAcube® was quantified in triplicate using Promega® (Madison, WI) Plexor® HY on an Applied Biosystems® (Grand Island, NY) 7500 Real-Time PCR System and analyzed using Plexor® Analysis Software to determine the concentration of DNA in the original sample. The average concentration was calculated as 7.029 ng/µL for the original sample. A dilution series consisting of six samples was prepared with each sequential sample diluted at a ratio of 1:3 with TE<sup>-4</sup>. The dilutions in the series began with a concentration of approximately 1.5 ng/µL and ended with a concentration of approximately 0.006 ng/µL. 1,067 µL of the original semen sample was added to 3,933 µL of TE<sup>-4</sup> to make dilution A, the first dilution in the series with a concentration of approximately 1.5 ng/µL. The remaining dilutions were prepared as shown in Table 1.

Table 1: Serial Dilution Preparation

<b>Dilution ID</b>	<b>Volume of Dilute Semen</b>	<b>Volume of TE<sup>-4</sup></b>	<b>Estimated DNA concentration</b>
A	1,067 $\mu\text{L}$ of Neat	3,933 $\mu\text{L}$	1.5 ng/ $\mu\text{L}$
B	300 $\mu\text{L}$ of A	600 $\mu\text{L}$	0.5 ng/ $\mu\text{L}$
C	300 $\mu\text{L}$ of B	600 $\mu\text{L}$	0.167 ng/ $\mu\text{L}$
D	300 $\mu\text{L}$ of C	600 $\mu\text{L}$	0.056 ng/ $\mu\text{L}$
E	300 $\mu\text{L}$ of D	600 $\mu\text{L}$	0.019 ng/ $\mu\text{L}$
F	300 $\mu\text{L}$ of E	600 $\mu\text{L}$	0.006 ng/ $\mu\text{L}$

This dilution series was used as the sperm fraction for all studies except for the substrate study and contamination study. When epithelial cells were required for sperm/epithelial mixed samples a small hole-punch 1/8" in diameter was taken from a General Electric Healthcare® (Pittsburgh, PA) Whatman® FTA saliva card obtained from a couple of the female analysts working in the DNA lab. The punches were added to each of the sperm dilutions in order for each sample to contain approximately the same amount of epithelial DNA. Each punch was expected to contain approximately 1.0 ng, however, this could vary depending on the area where the punch was taken and how vigorous the individual was with the sample applicator.

#### *Automated Separation*

Before the samples were placed on the QIAcube®, the samples vortexed for 10 seconds with a mixture of 500  $\mu\text{L}$  of a digest buffer and 15  $\mu\text{L}$  of Proteinase K. The samples were then incubated at 56 °C for 1 hour at 550 rpm on a thermomixer. After incubation the samples were again vortexed and transferred to the appropriate 1.5 mL elution tube placed in the collection tube position of a rotor adapter, shown in Figure 1.



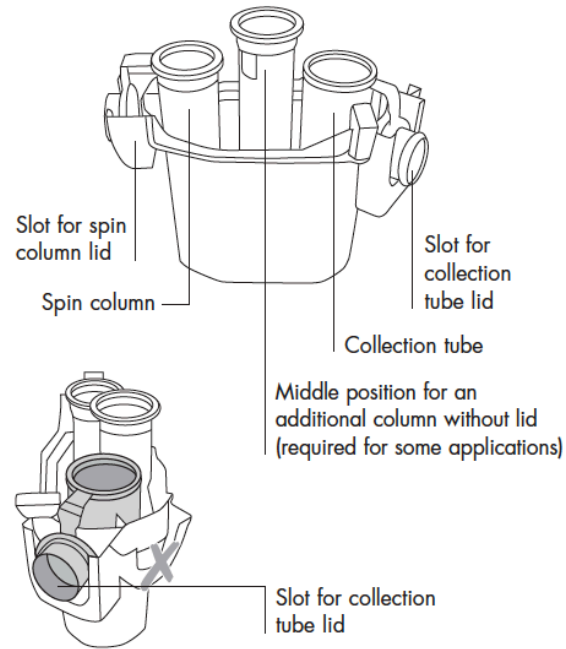


Figure 1: Rotor Adapter Loading Diagram  
Qiagen - QIAcube® Quick-Start Guide

The rotor adapters are placed in the centrifuge and empty 2 mL elution tubes are placed in shaker of the QIAcube® based on the loading chart, shown in Figure 2.

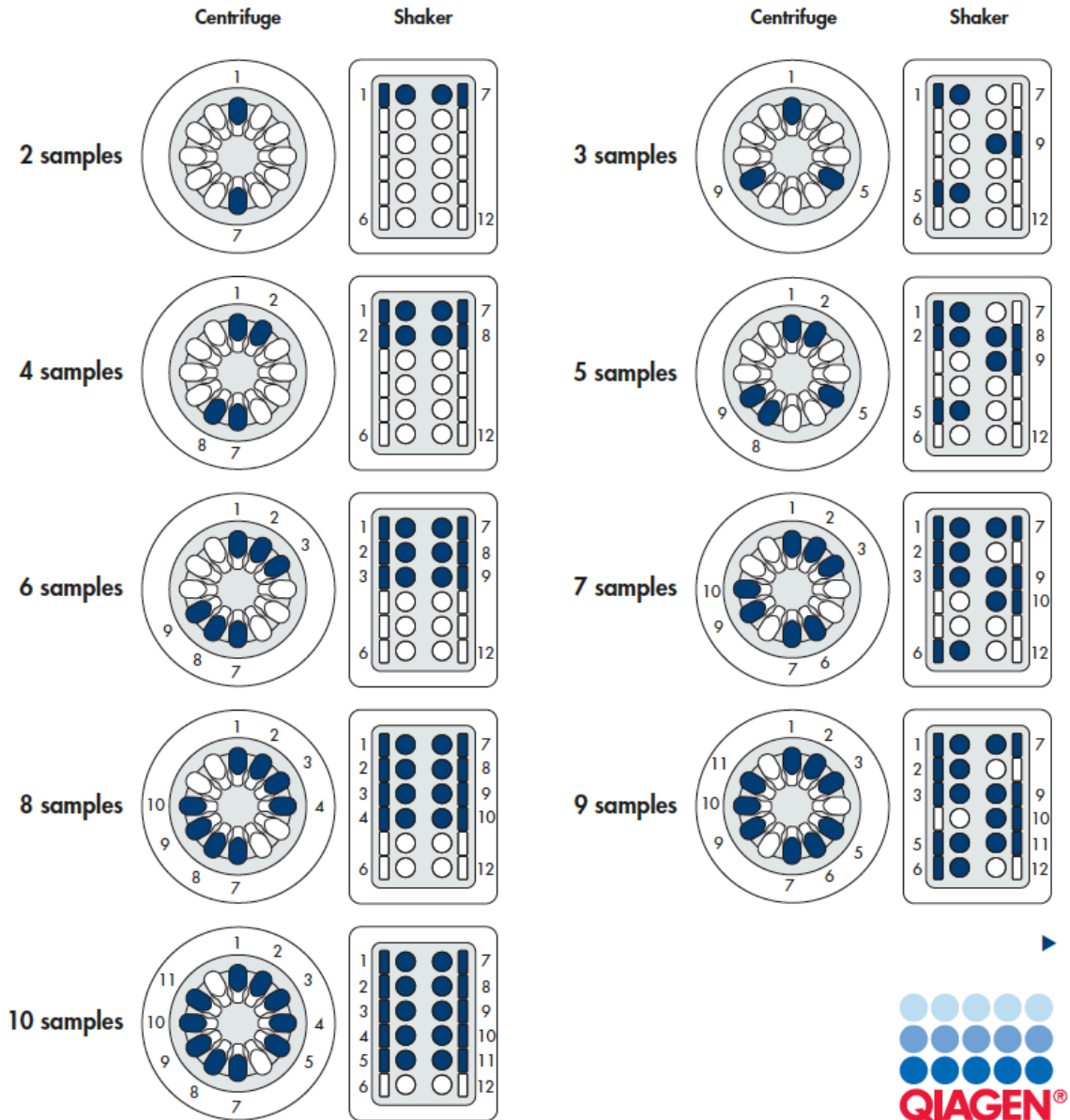


Figure 2: Centrifuge and Shaker Loading Chart  
 Qiagen - QIAcube® Loading Chart

The first protocol was selected using the touch screen on the QIAcube® for centrifugation of the samples and separation of the epithelial fraction from the samples. This protocol also performs two sperm washes. After the first protocol was complete, the epithelial fractions, transferred to the elution tubes in the shaker, were removed, capped and stored until further processing could be done. The second protocol was selected on

the QIAcube® touch screen for two additional sperm washes and the addition of the sperm lysis buffer, which was prepared according to the sperm lysis preparation chart, shown in Table 2.

Table 2: Sperm Lysis Reagent Chart  
MUFSC DNA Lab- EZ1 Differential Sample Extraction

Number of Samples	Buffer G2 (uL)	QIAGEN Pro. K (uL)	1M DTT (uL)
2	2880	180	720
3	3040	190	760
4	3200	200	800
5	3360	210	840
6	3520	220	880
7	3680	230	920
8	3840	240	960
9	4000	250	1000
10	4160	260	1040
12	4480	280	1120

Once the second protocol was finished the sperm fractions were vortexed for 10 seconds and incubated at 70 °C for 10 minutes at 900 rpm or at 56 °C for 1 hour at 550 rpm.

The protocol provided by Qiagen® utilized Buffer G2 for the epithelial lysis buffer, the wash buffer and the sperm lysis buffer. The WCSO Crime Lab wanted to explore the possibility of using ATL, which was suggested by the Maryland State Police Department, or their in-house digest buffer since they are currently being used in the QIAcube® QIAamp® DNA Blood Mini Kit purification protocol and the QIAamp® DNA Blood Mini Kit manual differential extraction protocol, respectively. Therefore, an internal study was run on the QIAcube® comparing Buffer ATL, Buffer G2 and the in-house digest buffer. Two different procedures were tested using Buffer ATL. The first ATL runs, labeled ATL1, used Buffer G2 for the epithelial lysis and wash buffer followed by ATL for the sperm lysis. The second ATL runs, labeled ATL2, used ATL for the

epithelial lysis, wash buffer, and sperm lysis. The temperature and time of the sperm lysis incubation was also tested with an internal study to determine if the differences in temperature and time combinations had a substantial impact on the DNA recovered from sperm. Qiagen's® protocol recommended incubating at 70 °C for 10 minutes at 900 rpm, however, in the WCSO Crime Lab manual differential protocol, the incubation was at 56 °C or 1-24 hours at 550 rpm. The most favorable results were obtained with samples that used the in-house digest buffer, which was used for the remaining validation studies performed. The two different temperature and time combinations were found to be consistent with one another so either could be used, however, to save time 70 °C for 10 minutes at 900 rpm was used for the remaining studies.

Both the epithelial fractions and sperm fractions had 500 µL of Buffer AL added to the sample, were vortexed for 10 seconds and incubated at 56 °C for 10 minutes at 550 rpm on a thermomixer. Once this incubation was complete the samples were transferred to 1.5 mL elution tubes and placed in the shaker according to the loading chart, seen in Figure 2. To collect the purified DNA solution, a rotor adaptor was placed in the centrifuge according to the loading chart, seen in Figure 2, with an empty collection tube placed in the collection tube slot and a spin column in the spin column slot of the rotor adaptor, seen in Figure 1. The purification was performed using the QIAamp® DNA Blood Mini Kit protocol that is automated for the QIAcube®.

### *Manual Separation*

Manual samples followed the validated QIAamp® DNA Blood Mini Kit manual procedure currently validated in the WCSO Crime Lab. 500 µL of the in-house digest

buffer along with 15  $\mu\text{L}$  of Proteinase K was added to the samples and incubated at 56  $^{\circ}\text{C}$  for 1 hour at 550 rpm on a thermomixer. After incubation, the samples centrifuged for 5 minutes at maximum speed, which was 15,000 rpm for the Eppendorf<sup>®</sup> (Hauppauge, NY) Centrifuge 5424 used at the WCSO Crime Lab. All but approximately 50  $\mu\text{L}$  of the resulting supernatant was removed and transferred to a new tube as the epithelial fraction. Between 500 and 1,000  $\mu\text{L}$  of digest buffer was added to suspend the sperm pellet, vortexed vigorously and again the tube was centrifuged at maximum speed for 5 minutes. All but 50  $\mu\text{L}$  of the supernatant was discarded. The wash step was repeated a minimum of 3 times and a maximum of 5 times before the sperm were lysed. The sperm lysis buffer, consisting of 500  $\mu\text{L}$  of digest buffer, 15  $\mu\text{L}$  Proteinase K and 20  $\mu\text{L}$  of DTT, was added to the samples and incubated at 56  $^{\circ}\text{C}$  for at least 1 hour and no longer than 24 hours at 550 rpm.

After separation, both the epithelial and sperm lysed cell fractions had 500  $\mu\text{L}$  of buffer AL added followed by incubation at 56  $^{\circ}\text{C}$  for 10 minute at 550 rpm. 525  $\mu\text{L}$  of Ethanol was added to each sample, vigorously vortexed and the samples were each transferred to a QIAamp<sup>®</sup> spin column before being centrifuged at 8,000 rpm for 1 minute. The spin column was placed in a clean tube and the filtrate was discarded. This step was repeated with the same spin column until the entire lysed fraction had been added. 500  $\mu\text{L}$  of AW1 buffer was added to the spin column and the tube was centrifuged at 8,000 rpm for 1 minute. The spin column was again placed in a new tube and the filtrate was discarded. 500  $\mu\text{L}$  of AW2 buffer was added and centrifuged at 14,000 rpm for 3 minutes. The spin column once again was placed in a new tube and the filtrate was discarded. Between 50 and 200  $\mu\text{L}$  of AE buffer was, incubated for at least 1 minute and centrifuged at 8,000

rpm for 1 minute. These final filtrates contained the purified DNA from both the epithelial fractions and sperm fractions. The spin column was removed and discarded. All samples, both automated and manual, were quantified using Promega® Plexor® HY on an Applied Biosystems® 7500 Real-Time PCR System. Due to complications during the sensitivity studies, time did not permit for samples to be amplified and carried through capillary electrophoresis analysis.

#### *Buffer Study*

Twelve replicates of the semen serial dilution were extracted using the automated separation method, incorporating the different buffers being tested: three ATL1, three ATL2, three G2 and three in-house digest buffer. The sperm fraction was incubated at 70 °C for 10 minutes at 900 rpm. 20 µL of each dilution was used in the samples. Eight replicates of the semen serial dilution along with a saliva punch were also run with the automated separation method, incorporating the different buffers being tested, two ATL1, two ATL2, two G2 and two in-house digest buffer and incubating the sperm fraction at 70 °C for 10 minutes at 900 rpm. One 1/8" diameter punch was added to each of the 20 µL semen dilution being tested. The average of each type of buffer for both the semen only samples as well as the semen and epithelial mixed samples were compared to determine the optimal digest buffer.

#### *Temperature Study*

Six replicates of the semen serial dilution were extracted using the automated separation method, incorporating the different digest buffers being tested: two ATL2, two G2 and two in-house digest buffer. The sperm fraction was incubated at 56 °C for 1 hour at 550 rpm and 20 µL of semen was used for each sample. Three replicates of the semen serial

dilution, 20  $\mu$ L each, was used for the semen and epithelial mixed samples along with a 1/8" diameter saliva punch. The samples were extracted using the automated separation method, incorporating the different buffers being tested: one ATL2, one G2 and one in-house digest buffer. The sperm fraction was incubated at 56 °C for 1 hour at 550 rpm. The results were averaged and compared to the average results from the Buffer Study to determine the optimal temperature. In this study only the ATL2 procedure was used because the buffer study showed the worst results with the ATL1 procedure so it was excluded as a potential candidate.

#### *Sensitivity and Reproducibility*

Five replicates of the serial semen dilution were prepared, three with semen only and two containing a mix of semen and epithelial cells. Each mixed sample consisted of 20  $\mu$ L semen and one 1/8" diameter saliva punch . These were run using the manual separation procedure. The results were averaged and compared to the averaged results of the optimal buffer and temperature samples.

#### *Contamination Study*

24 samples containing 20  $\mu$ L of the neat in-house semen and 20  $\mu$ L of neat in-house female blood along with 24 blank samples were prepared and extracted using the automated separation procedure. All four QIAcubes® were used, with the 1<sup>st</sup> and 3<sup>rd</sup> containing even samples with odd blank samples and the 2<sup>nd</sup> and 4<sup>th</sup> containing odd samples with even blank samples. The results were used to show that there was no cross-contamination between the samples, i.e. samples contained DNA and blanks remained blank.

#### *Substrate Study*

Five different types of substrates were chosen, including white cotton, colored cotton, cotton swabs, jeans and leather. The white cotton samples were cut squares of 100% cotton white fabric purchased from a fabric store, the colored cotton samples were cut squares of 100% cotton fabric containing a blue flower pattern purchased from a fabric store, the cotton swabs were pre-packaged swabs taken from the lab, the jean samples were cut squares of denim fabric purchased from a fabric store, and the leather samples were cut squares from a pair of newly purchased leather work gloves. Four separate samples from each type of substrate were analyzed, 2 with a semen saliva mixture, 1 with saliva only and 1 with semen only. The semen used was from the in-house semen sample used in the previous studies and the neat saliva was acquired from a in-house female lab donor. These samples were cut and extracted using the automated separation procedure. The results were averaged and compared to determine if any inhibition resulted from the different fabrics.

**Results**

*Buffer Study*

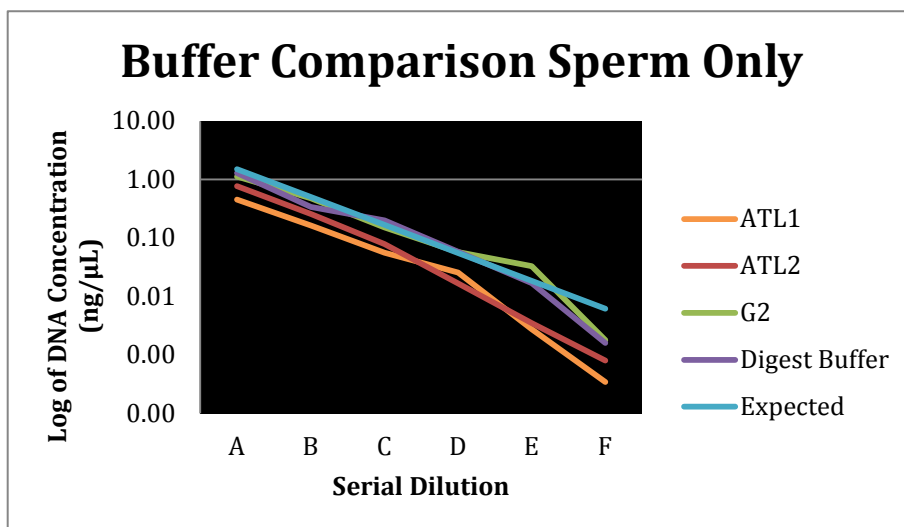


Figure 3: Line Graph of Buffer Study.



Figure 3 shows the average of the six serial dilutions from the three runs of each buffer used with semen only samples. It can be seen that G2 and the in-house digest buffer produce results more consistent with the expected results than either of the two ATL procedures.

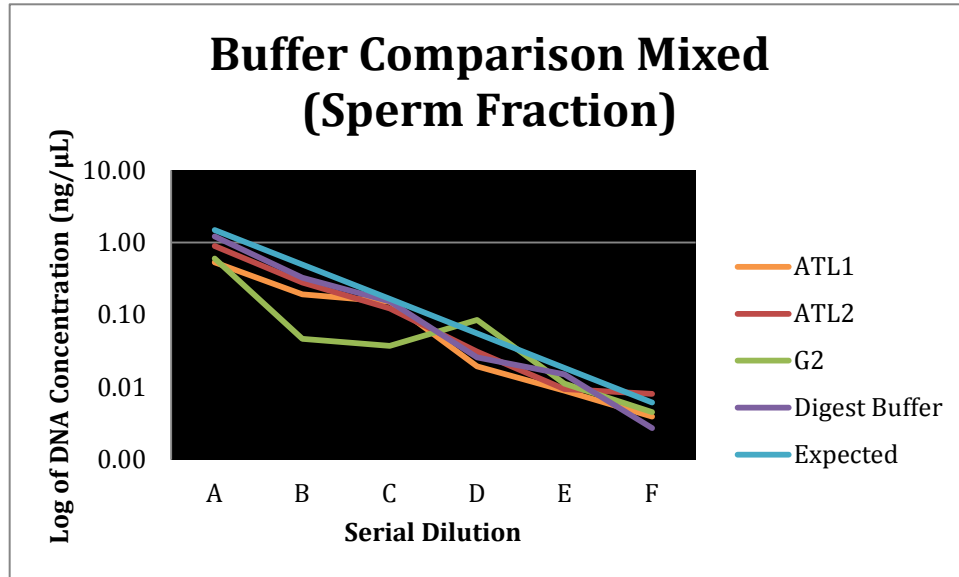


Figure 4: Sperm Fraction Comparison of Buffer Study for Mixed Samples.

Figure 4 shows that the in-house digest buffer continued to show consistent results with the expected concentrations. G2 shows an inconsistency in the mixed samples that was not seen in the semen only samples. Although both ATL procedures show improved consistency with the expected results, they are not as consistent as the digest buffer samples. ATL1 also continues to show the worse consistency; therefore it was eliminated for the remaining studies.

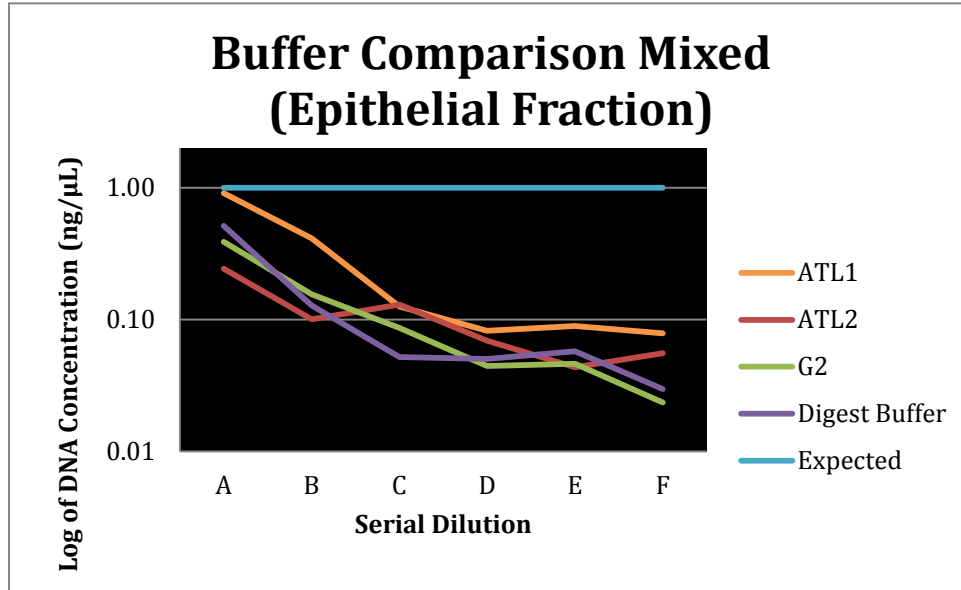


Figure 5: Epithelial Fraction Comparison of Buffer Study for Mixed Samples.

Figure 5 shows that even though all the samples were supposed to contain approximately the same amount of DNA, all the buffers showed a decrease in the epithelial DNA concentration consistent with the sperm fractions. The epithelial concentrations were expected to remain constant across the dilutions because they contained the same amount of epithelial DNA throughout the series based on the FTA card punch. There is a possibility that the FTA card used for this study had an insufficient amount of epithelial cells from a lack of vigorous collection, producing results that are only indicating the amount of epithelial DNA found in the semen dilutions, which would decrease along with the amount of sperm.

Temperature Study

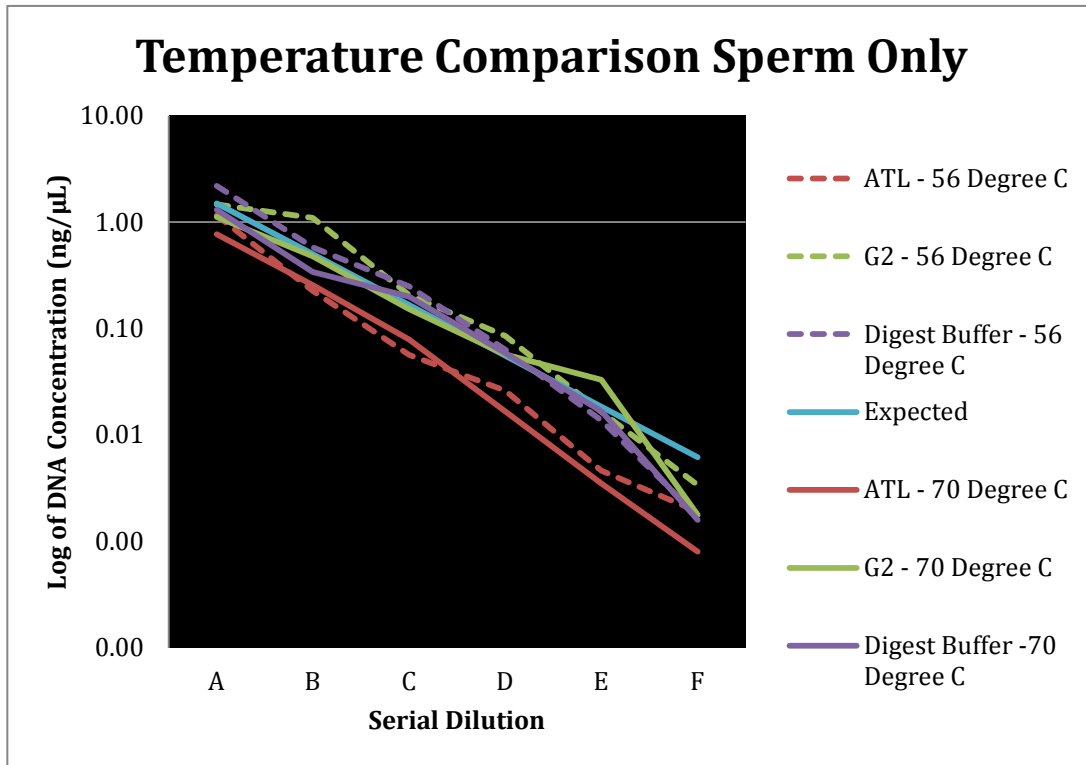


Figure 6: Temperature Chart Showing the Comparison of each Buffer at 70°C and 56°C.

Figure 6 shows that the 56 °C incubation for 1 hour at 550 rpm is comparable to the 70 °C incubation for 10 minutes at 900 rpm within each buffer. The in-house digest buffer shows to be the most consistent of the buffers at either incubation condition.

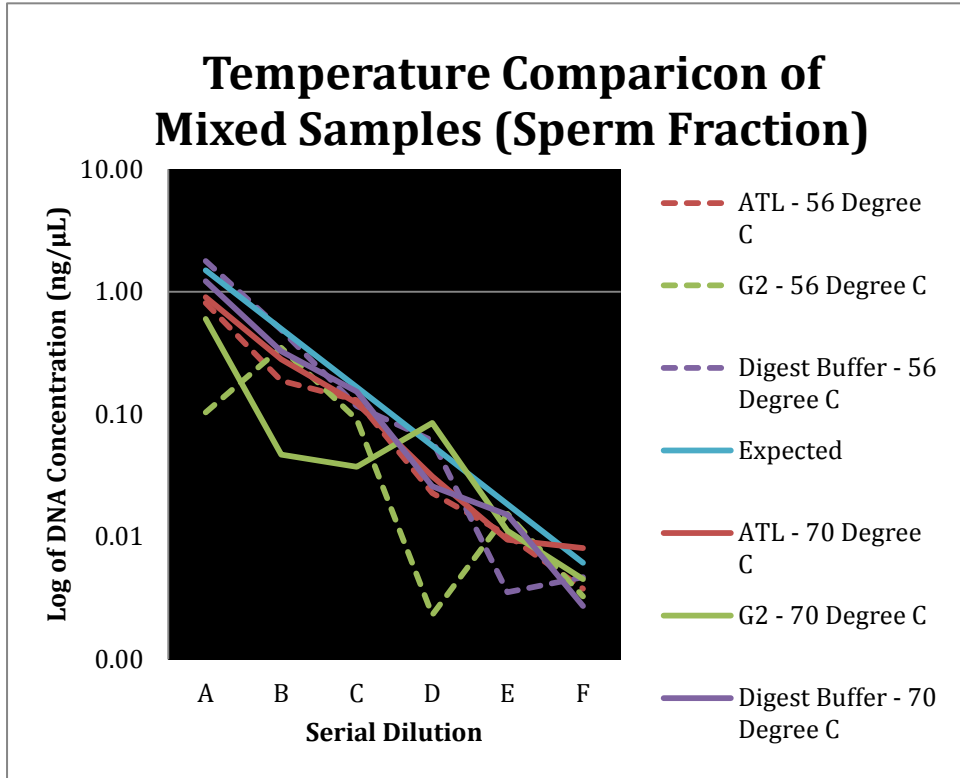


Figure 7: Sperm Fraction Temperature Comparison of Mixed Samples.

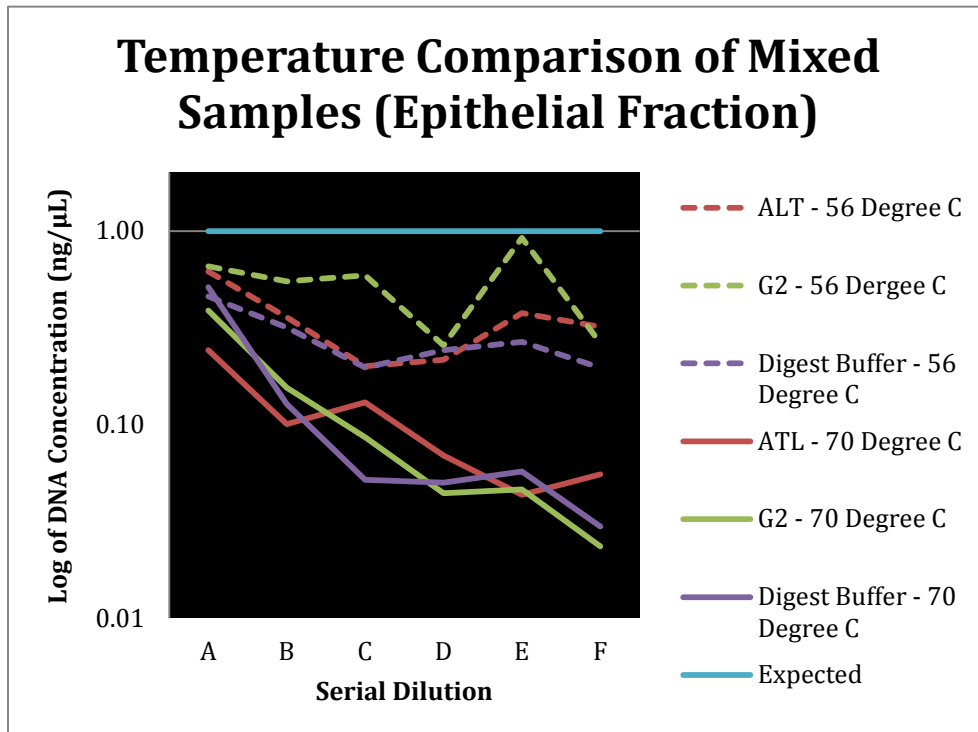


Figure 8: Epithelial Fraction Temperature Comparison of Mixed Samples.

From Figure 7, it can be seen that the 56 °C incubation for an hour at 550 rpm is consistent with the 70 °C incubation for 10 minutes at 900 rpm for the samples run with ATL and the in-house digest buffer. The G2 samples at both incubation combinations showed the most inconsistency. Figure 8 shows the average epithelial cell fraction quantity from the mixed sample for each incubation test. Although it appears that the extraction of the epithelial fraction improves with the 56 °C incubation for 1 hour at 550 rpm, this cannot be the case since the different incubation combinations were only tested with the sperm fractions after the epithelial fraction had been removed, which makes the epithelial fractions unaffected by the change in incubation. The two different quantities obtained for the epithelial fractions from the two different incubation combinations indicate that the saliva card used for the mixed samples tested using the 70 °C incubation for 10 minutes at 900 rpm had less DNA than the mixed samples tested using the 56 °C incubation for 1 hour at 550 rpm. This could have been due to differences in how vigorously the samples were collected or how much DNA was available at the time the samples were collected.

These temperature results indicate that the incubation of the samples can be run at either 56 °C for 1 hour at 550 rpm or at 70 °C for 10 minutes at 900 rpm, which would result in consistent results. To save time on the continuing studies the 70 °C incubation for 10 minutes at 900 rpm was chosen using the in-house digest buffer as epithelial lysis buffer, wash buffer and sperm lysis buffer.

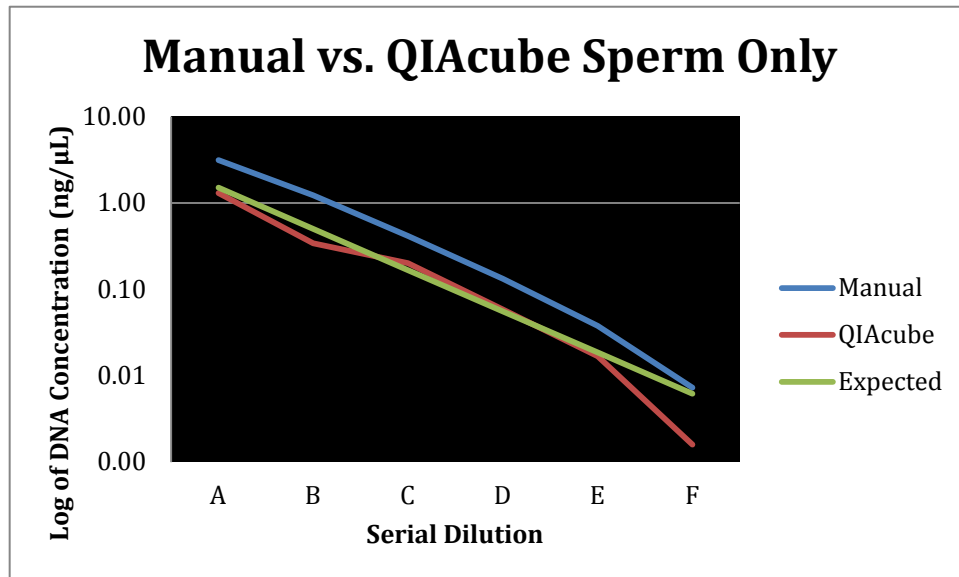
*Sensitivity Study*

Figure 9: Sensitivity Between Manual and QIAcube®.

Figure 9 shows the comparison of the dilution series run manually using the QIAamp® DNA Blood Mini Kit with the same dilution series run using the QIAcube® when utilizing the in-house digest buffer and the 70 °C incubation for 10 minutes at 900 rpm. It can be seen that the manual method significantly out performs the QIAcube® by obtaining approximately twice as much DNA. The manual method can also be seen to be approximately twice as high as the expected quantities. The low expected values are likely a result of the concentration of the neat in-house semen sample being determined using the QIAcube® for extraction and purification. Although the expected values are not accurate, based on the higher manual concentrations, the results are still relevant to show the currently used manual differential extraction method is superior to the automated differential method of the QIAcube® utilizing the available reagents and kits.

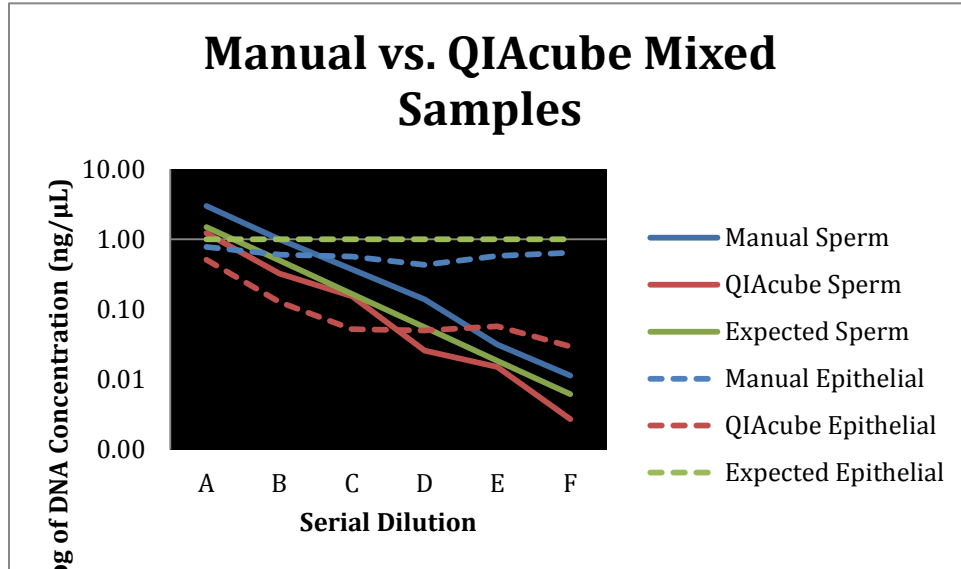


Figure 10: Sensitivity Between Manual and QIAcube® of Mixed Samples.

Figure 10 confirms the conclusions made from Figure 9. The manual sperm samples contain approximately twice as much DNA as both the QIAcube® samples and the expected values. This again shows that the expected values were not accurate because they were determined using the QIAcube® method. The epithelial differences may be from a combination of the inefficiency of the QIAcube® method and/or the possibility of different quantities of DNA being on the saliva FTA cards sampled.

Due to the fact that the sensitivity study showed that the QIAcube® automated differential extraction using the in-house digest buffer and the 70 °C incubation for 10 minutes at 900 rpm in combination with the automated purification in the QIAcube® using the QIAamp® DNA Blood Mini Kit was not comparable to the manual QIAamp® DNA Blood Mini Kit differential extraction currently being used in the lab, it was concluded that a reproducibility study would be trivial and was not done.

*Contamination Study*

The expected results were obtained for this study. Most of the blank samples from both the sperm and epithelial fraction contained a concentration of 0.00 ng/ $\mu$ L after quantification using Plexor<sup>®</sup> HY. The few blank samples that showed low amounts of DNA were quantified a second time and resulted in a concentration of 0.00 ng/ $\mu$ L. The samples containing the male semen and female blood mixture resulted in a range of DNA concentrations between 7.45 and 1.44 ng/ $\mu$ L after quantification using Plexor<sup>®</sup> HY. Due to time limitation the samples were not amplified or typed using a genetic analyzer. The 0.00 ng/ $\mu$ L quantification results were sufficient in concluding that the instrument was capable of preventing cross-contamination of samples.

*Substrate Study*

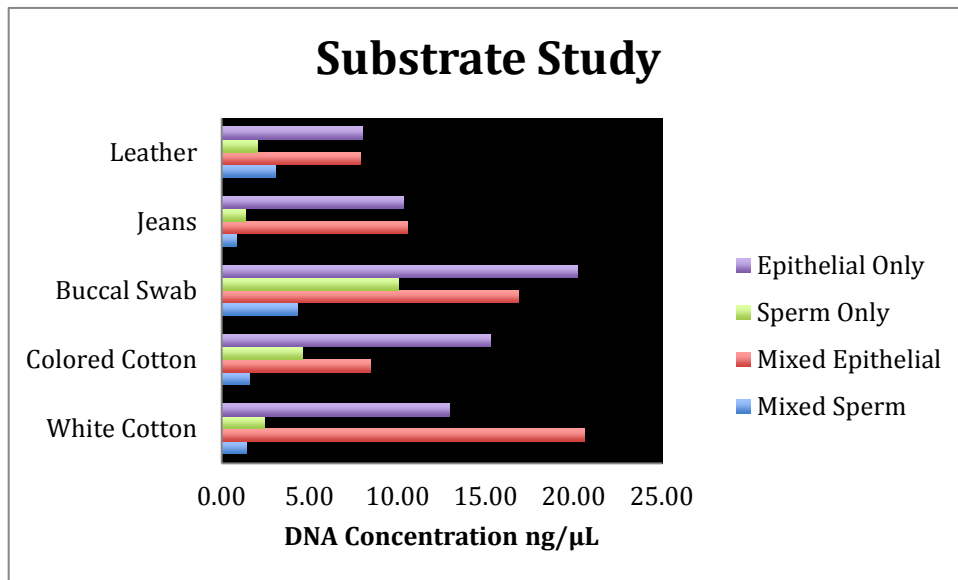


Figure 11: Substrate Comparison

Figure 11 shows the differences in the DNA extracted from different types of samples. Buccal swabs seem to be the substrate that the QIAcube<sup>®</sup> extracts the most sperm from out of the different types tested. From this study it appears that both cotton swabs and



white cotton are the best choices when it comes to the QIAcube® extracting the most epithelial DNA from a sample.

### *Discussion and Conclusion*

From the results it was concluded that the QIAcube® could perform differential extraction without cross-contamination of adjacent samples. It was also concluded that although an analyst cannot choose the samples submitted in casework, the substrates most likely to produce a DNA profile from the sperm fraction are buccal swabs followed by colored cotton and white cotton. This conclusion could assist in the collection of sexual assault evidence by informing the hospitals and investigators of the most probative samples. In addition, the results showed that the optimal buffer to use on the QIAcube® as an epithelial lysis buffer, wash buffer and sperm lysis buffer was the in-house lysis buffer of the WCSO DNA Lab. It was determined that the difference between the 70 °C incubation for 10 minute at 900 rpm and the 56 °C incubation for 1 hour at 550 rpm was not great enough to choose one over the other. The combination chosen would depend on the analysts preference and the condition of the original sample, for instance if the sample was suspected of being degraded or have a minimal amount of sperm DNA the sample may be incubated with the lower temperature for a longer time to insure all possible sperm were lysed to release their DNA.

Although finding the appropriate buffer to use as well as the proper incubation combinations for the sperm lysis step seemed to optimize the extracting power of the QIAcube®, the results from the sensitivity study show the manual method using the QIAamp® DNA Blood Mini Kit far out performs the automated method. There have been several validation studies of the QIAcube® differential wash protocol that have

shown it is comparable to manual methods performed in various labs. However, these studies indicated that the two differentiated fractions were purified using the QIAamp® DNA Investigator Kit manually or automated on the QIAcube® (2) or with the EZ1® Advanced instrument using the EZ1® DNA Investigator Kit (5). This is confirmed by the Qiagen® website and the pamphlet advertising the automated differential wash protocols for the QIAcube® (8). It is unclear why the automated QIAamp DNA Blood Mini Kit protocol on the QIAcube® did not show similar results from the previous studies.

Although the chemistries in the QIAamp® DNA Blood Mini Kit are almost identical to those in the QIAamp® DNA Investigator Kit, there seems to be something that allows the QIAamp® DNA Investigator Kit to work with the QIAcube® differential wash protocol while leaving the QIAamp® DNA Blood Mini Kit incompatible with it. More studies should be done to determine the differences between the two kits and how to make the QIAamp® DNA Blood Mini Kit compatible with the QIAcube® differential wash protocol.

The WCSO DNA lab has determined that the QIAcube® differential wash protocol will not be utilized for differential extractions unless future modifications of the standard protocols result in higher male yields. Options for utilizing the QIAcube® differential wash protocol include investing in an EZ1® Advanced instrument or switching to the QIAamp® DNA Investigator Kit for purification following differential extraction, however, both options require multiple validations to be done before introduction into casework workflow. For the present time the lab will continue using the QIAamp® DNA Blood Mini Kit manually for differential extractions.

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