INVESTIGATIONS ON THE USE OF SAMPLEMATRIX TO STABILIZE CRIME SCENE BIOLOGICAL SAMPLES FOR OPTIMIZED ANALYSIS AND ROOM TEMPERATURE STORAGE

Award Number: 2007-DN-BX-K172

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Abstract

Storage and preservation of biological evidence are key forensic issues that are currently under investigation in anticipation of more cost saving and effective stabilization methods. Once evidence has been identified and collected at a crime scene, it is typically stored for analysis later. Storing biological evidence at subzero temperatures is one of the current methods employed to prevent DNA degradation until time of analysis; however, previous studies have shown that freezing biological evidence does not completely cease the degradation process. In addition, it has been shown that repeated freezing and thawing of biological evidence may add to the degradation effects. The research presented here evaluates a proprietary platform technology for the dry storage of biological materials at ambient temperature. The technology behind this room temperature stability is primarily adopted from natural principles and is based on the DNA stabilizing properties of small molecules such as trehalose. Three non-lysis formulations (SM1, SM2, and SM3) and one lysis formulation were evaluated with approximately 7,500 samples.

The coating agent study evaluated the total amount of DNA recovered from blood, saliva, and semen samples deposited on a cotton swab following a 6-24 month storage period. The samples were subjected to the following experimental variables: protection with one of two SampleMatrix™ formulations (SM1 and SM2) as a post-collection treatment of the sample, extraction method, storage medium, dilution factor, and the method used to apply SampleMatrix™ as a coating agent (applied to wet sample versus dry). The wetting agent study also evaluated the performance of the two formulations of SampleMatrix™ in comparison to water in the collection and preservation of DNA recovered from blood, saliva, and semen samples. These samples were subjected to the following experimental variables: the extraction method, the storage medium, dilution factor, and the substrate used to deposit the samples prior to recovery with cotton swabs. Two environmental insult studies were performed. The first insult study evaluated the DNA recovery from blood, semen, and saliva samples deposited on a cotton swab following storage under accelerated aging conditions (elevated temperature at 50 ºC) for 1-19 weeks. The second insult study determined the recovery of DNA from blood swabs in relation to storage condition (frozen, room temperature, unprotected at 55 ºC/60% humidity) and composition of the protective coating (SM1, SM2, and lysis formulation) over a 1-4 week period. Further studies were designed to assess the ability of SampleMatrix™ to store and preserve blood, semen and saliva stains relative to conventional forensic methods that maintain the samples in cold storage. The cell morphology study was designed to test the effectiveness of the SM3 formulation in maintaining the structural integrity of red blood cells (RBC) and white blood cells (WBC) when stored at room temperature. The primary focus was to evaluate the preservation of SM3 treated blood relative to untreated blood as a function of storage time. The

final studies investigated the effects of the collection methods and storage conditions for the wetting and coating agent studies on the quality of the DNA genotyping profiles.

The findings support that SampleMatrix™ was compatible with each extraction chemistry evaluated and with the conventional serology tests when used as a wetting or coating agent to protect biological samples. While clear differences in the mean DNA yield are evident when comparing the collection and storage conditions, in the majority of cases these differences were not statistically significant. For example, the results of the wetting studies indicate that SampleMatrix™ protected samples consistently gave greater mean DNA yields compared with unprotected control samples. However, the ANOVA and t test analyses determined that the differences observed are not statistically significant. This attributed to the large standard deviation associated with each mean. The factors contributing to the large standards deviation include the small number of replicate samples, the variation introduced during sample preparation and DNA extraction, and the use of multi-step analytical techniques that required extensive manual manipulations. For the wetting agent studies, SampleMatrix™ outperformed water in recovering DNA from blood or semen and gave a comparable DNA recovery to water from saliva samples. For the coating agent study, preliminary findings indicate that the SampleMatrix™ protected blood samples result in a higher DNA recovery than samples stored at -20ºC but were lower than the unprotected control samples that were also stored at ambient temperature. The coating agent studies for semen suggest that storage at room temperature (unprotected) or at -20ºC provides the better DNA recovery in the short-term but there is an advantage when storing these samples at -20ºC for more extended times. The coating agent studies for saliva support that storage at room temperature with SampleMatrix™ provides the better DNA recovery in the short-term but that there is an advantage when storing these samples at -20ºC for more extended times. The results of the accelerated aging studies demonstrate an advantage to SampleMatrix™ protected samples, regardless of the body fluid or dilution. Based on a comparison of the mean yield, the data indicate that SampleMatrix™ SM1 outperforms SM2 and applying either formulation following a 24-hour drying period results in a higher recovery of DNA as compared with the immediate application of SampleMatrix™. The results of the cell morphology studies indicate that SM3-treated blood preserves red blood cell morphology at intervals substantially greater than that of untreated blood. Finally, the DNA genotyping results support that the SampleMatrix™ formulations evaluated in the studies reported here do not interfere with the quality of the STR profiles.

In summary, it is important to note that when considering the experimental variables for the wetting and environmental insult studies, a comparison of the various storage conditions shows that SampleMatrix™ protected samples provide mean DNA yields that are equivalent or greater compared with the unprotected controls. Further, when comparing mean recovery as a function of substrate or dilution series, significant differences in the mean recovery are observed. While these comparisons were not the primary focus of this study, they lend support to the fact that the data are not randomly distributed. The data also support that there is no disadvantage to storing samples at room temperature as compared with the frozen storage. However, statistically analyses were performed in order to assess the differences in the mean DNA yield for the protected and unprotected samples. The results, with few exceptions, indicate that the differences are not statistically significant and lead us to conclude that we cannot support any of our original hypotheses. Clearly, a larger number of replicates are required in order to determine the significance of the treatment methods.

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EXECUTIVE SUMMARY

Problem Statement

The collection and storage of biological evidence are key forensic issues that are currently being investigated in anticipation of cost saving and more effective stabilization methods. Once evidence has been identified and collected at a crime scene, it is typically stored for analysis later. Storing biological evidence at subzero temperatures is one of the current methods employed to prevent DNA degradation until time of analysis; however, previous studies have shown that freezing biological evidence does not completely cease the degradation process. In addition, it has been shown that repeated freezing and thawing of biological evidence may contribute to degradation. In an attempt to circumvent these issues, several companies have developed products that allow biological evidence to be stored at ambient temperature.

The study presented here is relevant to the field of forensic science because the ability to recover a DNA profile is contingent on the quality and quantity of DNA recovered. Despite a field investigator's best attempt to properly collect and package biological evidence, the storage conditions will have a significant effect on the final recovery of a DNA profile. Many samples are collected in less than desirable conditions. This may be because samples have been exposed to harsh environmental conditions such as direct sunlight or high humidity, which both promote DNA degradation. Therefore, the storage conditions of the sample should ideally prevent any further degradation. Degraded or low copy number DNA can significantly reduce the discriminating power of a DNA profile. Partial profiles and allelic dropout are common effects of analysis of a degraded DNA sample.

Although a sample may have been collected appropriately and is considered of high quality, it may not be analyzed immediately by the laboratory. The analysis timeframe is largely dictated by current case backlogs in many forensic laboratories. Due to the high demand for DNA testing and less than adequate forensic staffing, samples are often stored for several months if not years before analysis. This is particularly true in jurisdictions where agencies collect biological samples for non-violent property crimes. Therefore, storage of biological samples is of equal importance to obtaining a DNA profile as is collection, analysis, and interpretation. Although current storage methods have succeeded in slowing the process of degradation, alternative methods must be investigated to improve the recovery of DNA from stored biological evidence. The study reported here investigates the preservation properties of SampleMatrix™ as a sustainable room temperature stability product. Incidental benefits include the potential to positively impact financial and space concerns of forensic laboratories. Currently, forensic laboratories must purchase and maintain large freezers to store biological evidence, which results in high costs and a growing need for space. Room temperature storage could essentially eliminate the need for multiple freezers and significantly reduce a laboratory's annual overhead costs.

This research examined four SampleMatrix™ formulations: SM1, SM2, SM3, and a lysis formulation for the capture and preservation of biological samples collected on swabs. The formulations were specifically designed for this research. SM1 and SM2 were general-purpose formulations; SM3 was formulated to preserve red blood cells and white blood cells in whole blood; and the lysis formulation was designed to rupture cells for the immediate stabilization of the native DNA.However, the SampleMatrix™ technology was originally designed for the room

temperature storage of DNA extracts. We saw its possible application for the capture and preservation of biological samples collected with swabs. Swab samples are subject to numerous and diverse types of testing, and laboratories can follow different procedures as to the overall processing of swab samples. Therefore, many issues must be considered in order to evaluate the SampleMatrix™ technology for this application. We decided on a comprehensive approach to assess the technology. We examined the compatibility of the technology with current methods, and compared its performance to that of standard practices, in relationship to the many facets of sample collection, storage, and testing. We reasoned that a general view was the best approach to take first, in that it would identify any fundamental problems with the technology that needed to be corrected at the start. Once the technology was optimized for general use, we reasoned, more focused and detailed studies would follow to better quantify the specific performance characteristics of the technology. Our survey approach did prove to be enlightening, but it also had its shortcomings. Given the many different experiments, the sample size for any one variable was often small. This complicated the statistical analysis of the results. Additionally, we tested the technology against standard methods used by forensic laboratories. While this directly measured the technology's compatibility with present practices, it also introduced higher degrees of variance, because of the large amount of manual manipulation associated with these methods. Notwithstanding, as a first approximation, the results of this research are informative and provide a foundation for the further investigation of this promising technology.

Purpose, Research Design and Hypotheses

i. Conventional Serology

These studies were designed to assess the effect of SampleMatrix™ on the conventional serological testing of blood, saliva, and semen with respect to the following independent variables: sample dilutions, storage conditions, and storage time. The effect of each independent variable on the dependent variables (presumptive and/or confirmatory test results) was measured based on the biochemical reaction obtained.

The specific objectives relating to blood were to evaluate the following research questions:

1. How do the sample dilutions affect the results of the presumptive tests for blood and species of origin test?
2. Does the storage time affect the results of the presumptive tests for blood and species of origin test?
3. Does the storage condition affect the results of the presumptive tests for blood and species of origin test?
4. With respect to SampleMatrix™ storage at room temperature:
    a. How do the specific SampleMatrix™ formulations compare in terms of the results of the presumptive tests for blood and species of origin test?
    b. Are the two SampleMatrix™ formulations compatible with the presumptive tests and species of origin test as compared with conventional freezer storage methods?

The specific objectives relating to semen were to evaluate the following research questions:

1. Does sample dilution affect the results of the AP test, the PSA test, and the morphological evaluation?
2. Does the storage time affect the results of the AP test, the PSA test, and the morphological evaluation?
3. Does the storage condition affect the results of the AP test, the PSA test, and the morphological evaluation?
4. With respect to SampleMatrix™ storage at room temperature:
    a. How do the specific SampleMatrix™ formulations compare in terms of the results of the AP test, the PSA test, and the morphological evaluation?
    b. Are the two SampleMatrix™ formulations compatible with the AP test, the PSA test, and the morphological evaluation?

The specific objectives relating to saliva were to evaluate the following research questions:

1. Does sample dilution affect the results of the alpha-amylase test, the SALIgAE® test, and the morphological evaluation?
2. Does the storage time affect the results of the alpha-amylase test, the SALIgAE® test, and the morphological evaluation?
3. Does the storage condition affect the results of the alpha-amylase test, the SALIgAE® test, and the morphological evaluation?
4. With respect to SampleMatrix™ storage at room temperature:
    a. How do the specific SampleMatrix™ formulations compare in terms of the results of the alpha-amylase test, the SALIgAE® test, and the morphological evaluation?
    b. Are the two SampleMatrix™ formulations compatible with the alpha-amylase test, the SALIgAE® test, and the morphological evaluation?

Hypotheses

Blood, semen and saliva samples stored at room temperature protected by SampleMatrix™ were predicted to give a greater number of positive test results, particularly at higher dilutions and at longer storage time periods, as compared with the control samples stored without SampleMatrix™, either at room temperature or frozen. Additionally, it was expected that SampleMatrix™ will neither interfere nor compromise the results of the presumptive tests Given that the specific formulations of SM1 and SM2 are proprietary, there was no expectation as to which would provide better protective properties; this particular evaluation was considered a blind test for the purpose of this study.

ii. Wetting Agent Studies

These studies were designed to compare the use of water versus SampleMatrix™ as a swab wetting agent in recovering DNA (dependent variable) from blood, semen, and saliva with respect to the following independent variables: storage conditions, substrate used to deposit stain, extraction chemistry, stain dilution factor, and specific SampleMatrix™ formulation.

The specific objectives of these studies were to evaluate the following research questions:

1. Are the different extraction methods compatible with the use of SampleMatrix™ as a wetting agent?
2. How does SampleMatrix™ compare to water in terms of recovering DNA from various substrates?
3. How does SampleMatrix™ compare to water in terms of recovering DNA at different dilutions?
4. How do the specific SampleMatrix™ formulations compare in terms of effective recovery of DNA?

iii. Coating Agent Studies

These studies were designed to assess the performance of SampleMatrix™ as a post-collection swab treatment by measuring the recovery of total human nuclear DNA from blood, semen and swabs with respect to the storage conditions, extraction chemistry, stain dilution factor, specific SampleMatrix™ formulation, and time delay prior to application of SampleMatrix™ to the sample.

The specific objectives of these studies were to evaluate the following research questions:

1. Are the different extraction methods compatible with the use of SampleMatrix™ as a coating agent?
2. How does SampleMatrix™ compare to the unprotected control samples in terms of recovery of DNA?
3. How do the specific SampleMatrix™ formulations compare in terms of effective recovery of DNA?
4. Does the time delay in applying SampleMatrix™ to blood swab affect or affect recovery of DNA?

Hypotheses

SampleMatrix™ biotechnology is designed to preserve biological sample by simulating anhydrobiosis. Anhydrobiosis stabilizes molecules by applying a vitrifying element to the molecules in order to stabilize it in the absence of water. The traditional method of freezing samples simply retards the degradation process, but does not provide specific stabilizing factors. Therefore, was predicted that SampleMatrix™ would provide greater DNA recovery than both the unprotected samples stored at room temperature and frozen samples. There was no expectation that SampleMatrix™ would interfere with any of the three extraction methodologies (Qiagen, Chelex, and Organic). Given that the SM1 and SM2 formulations are proprietary, there was no expectation as to which would provide better DNA protective properties. This particular evaluation was considered a blind test for the purpose of this study. With respect to the coating agent study, blood, semen and saliva samples stored at ambient temperatures protected by SampleMatrix™ were predicted to result in higher DNA recovery as compared to control samples that were stored without SampleMatrix™, either at ambient temperature or at -20°C (frozen). Since DNA degradation is more readily observed at concentrations below 1-2ng, the benefits of SampleMatrix™ should have a greater impact with lower amounts of starting DNA. However, it was hypothesized that the addition of SampleMatrix™ immediately following the

deposition of a biological fluid (wet application) would afford greater homogeneity, and thus would offer greater protection relative to delaying the application for a 24-hour period.

iv. Environmental Insults

a. Elevated Temperature

This study was designed to evaluate the recovery of DNA from blood, semen, and saliva swabs with respect to time exposed to accelerated aging conditions, stain dilution factors and specific SampleMatrix™ formulation.

The specific objectives of this study were to evaluate the following research questions:

1. Do the SampleMatrix™ formulations protect DNA that is exposed to the effects of accelerated aging as compared to untreated samples?
2. How do the specific protective properties of SampleMatrix™ formulations compare in terms to the effective recovery of DNA?
3. Does SampleMatrix™ protect DNA associated with different biological fluids?
4. Assuming SampleMatrix™ affords protection, do the protective properties of SampleMatrix™ provide advantages as the amount of DNA present in a biological sample decreases?
5. If SampleMatrix™ does protect nuclear DNA, how long will the biostability suffice under accelerated aging conditions?

Hypotheses

Regardless of the biological fluid, all of the samples that are protected by SampleMatrix™ were predicted to yield a higher DNA recovery in comparison with unprotected samples when subjected to accelerated aging conditions. The performance of SampleMatrix™ at lower concentration was anticipated to be more important because protecting DNA at lower levels may be critical to obtaining a STR profile.

b. Elevated Temperature and Humidity

This study was designed to determine the recovery DNA from blood swabs in relation to storage condition (frozen, room temperature, unprotected at high temperature), composition of the protective coating (SM1, SM2, and the lysis formulation), dilution factor, and length of time exposed to elevated temperature (55 °C) and humidity (60%). The objectives of this study were to address the following research questions:

1. Does SampleMatrix provide better protection to DNA to blood samples that have been exposed to the effects of elevated temperature and humidity as compared to untreated control samples?
2. How do the specific SampleMatrix formulations compare in terms of the effective recovery of DNA?
3. Assuming SampleMatrix affords protection, do the protective properties provide advantages as the amount of DNA present in a blood sample decreases?
4. If SampleMatrix does protect DNA, how long will the biostability persist at elevated temperature and humidity?

Hypotheses

Based on information provided in the relevant literature, it was predicted that SampleMatrix™ would withstand high temperatures and that blood samples protected with SampleMatrix™ would allow for greater recovery of DNA relative to the unprotected control samples. Additionally, it was expected that samples stored unprotected at room temperature or frozen would allow for greater recovery than unprotected samples exposed to high temperature. Further, it was also predicted that SampleMatrix would assist in the protection of DNA at moderate humidity levels. No predictions were made in relation to the comparative properties of the two non-lysis formulations: SM1 and SM2. However, it was predicted that the lysis formulation would provide increased protection as compared to both non-lysis formulations because the lysis formulation is designed specifically to cause cell lysis, providing direct protection to free DNA. In contrast, the non-lysis formulations (SM1, SM2, SM3) are designed to protect the intact cell as a whole.

v. Red Blood Cell Morphology

In this study, the protective properties of the SM3 non-lysis formulation were evaluated with respect to the following dependent variables: cell morphological integrity and quantity of DNA recovered as a function of time. The research examined the morphology of aged red blood cells, white blood cells, and nuclei treated with SM3 over the course of 31 weeks.

Hypotheses

The study broadly surveyed the effectiveness of the SM3 formulation in preserving whole blood cells at room temperature for the purposes of downstream forensic analysis. Aged SM3-treated samples were assessed both qualitatively and quantitatively for preservation of characteristics relevant to applications in forensic science. Based upon the established viability of SampleMatrix™ in preserving purified DNA at room temperature for extended periods, it was hypothesized that similarly stored SM3-treated whole blood will demonstrate preserved integrity of component morphology to a degree comparable with that of freshly drawn, untreated samples.

vi. DNA Analysis

This study was designed to evaluate the quality of DNA recovered from semen, saliva, and blood swabs stored for 17-24 months with respect to the following independent variables: storage conditions, extraction method, several different dilution factors, specific SampleMatrix™ formulation, and the method (wet *vs*. dry) used to apply SampleMatrix™.

This study evaluated the following research questions:

1. Does the storage condition (frozen, room temperature unprotected, room temperature protected) affect the quality of the DNA profile recovered?
2. Which SampleMatrix™ formulation (SM1 vs. SM2) is more effective in protecting DNA in order to optimize STR typing?
3. Which coating application method (applied to wet vs. dry sample) is more effective in protecting DNA in order to optimize STR typing?

Hypotheses

Samples stored at room temperature, protected by SampleMatrix™ are predicted to result in the recovery of higher quality DNA and are therefore expected to produce more complete STR profiles as compared to samples stored without SampleMatrix™, stored at room temperature or frozen. In addition, it was predicted that SampleMatrix™ would not interfere with the amplification reactions or STR genotyping analysis.

Findings

Assessment of DNA Yields

The conclusions are based on a comparison of the mean DNA yields of the various experimental variables. While clear differences in the mean DNA yield are evident when comparing the collection and storage conditions, in the majority of cases these differences were not statistically significant. For example, the results of the wetting studies indicate that SampleMatrix™ protected samples consistently gave greater mean DNA yields compared with unprotected control samples. However, the ANOVA and t test analyses determined that the differences observed are not statistically significant. The authors attribute this to the large standard deviation associated with each mean. The factors contributing to the large standard deviation include the small number of replicate samples, the variation introduced during sample preparation and DNA extraction, and the use of multi-step analytical techniques that required extensive manual manipulations. For the wetting agent studies, SampleMatrix™ outperformed water in recovering DNA from blood or semen stains and gave a comparable DNA recovery to water from saliva stains. For the coating agent study, preliminary findings indicate that the SampleMatrix™ protected blood samples result in a higher DNA recovery than samples stored at -20ºC but were lower than the unprotected control samples that were also stored at ambient temperature. The coating agent studies for semen suggest that storage at room temperature (unprotected) or at -20ºC provides the better DNA recovery in the short-term but there is an advantage when storing these samples at -20ºC for more extended times. The coating agent studies for saliva support that storage at room temperature with SampleMatrix™ provides the better DNA recovery in the short-term but that there is an advantage when storing these samples at -20ºC for more extended times. The results of the accelerated aging studies demonstrate a clear advantage to SampleMatrix™ protected samples, regardless of the body fluid or dilution. Overall, the data indicate that SampleMatrix™ SM1 outperforms SM2 and applying either formulation following a 24-hour drying period results in a higher recovery of DNA as compared with the immediate application of SampleMatrix™.

Summary of Conclusions and Implications for Policy and Practice

There are some clear trends in the final analyses. The data indicates that SampleMatrix™, specifically the SM1 formulation, presents advantages when used as a wetting agent for the recovery blood and semen stains deposited on several substrates. This is in comparison with the standard practice of forensic laboratories to collect blood and semen by wetting the swab with water and storing the swab in a frozen condition. The results obtained with saliva stains are more ambiguous; however, the data suggest that the performance of SampleMatrix™ is comparable to the standard method. The optimization of a formulation that improves the stabilization of saliva

should be considered for future research. In phase II of our original proposal (not originally funded), we considered the development of a crime scene collection kit based on the SampleMatrix™ technology. Our research indicates that none of the SampleMatrix™ formulations interferes with conventional serological testing. In fact, enzymatic-based testing showed improved results with SampleMatrix™-treated samples.

Research on the optimization of SampleMatrix™ as a coating agent should be pursued given that evidentiary samples may be submitted which were collected by the standard method (an example includes the collection of sexual assault kits). The findings of our accelerated aging study support the advantage of SampleMatrix™ as a coating agent relative to unprotected samples. One approach to the treatment of swabs would be to consider the optimization of the coating formulation. Again, our data supports an advantage of the formulation in protecting blood samples. However, the current formulation appears to favor more concentrated blood samples; therefore, the formulation may need to be modified to extend the benefits to dilute samples.

Our findings show a clear advantage in the ability of the SM3 formulation in maintaining the morphology of WBC and RBC. Few forensic methods are available for the positive identification of blood. Future research should explore the potential of SM3 as a wetting agent to recover bloodstains for the dual purpose of identifying the stain as blood and determining the genotype of the stain.

In summary, it is important to note that when considering the experimental variables for the wetting and environmental insult studies, a comparison of the various storage conditions shows that SampleMatrix™ protected samples provide mean DNA yields that are equivalent or greater compared with the unprotected controls. Further, when comparing mean recovery as a function of substrate or dilution series, significant differences in the mean recovery are observed. While these comparisons were not the primary focus of this study, they lend support to the fact that the data are not randomly distributed. The data also support that there is no disadvantage to storing samples at room temperature as compared with the frozen storage. However, statistically analyses were performed in order to assess the differences in the mean DNA yield for the protected and unprotected samples. The results, with few exceptions, indicate that the differences are not statistically significant and lead us to conclude that we cannot support any of our original hypotheses. Clearly, a larger number of replicates are required in order to determine the significance of the treatment methods.

CHAPTER 1: INTRODUCTION

1. Statement of the problem

The collection and storage of biological evidence are key forensic issues that are currently being investigated in anticipation of cost saving and more effective stabilization methods. Once evidence has been identified and collected at a crime scene, it is typically stored for analysis later. Storing biological evidence at subzero temperatures is the current method employed to prevent DNA degradation until time of analysis; however, previous studies have shown that freezing biological evidence does not completely cease the degradation process. In addition, it has been shown that repeated freezing and thawing of biological evidence may contribute to degradation. In an attempt to circumvent these issues, several companies have developed products that allow biological evidence to be stored at ambient temperature.

The study presented here is relevant to the field of forensic science because the ability to recover a DNA profile is contingent on the quality and quantity of DNA recovered. Despite a field investigator's best attempt to properly collect and package biological evidence, the storage conditions will have a significant effect on the final recovery of a DNA profile. Many samples are collected in less than desirable conditions. This may be because samples have been exposed to harsh environmental conditions such as direct sunlight or high humidity, which both promote DNA degradation. Therefore, the storage conditions of the sample should ideally prevent any further degradation. Degraded or low copy number DNA can significantly reduce the discriminating power of a DNA profile. Partial profiles and allelic dropout are common effects of analysis of a degraded DNA sample.

Although a sample may have been collected appropriately and is considered of high quality, it may not be analyzed immediately by the laboratory. The analysis timeframe is largely dictated by current case backlogs in many forensic laboratories. Due to the high demand for DNA testing and less than adequate forensic staffing, samples are often stored for several months if not years before analysis. This is especially the case in areas where agencies collect biological samples for non-violent property crimes. Therefore, storage of biological samples is of equal importance to obtaining a DNA profile as is collection, analysis, and interpretation. Although current storage methods have succeeded in slowing the process of degradation, alternative methods must be investigated to improve the recovery of DNA from stored biological evidence. The study reported here investigates the preservation properties of SampleMatrix™ as a sustainable room temperature stability product. Incidental benefits include the potential to positively impact financial and space concerns of forensic laboratories. Currently, forensic laboratories must purchase and maintain large freezers to store biological evidence, which results in high costs and a growing need for space. Room temperature storage could essentially eliminate the need for multiple freezers and significantly reduce a laboratory's annual overhead costs.

DNA Stability

DNA is a nucleic acid that exists in nature as a double stranded molecule consisting of an organic base, a five-carbon sugar and a phosphate group (Figure 1). The bases occupy the core of the helix and sugar-phosphate chains are coiled about its periphery, thereby minimizing the repulsions between charged phosphate groups [1]. Each base is hydrogen bonded to a base on the opposing strand to form a planar base pair. These hydrogen-bonding interactions result in the specific association of the two chains of the double helix. DNA does not possess the structural

integrity of proteins, mainly because it lacks a tertiary or quaternary structure; however, DNA does possess several stabilizing properties. For one, DNA is capable of denaturation and renaturation under certain conditions. When DNA is heated its structure collapses and its two complementary strands separate and assume a random conformation. This can be monitored by ultraviolet (UV) spectrometry, as the absorbance value increases by approximately 40% when DNA is denatured, an effect that is attributed to the molecule's aromatic bases. The stability of the native structure of DNA depends on several factors, including temperature and pH conditions. However, under the appropriate conditions, DNA is capable of renaturation [1].

Base pairing is another factor in the stability of double stranded nucleic acids. The nucleotides in DNA specifically pair to one another, adenine (A) to thymine (T) and guanine (G) to cytosine (C), the former via a double bond and the latter via a triple bond. Although hydrogen bonding confers some stabilizing properties it contributes little to the overall stability of the double helix [1]. For instance, if non-polar ethanol is added to an aqueous DNA solution it will strengthen the hydrogen bonds but it will also destabilize the double helix. This is due to hydrophobic forces that are disrupted by non-polar solvents. In contrast, the hydrogen bonds between the base pairs of native DNA are replaced in denatured DNA by equivalent hydrogen bonds between the bases and water [1].

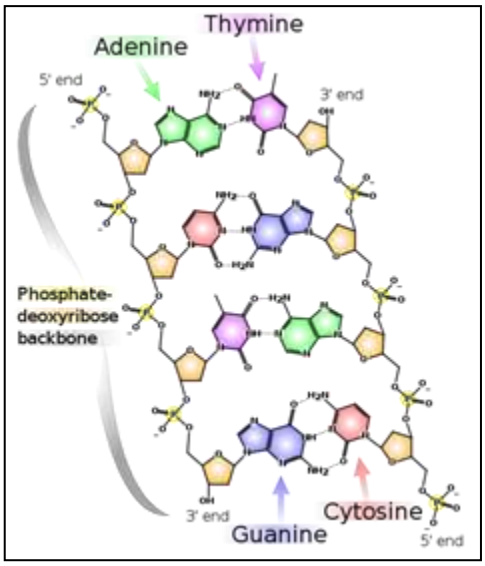


Fig. 1: DNA Chemical Structure [2].

Base stacking also affects DNA stability; this phenomenon refers to the fact that purines and pyrimidines tend to form extended stacks of planar parallel molecules. Stacking interactions also have some specificity but not to the same degree as base pairing. Stacking associations are largely stabilized by hydrophobic forces; however, the mechanism is not completely understood [1]. One final factor in relation to DNA stability concerns the electrostatic interactions of the charged phosphate groups. The melting temperature of the double helix increases with cation concentration because these ions electrostatically shield the anionic phosphate groups from each other [1]. Monovalent cations such as Na^+, Li^+, and K^+ all have similar nonspecific interactions with phosphate groups. In contrast, divalent cations such as Mg^{2+} and Mn^{2+} specifically bind to phosphate groups and are far more effective shielding agents for nucleic acids than are

monovalent cations. Enzymes that mediate reactions with nucleic acids usually require Mg^{2+} for activity and play an essential role in stabilizing the complex structures assumed by many ribonucleic acids (RNA) [1].

Factors that Compromise Biostability

Although DNA possesses several properties that aide in its chemical stability, there are several opposing factors, including elevated temperatures, increased humidity, changes in pH, UV exposure and the effect of multiple freeze-thaw cycles. DNA in a neutral (pH~ 7.0) solution will change structurally when exposed to high temperatures. With a small increase in temperature, the macromolecular structure of DNA will denature and the strands will separate once the linking hydrogen bonds have broken; however, within a larger temperature interval, the heat will induce a degradation of the primary structure of the DNA that results in irreparable damage due to depurination [3]. In addition, the amount of water present or humidity levels can have a negative effect on the stability of DNA. DNA is unstable in the presence of water because of its sensitivity to the addition of water across its phosphodiester bonds, which produces stand breakage. If water is absent, the rate of strand breakage becomes very slow. In addition, the hydrated state limits the long-term storage of DNA samples because it promotes the growth of yeast, mold, and bacteria that can degrade DNA [4].

Ultraviolet exposure has been shown to decrease DNA stability, which can cause a change in the DNA sequence. Ultraviolet light damages DNA by producing thymine dimers, which is the covalent bonding of two adjacent thymine residues within a DNA molecule. UV light is also known to induce apurinic/apyrimidinic sites in DNA, which is a spontaneous lesion in the DNA chain [5]. Apurinic/apyrimidinic sites in DNA result from the cleavage of the N-glycosylic bond that connects the purine or pyrimidine base to the deoxyribose sugar [6].

Additionally, the storage conditions can have a drastic effect on the chemical stability of DNA. Current storage practices involve freezing samples; however, current protocols mandate that the samples must be thawed prior to analysis. Samples are often refrozen after initial analysis for use later and this process may occur multiple times. Over time, multiple freeze-thaw cycles has shown to cause DNA degradation on the stored samples. Further, biological samples intended for long-term storage are sometimes maintained at temperatures reaching -80°C; although water activity is reduced at these temperatures, it persists. While water activity is present, biological and chemical changes can still occur, causing profound changes in DNA structure [7].

In Vitro Biostability

Proper storage and preservation conditions are vital in ensuring the stability and utility of biological samples. Storing biological samples, such as blood, in airtight containers before they are properly dried will ultimately accelerate the deterioration of the sample. Therefore, samples must be air-dried prior to freezer storage to retain the sample's integrity [8]. This will ensure the least amount of sample degradation and allow the laboratory to obtain the most definitive results possible. Dried biological stains are typically collected with a wetted absorbent material such as a cotton-tipped applicator (swab). The applicator is placed (swab side-up) in a container such as a test tube and allowed to dry [8]. For larger items containing biological evidence, laboratories currently use drying sheds to ensure evidence is completely air dry prior to storage. The items are air-dried by exposing to indirect sunlight. Once the items have been sufficiently dried, they are placed in a porous, ventilated container such a paper bag and stored at cold temperatures.

Depending on the estimated time of analysis the items or samples can be refrigerated (4°C) frozen (-20°C or below for short-term storage and 70°C to -80°C for long-term storage).

Biostability at Room Temperature

Preservation of DNA is the key issue relating to DNA storage. The need for alternative methods of storage has given rise to a relatively new form of storage that no longer requires freezing temperatures. FTA™ paper is one example of a substrate that is designed for DNA storage at ambient temperatures that was developed in the 1980s. It is an absorbent cellulose-based paper that contains four chemical substances to protect DNA molecules from nuclease degradation while also preserving the paper from bacterial growth. FTA™ paper products allow samples to be collected, shipped, and stored at room temperature. According to the manufacturer, sample volume requirements are minimal and sample processing requires a simple water elution procedure to isolate the DNA. Use of FTA™ paper requires a small sample (blood) to be applied to the paper and allowed to dry at room temperature. The cells are lysed upon contact with the paper and DNA from the white blood cells is immobilized within the matrix of the paper [9]. At this point, the FTA™ paper cards can either be stored for future extraction or immediately extracted. The extraction procedure requires a small disc to be removed from the card and then placed in a tube. The DNA attached to the card is washed several times, heated, vortexed and centrifuged. Upon removal of the FTA™ disc, the eluent is ready for further analysis such as polymerase chain reaction (PCR).

GenVault incorporates FTA™ paper technology into their products; this company has created high-density, 384-well plates (GenPlates) that contain 6-mm discs of FTA™ paper molded into a hemispherical shape [4]. Approximately 10µL of DNA or blood is added directly to the paper hemisphere, the cells are lysed, and cellular enzymes are inactivated. DNA is then released and becomes entwined in the fibrous cellulose pores. The GenPlate is air dried at room temperature and sealed with an adhesive cover prior to storage or transport. GenVault manufactures another product, GenTegra DNA Tubes, which is a proprietary technology for storing purified DNA in a dry, water free environment. When the DNA is required for analysis, the tubes are simply rehydrated and ready for use. Although FTA™ paper technology is beneficial for reference samples such as whole blood; its application to the treatment of forensic casework samples is problematic. Loss from the process of extracting sample from a swab to a liquid extract of the stain can result in lower DNA yields.

The focus of the study presented here is based on technology from Biomatrica, Inc.; this company has developed a proprietary platform technology for the dry storage of biological materials at ambient temperatures and a lower humidity range. Biomatrica's stabilization technology allows for storage of samples outside cold environments (4ºC, -20ºC, -80ºC and liquid nitrogen) while preserving sample integrity. Samples are preserved through formation of a protective thermo-stable barrier during a simple air-drying process. According to the manufacturer, samples are protected from degradation from heat and UV light. The key component of this technology is SampleMatrix™, which consists of protective agents developed from combining small molecule chemistry with advanced polymer chemistry.

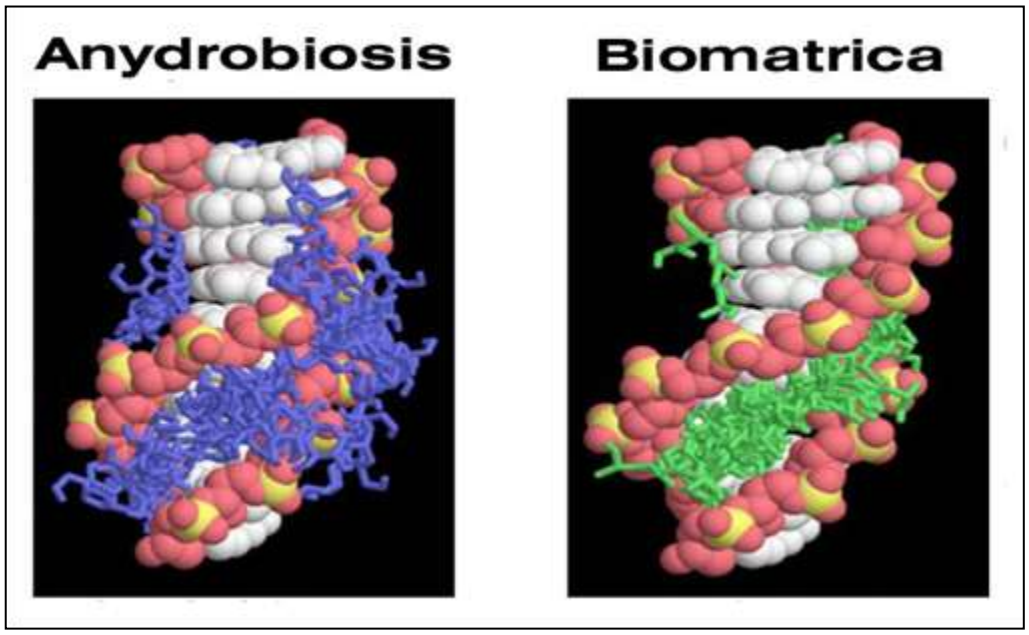


Fig. 2: Comparison of DNA with SampleMatrix™ to DNA Undergoing Anyhydrobiosis [10]

The underlying theory guiding the formulation of SampleMatrix™ was derived from studies on extremophile organisms, some of which may be reduced to anhydrous conditions, a state known as anhydrobiosis (Figure 2). Anhydrobiosis, or life without water, is a phenomenon in which organisms can naturally survive dehydration. Human cells are not desiccant-tolerant; therefore, the removal of intracellular water causes drastic changes in inter- and intra-molecular interactions. This results in forced intermolecular interactions between molecules that normally would not interact with each other in the presence of bulk water [11]. This can cause irreversible changes, which may result in a loss of enzymatic activity as well as fusion of cellular organelles [11]. Several bacterial strains, baker's yeast, cysts of brine shrimp, and tardigrades are examples of organisms that survive because of anhydrobiosis. The tardigrade lives in the water film around soil grains, which dries up as the water in the soil evaporates [12]. The tardigrade can persist in this dry state for decades, during which time they are remarkably resistant to environmental extremes (Figure 3). A better understanding of how these organisms achieve such durability allows the development of the preservation of cells and organisms that are normally incapable of surviving in the dried state [12].

The ability of these organisms to survive in a state of dehydration is partly due to the synthesis of sugars, specifically trehalose, which is often found in high concentrations within their metabolism. The presence of trehalose has been shown to replace the water around the polar residues of membranes and proteins, thus stabilizing these structures in the absence of appreciable amounts of water [13]. In combination with this function, trehalose can also stabilize anhydrobiotic organisms through vitrification, which is the ability to form a glass-like state. A glass is a liquid of such high viscosity that it is capable of slowing chemical reactions or even stopping them altogether [13]. The high viscosity coupled with a low mobility leads to the increased stability of the material being preserved [14]. Most importantly, the glass can be readily melted by addition of water, thus restoring conditions permissive for normal metabolism.

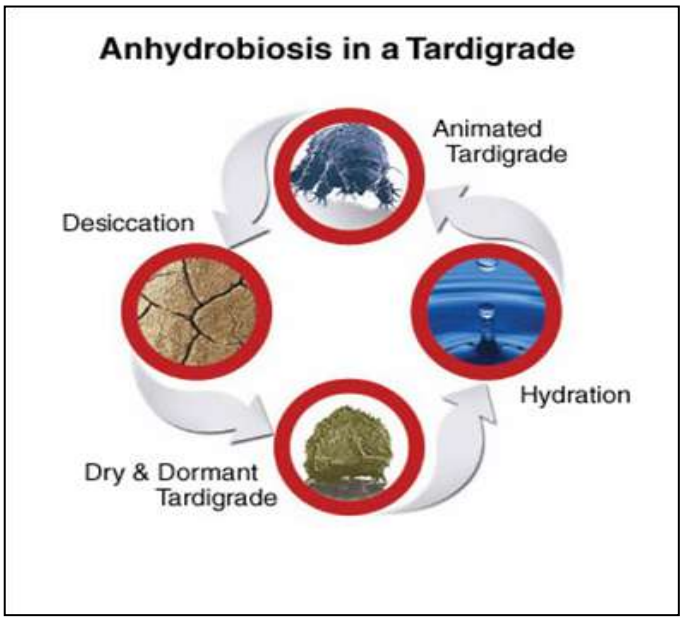


Fig. 3: Anhydrobiosis Cycle in a Tardigrade [10]

SampleMatrix™ combines this technology into a multi-component system including a dissolvable polymer in a stabilization buffer adjusted for different biological samples and a stabilizing solution containing small synthetic molecules. During the dry state, a water dissolvable polymer mix provides the stabilizing structure (Figure 4). The polymer completely dissolves during rehydration and presumably allows for complete DNA recovery without degradation or loss of sample activity. DNA extracts can be directly used in downstream applications such as enzymatic activity assays and STR analysis without further purification. Although this technology was designed for extracted DNA, it remains to be seen whether it also has applicability in protecting biological samples commonly collected from crime scenes.

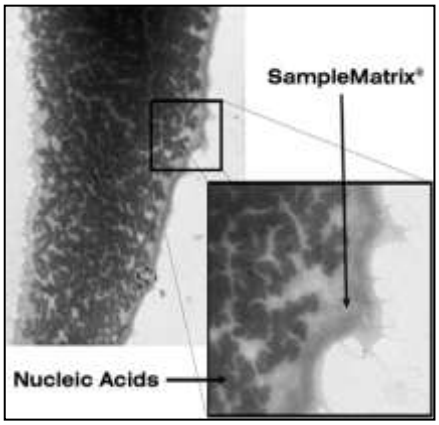


Fig. 4: SampleMatrix™ Forming a Barrier Around Nucleic Acids [10]

Long-Term Biostability at Room Temperature and Accelerated Aging

Besides the applicability of SampleMatrix™ to pre-extraction samples, the long-term stability of DNA protected by SampleMatrix™ is a major concern especially to the forensic community. Very few studies detail the long-term performance of SampleMatrix™. An accelerated aging study is one approach to evaluating the long-term stability of DNA in biological fluids protected by SampleMatrix™. Accelerated aging allows a prediction of the performance of a product over a short period. There are several different accelerated aging methods, including general thermal and oxidation approaches, hydrolysis, reaction with reactive excipient impurities, photolysis and protein denaturation.

Accelerated aging employed in the pharmaceutical industry generally involves increasing temperatures to accelerate chemical reactivity. The stability at a desired temperature is extrapolated by estimating the reaction rates at the different temperatures. The following summarizes the mathematical theory approach to accelerated aging studies using elevated temperature, referred to as the 10°C rule:

$$f = 2\Delta T/10, \text{ where } \Delta T = T - T_{ref}$$

T_{ref} represents a reference temperature for which the aging effect must be determined whereas T designates the elevated temperature used to accelerate the aging effects. The 10°C rule is an empirical observation that describes how an increase in temperature by 10 ºC approximately doubles the reaction rate. Hukins *et al.* argue that the use of an empirical rule is not as accurate and should be replaced by mathematical equations that are based on the principle of chemical kinetics. However, when applying chemical kinetics the primary assumption is that the aging process follows first order chemical kinetics. The order of chemical kinetics is governed by the involvement of reactants and spontaneity of the reaction. If the concentration of reactants does not affect the rate of reaction, the reaction follows zero order chemical kinetics. If the rate of reaction only depends on concentration of one of the reactants and the final product forms spontaneously, the reaction follows a first order chemical kinetics. In reality, most reactions in the human body are dependent on the concentration of a number of reactants, and consequently the chemical kinetics approach is not always feasible to adopt. Therefore, it is important to understand the conditions where the 10-degree rule is valid. It was discussed in several published articles that the 10-degree rule is only valid at temperatures below 60 ºC [15-17]. Normally, a set of fixed temperatures are used in accelerated pharmaceutical stability studies utilizing this method, yet since the temperature set is usually within a small range, considerable error can be associated with the temperature extrapolations [15].

A better approach to accelerated aging is the non-isothermal aging method where the use of a temperature set is replaced by a single temperature where samples are placed in a temperature ramping oven and removed at various time frames. The temperature profile for this non-isothermal aging method can be described by the following equation:

$$T = T_0 - 10\ln\left[\frac{1 - t/t_{final}\left(1 - d^{-\frac{\Delta T}{10}}\right)}{\ln d}\right]$$

In the above equation, T is the temperature at time t, T_0 is the initial temperature, t_{final} is the experiment duration, ΔT is the temperature range of the experiment, and d is a factor based on activation energy of the process that ranges in value from 1-4. This method allows samples to experience many time points from a single oven; however, the disadvantage of this approach is that an oven can only be dedicated to one single experiment set at any given time [18].

Serological Testing

Seminal Fluid

The ultimate purpose of analyzing semen in the majority of criminal cases is to compare a DNA profile obtained from the evidential stain to a known source. The known source is typically the DNA of a suspect alleged to be the perpetrator of the crime in question. Consequently, an association can be used to support the prosecution of the perpetrator in a court of law. The primary cellular component of seminal fluid that is analyzed for genetic identification is the spermatozoon. Other components of seminal fluid include a mixture of cells, amino acids, sugars, salts, ions, and other organic and inorganic materials [19]. Two important proteins, for the purpose of the present study, that are found in semen are prostatic acid phosphatase (AP) and prostate-specific antigen (PSA).

In sexual assault investigations, there are accompanying chemical tests that are used in the presumptive and confirmatory identification of semen stains. These tests are performed prior to developing a DNA profile. For example, AP is a presumptive test, oftentimes used in the field to locate and detect semen stains. Further, two forms of confirmatory tests are performed in many crime laboratories. These include the detection of PSA or the microscopic identification of spermatozoa (observed intact or based on the presence of heads) in a stain. Although PSA has been detected in other bodily fluids and tumors, the elevated levels found in semen forms the basis of a standard confirmatory test for semen in many forensic laboratories [20-22]. The second confirmatory test used as common practice is the microscopic examination of a stain extract. This morphological examination requires the identification of spermatozoa in the extract in order to confirm the presence of semen.

Blood

One of the principal forms of biological evidence encountered in forensic casework is blood. Blood is a complex mixture of cells, enzymes, proteins, and inorganic substances. The fluid portion of blood is referred to as plasma, which is mainly composed of water and comprises 55% of whole blood. The remaining 45% consists mainly of formed elements, including platelets (responsible for clotting), red blood cells, and white blood cells. Red blood cells are by far the most abundant cells found within blood and contain hemoglobin, which facilitate in distributing oxygen throughout the body. These cellular components also contain antigens along the cell membrane surface that distinguish an individual's ABO and Rhesus blood type. However, mature red blood cells lack a nucleus and, thus, lack nuclear DNA (nuDNA). The nuDNA present in blood samples is located within white blood cells [23].

Saliva

Saliva is commonly encountered as evidence in forensic casework. Potential sources include bite marks, sexual assault kits, cigarette butts, stamps, envelopes, beverage containers, and motor vehicle air bags. Given that saliva may provide a crucial role in solving a crime, improved

storage and handling would reduce the number of samples that would otherwise be considered untestable evidence. It has been suggested that samples should be kept cold as soon as possible after collection to prevent degradation and growth of bacteria [24], which may be impractical in many situations. For this reason, it is important to find alternative ways that samples can be collected and stored while maintaining biostability. Saliva can be of great evidentiary value in identifying suspects and victims. In fact, buccal swabs are now routinely collected to serve as a reference source due to the simple and non-invasive collection method.

Salivary Production and Composition

Human saliva is a viscous fluid possessing several functions that play an active protective role in maintaining oral health and homeostasis. It is comprised of secretions from the parotid, sublingual and sub maxillary glands and a large number of smaller salivary glands.

Saliva helps bolus formation by moistening food, protects the oral mucosa against damage, and plays a role in the preliminary digestion of food through the presence of amylase and other enzymes [24]. Healthy adult subjects normally produce 500-1500 mL of saliva per day, at the rate of approximately 0.5mL/min. However, several physiological and pathological conditions such as smell and taste stimulation, hormonal status, hereditary factors and oral hygiene can influence the quality and quantity of saliva production [24].

The term "whole saliva," "mixed saliva" and "oral fluid" is used to describe the combined fluids present in the oral cavity. This fluid is mainly composed of water (99.5%), proteins (0.3%), and inorganic and trace substances (0.2%-0.5%). Glycoproteins, enzymes (e.g., α-amylase), immunoglobulins, and a wide range of peptides with antimicrobial activities [25] mainly constitute the proteins in saliva. Amylase is found in the body fluid of humans, as well as primates and most mammals [26]. Further, the enzymes have been detected in both plants and animals; however, mammalian amylases have been designated as α-amylases. The two forms found in human body fluids are salivary amylase (AMY 1) and pancreatic amylase (AMY 2). These structurally distinct amylases are encoded by separate loci on chromosome 1 [27]. Although the distribution of pancreatic and salivary amylase can vary in body fluids, it has been reported that salivary amylase is found in saliva, perspiration, and breast milk while pancreatic amylase had been detected in semen, feces, and vaginal secretions [27]. Salivary amylase is the form that is targeted in the forensic identification of saliva. Detection of amylase, and in some cases the quantitative determination of its activity, forms the basis for most extensively used tests for the identification of saliva stains [28].

Table 1: Amylase Levels in Body Fluids [29]

Body fluid	Mean Amylase Concentration	S.D.	Range	No. of Samples
Saliva	350,000	240,000	72,000-1,300,000	50
Serum	160	60	84-300	49
Urine	850	763	130-3500	18
Semen (normal)	95		28-200	3
Semen (vasectomised)	22	33	3-150	93
	-	-	680-1300	3
Sweat	575		45-895	4
Tears	-	-	870-2150	2
Lip mucus	903	790	170-2900	45

Stability of Salivary Proteins

Saliva composition is influenced by several factors including: circadian rhythms, oral health status, and exercise [30]; however, microorganisms and proteases may also have a considerable effect on sample stability/protein degradation. Human saliva contains a large array of proteins that have important biological functions. Exploration of the salivary proteome allows for the identification of novel proteins and the examination of changes in protein levels under different physiological conditions or pathologic states. Salivary proteins have a remarkably diverse range of functions. These include, but are not limited to, digestive activities, protection of mucous membranes, antimicrobial activities, calcium and mineral homeostasis, inhibition of proteolytic enzymes, lubrication of oral tissues and binding of food tannins [31].

Factors Compromising Stability

Compared to plasma, saliva contains elevated bacterial loads and higher enzymatic levels, which may contribute to the degradation of proteins when stored in unfavorable conditions [32]. Whole saliva contains proteolytic enzymes originating from white blood cells, oral bacteria, and salivary glands all of which effectively break down proteins. In addition, slow freezing, long storage times, and freeze-thaw cycles may be problematic, since protein, induced precipitation may occur [25]. A prerequisite for measuring diagnostic protein markers in saliva is that the proteins are stable. However, according to Esser *et al.* proteins have been shown to degrade from the point of sample collection [33] particularly when held at room temperature. Degradation fragments have been shown to increase by as much as 7-fold over the course of a few hours when samples have been stored in these conditions, suggesting breakdown of larger proteins into peptides. Esser *et al.* state that six proteases have been found in saliva that may be contributing to the observed protein destruction. However, 13 protease inhibitor proteins were also identified that may counteract protease activity. Nevertheless, the overall balance is clearly in favor of degradation. Additionally, salivary amylase can lose much of its activity on drying and rehydration. Due to the loss of stability of samples at room temperature (and thus lost evidence), the recommended outcome is to store samples at temperatures frozen from collection until the time of analysis in order to improve in vitro biostability.

Literature Review

Anhydrobiosis Studies

Anhydrobiosis, or life without water, is a phenomenon in which organisms can naturally survive dehydration. A better understanding of how these organisms achieve such durability allows for the preservation of cells and organisms that are normally incapable of surviving in the dried state [12]. The ability of these organisms to survive in a state of dehydration is partly due to the synthesis of sugars, specifically trehalose, which is often found in high concentrations within the organism's metabolism. The presence of trehalose has been shown to replace the water near the polar residues of membranes and proteins, thus stabilizing these structures in the absence of appreciable amounts of water [13].

Wolkers investigated the preservation of human blood platelets by freeze-drying in the presence of trehalose. Given that platelets are activated by refrigeration, they must be stored at temperatures higher than 22ºC. However, this temperature significantly reduces the functionality after 5 days and may contribute to bacterial growth. Previous studies have used glucose in the freeze-drying process and found that metabolic activity remained active and that hemoglobin was

maintained in a functional physiologic state similar to fresh, non-freeze dried blood cells [11]. However, the use of glucose required storage at freezing temperatures, because it does not assume a glassy state at room temperature. This is in contrast to other disaccharides such as trehalose, which can form a glass-like state at room temperature. In their study, Wolkers freeze dried platelets using trehalose in combination with albumin in cryogenic vials that were frozen from 22ºC to -80 ºC. Reconstitution of platelets was either direct or included a prehydration step with subsequent direct rehydration. The results showed that the prehydration step led to a considerable reduction in the lysing of the platelets. In conclusion, the study found that the use of trehalose as the main lycoprotectant allows for storage at room temperature [11]. This study provides proof that trehalose is a major component in the process anhydrobiosis, which is key to providing biostability in room temperature storage.

This point is further expressed in a paper by Smith *et al*. [34], who indicate an improved DNA yield with the addition of trehalose as a storage medium. This study used human fecal and placenta samples as the source of DNA and stored the samples for a period of 12 months. Each concentration of DNA (100pg/µL, 20pg/µL, 5pg/µL, and 2pg/µL) was divided into three aliquots. Lambda HindIII DNA and TE Buffer were added to one aliquot, trehalose and TE Buffer were added to another aliquot, and TE buffer alone was added to the third aliquot. The samples were then stored at -80ºC, -20ºC, +4ºC or room temperature. At the completion of the study it was found that samples stored dry in trehalose retained a significantly higher concentration of DNA than those stored at + 4ºC or dry in the other treatments [34]. Specifically for human placental DNA, it was observed that samples treated with no more than TE buffer and stored dry degraded such that no PCR products were obtained for the 100 pg and 10pg starting quantities. There was no significant difference found in amplification yield between samples stored frozen and those stored dry in the presence of trehalose. In accordance with previous studies [13], Smith states that the improvement in PCR yield in samples treated with trehalose is due to its role as a biomolecule protector rather than its effect as a PCR enhancer. This finding is informative in gaining an understanding of the theory underlying current approaches to attaining biostability at room temperature.

A review by Crowe concludes that the presence of sugars alone is not fully responsible for the stabilization properties of anhydrobiosis. The paper states that vitrification may be required for preservation of labile components of the cells in the dry state; however, the water replacement hypothesis (a function of trehalose) seems to be more consistent with their data. Crowe also states that these two mechanisms, the water replacement theory and vitrification, are not mutually exclusive and that both are required. Numerous published studies concerning the nature of anhydrobiosis in providing biostability are in agreement with this statement; however, there are some researchers question whether anhydrobiosis alone can prevent DNA damage in the dry state.

Research performed by Neumann [35] poses the question as to whether tardigrades suffer DNA damage during anhydrobiosis and whether the time spent in the desiccated state adds to this damage. Specimens of M. tardigradum were used as test samples and subjected to desiccation for two days, six weeks, and ten months. They were then all rehydrated with spring water. For a positive control, a separate set of samples were exposed to UV-B radiation and rehydrated in the same manner as the experimental samples. As a result, only a small amount of DNA damage was found in either of the samples. Neumann performed a time-series experiment that indicated the level of DNA damage in time increments after rehydration. The results showed that the DNA damage level rises as the time post rehydration increases although once it reaches

90 minutes it begins to decrease. Neumann explains this occurrence by crediting the tardigrades' DNA repair mechanism. It is also stated that the longer an organism remains in the desiccated state, the more damage that accumulates. Neumann concludes that dehydration alone is not sufficient to protect nucleic acids from damage during the anhydrobiotic state and that an effective DNA repair system is required. These findings demonstrate the complexity of DNA stabilization and that there are several variables involved in preservation.

DNA Degradation and Low Copy Number

DNA degradation is a major obstacle in the characterization of biological evidence and results in the reduction or loss of the structural integrity of cells, the antigenicity, and enzymatic activity of proteins, and the quantity and quality of nuclear DNA. Degradation of DNA is an effect of its limited stability and is largely caused by hydrolysis, oxidation, and non-enzymatic methylation. A paper by Lindahl demonstrates how DNA has limited chemical stability. The author states that the chemical price paid for the increased resistance of the nucleic acid phosphodiester bond is a labile N-glycosyl bond. The difference between the depurination velocity between single-stranded and double-stranded DNA is only four-fold, so the double helical structure does not provide much protection. In addition to the fact that glycosyl bonds are labile, DNA base residues are susceptible to hydrolytic deamination where cytosine and its homologue 5-methylcytosine are the main targets for this reaction [36]. DNA oxidation is another concern that leads to limited chemical stability. This can occur as a DNA base lesion, which is a ring-saturated derivative of a pyrimidine. This lesion occurs in several forms, such as thymine and cytosine glycols and pyrimidine hydrates [36]. Other studies investigate beyond the structure of DNA and look at the effects of chemical stability on the protein structure.

A study by Allison [37] addressed the effects of hydrogen bonding on protein unfolding. In this experiment, lysozymes were used to observe the presence or absence of hydrogen bonding between protein and either water or disaccharide molecules. This was performed using infrared spectroscopy to detect the presence of the carboxylate band. Observation of the carboxylate band indicates the reduction in hydrogen bonding to protein carboxyl groups due to the removal of water. It was found that disaccharides hydrogen bond to the protein in place of lost water, which allows the protein structure to remain intact. This study demonstrates two important considerations: the integral role that disaccharides play in biostability and that hydrogen bonding is a primary factor in the mechanism of DNA degradation.

Forensic samples are often of low copy number, primarily because the DNA has degraded. This can make analyzing DNA more difficult. The lower limits of sensitivity recommended by manufacturers of short tandem repeat (STR) multiplex systems are in the region of 250pg; however, multiplexes usually work at their optimum efficiency when 1ng of DNA is analyzed and for 28-30 amplification cycles [36]. Some analysts have increased the number of PCR amplification cycles in an attempt to increase sensitivity when dealing with low copy number samples; however, this strategy is not without cost. The interpretation may be complicated due to the detection of additional alleles, an increase in stutter size, and allele dropout [36]. When present in low copy number, a molecule that is amplified by chance during the initial PCR cycles is likely to be preferentially amplified. This may result in several potential consequences: allele drop out may occur because one allele of a heterozygote locus can be preferentially amplified, stutter peaks may be preferentially analyzed (false alleles), and sporadic contamination may occur whereby alleles not associated with the target sample may preferentially amplify [37].

To circumvent the disadvantages associated with increased PCR amplification cycles, several studies have been conducted in an attempt to optimize sensitivity. Smith and Ballantyne explored the effects of increasing PCR sensitivity without increasing the amplification cycles by purifying the PCR product in an attempt to increase fluorescent allelic signal intensity [37]. STR PCR products are typically detected via electrophoresis. Prior to this, the sample is electro kinetically injected into the capillary, typically for 5 seconds, where a voltage is applied to the electrode, effectively drawing negatively charged molecules such as DNA into the capillary [39]. The short injection time permits a limited amount of sample to be taken into the capillary where uptake of smaller components is favored. During this process, STR amplicons compete with primers, unincorporated deoxynucleoside triphosphates (dNTPs), salts and other negatively charged PCR reaction components [38]. Smith and Ballantyne base their study on the theory that the removal of un-reacted amplification components should favor amplicon injection and lead to an increase in fluorescent signal intensity. Their study concluded that purification of the PCR product using the Qiagen MinElute silica column produced a fourfold increase in fluorescent signal intensity relative to unpurified product. The authors found that post-PCR purification could greatly enhance the sensitivity of the PCR process; full profiles were obtained down to 20 pg input template DNA while significant data was generated down to 5pg, without increasing the number of amplification cycles [38].

The manner in which DNA is deposited on a substrate (touch DNA or DNA collected from fingerprints) may also constitute low copy number samples. Trace amounts of DNA can originate from multiple sources, including single buccal cells or telogen hair, saliva stains on a cigarette butt, postage stamps, and envelope sealing flaps, epithelial cells shed from the hands due to excessive pressure during manual strangulation, or latent fingerprints applied to drinking glasses, clothes and other substrates [39]. Phipps and Petricevic performed a controlled study to investigate the likelihood of locating a DNA profile on an item that a person has touched. It was found that only 2 % of the samples resulted in a full profile when sampled from the volunteer's dominant hand while 12 % of the samples resulted in a full profile when sampling from the non-dominant hand. Thus, the likelihood of retrieving a full profile is small under these circumstances and the limited amount of DNA cannot afford to degrade.

Biostability

Several studies have investigated alternative storage methods that either prevent further degradation or preserve the limited amount of DNA present in a sample. Since it has been demonstrated that repeated freeze-thaw cycles and changes in temperature can lead to degradation and comprised results, many of these studies have focused on room temperature storage [40].

In one study filter paper, cards pretreated to retard bacterial growth, inhibit nuclease activity, and release DNA during processing were explored as a potential substrate for DNA storage [40]. These cards were stored at room temperature, -20ºC and -70ºC for two periods: 9 months and 7 years. The recovered DNA was washed with water and denatured twice at elevated temperatures prior to elution. A quantitative real-time PCR assay was performed on the samples to estimate the total DNA yield obtained for each elution. It was found that a higher DNA yield was recovered at room temperature; however, no suitable DNA quantity or quality of DNA was recovered after 7 years regardless of the storage conditions. The analysts conclude that the cause of the reduced DNA yield was due to DNA fixation to the filter paper card and degradation over time [41]. The use of FTA cards (Whatman BioSciences) is another alternative means of

preservation and storage of biological at room temperature. The technique involves the application of a biological fluid to the FTA treated paper; as a result, cells within the sample lyse to release DNA that becomes immobilized on the paper and stored at room temperature [42]. Harvey found that the cards successfully lysed cells from different insect life stages and provided DNA in a state suitable for use in PCR. One advantage that FTA paper offers is that samples are easily stored in a stable form on a card at room temperature, eliminating the need for freezing of samples [42]. Samples are also transportable at room temperature and most infectious agents are considered to be deactivated once they contact the card, thus removing potential biohazards [42]. Oragene-DNA is another product that has been developed for collecting and preserving DNA in saliva. A study performed by the manufacturer involved the collection and storage of saliva samples at room temperature (24ºC), 37ºC, or 50ºC for periods up to 187 days. The researchers also analyzed samples that were stored for 5 years. The results indicated that samples stored at room temperature and 37ºC showed no evidence of degradation; however, samples stored at 50ºC showed minimal degradation. Further, high molecular weight DNA was extracted from the samples stored for 5 years at room temperature [43].

Biomatrica, Inc. has developed a proprietary platform technology for the dry storage of biological materials at ambient temperatures. The R & D scientists at Biomatrica have performed long-term stability studies under accelerated aging conditions that are equivalent to greater than 11 years of room temperature storage. Aliquots of 20ng of human genomic DNA were applied to the DNA SampleMatrix™ or empty control wells and allowed to dry overnight. Accelerated conditions consisted of elevated temperatures at either 50°C or 60°C. The samples were analyzed after a 10-month storage period and rehydrated with 10 µL of water for 15 minutes at room temperature. As a control, identical samples were stored at 4°C or -20°C. All samples were analyzed by PCR using approximately 1/20th of the original starting sample. The study found that SampleMatrix™ did inhibit degradation of DNA and allowed recovery of DNA even after prolonged dry storage under fluctuating room temperature conditions [44].
Subsequent studies performed by Biomatrica, Inc. addressed the environmental conditions of high pressure and extreme temperatures above 120°C. This was performed by exposing samples protected by SampleMatrix™ to autoclaving conditions. The researchers found that the unprotected control sample resulted in complete degradation of DNA, with no visible amplified product. In comparison, SampleMatrix™ fully protected the DNA during the autoclave cycle; samples as low as 4ng of DNA remained viable and were amplified [45].

Extraction Chemistry

Currently there are several extraction methods available for use in forensic case samples. Organic extraction has traditionally been the method of choice for extracting DNA from a variety of forensic samples, including whole blood and bloodstains [46]. Chelex and other commercially available kits such as Qiagen are also commonly used in forensic laboratories. Vandenberg *et al*. performed a study to evaluate the recovery of DNA using several extraction methods, including organic, telex, and several commercial kits. They found the resin-based methods, including chelex, out-performed all other methods and that Chelex gave the highest average DNA yield from 25 µL whole blood and bloodstains [46]. Following Chelex in extraction efficiency were the organic and QIAamp Qiagen methods. Vandenberg *et al*. found a limitation with the QIAamp procedure for bloodstains in that the incubation of the bloodstain in lysis buffer prior to the first transfer of the supernatant may not be of sufficient length, which may contribute to reduced yields [46]. The other methods that were tested included: Definitive, Dynabeads, Ready Amp,

and Promega's Wizards™ all of these methods resulted in a lower DNA yield then the three Chelex, Qiagen or organic. Time and cost are important factors when considering an extraction method. Vandenberg *et al*. found that in terms of total time to perform the extraction, the organic method is considerably longer than the other methods evaluated. However, the organic method remains the "gold standard" for the extraction of the majority of crime scene samples in forensic laboratories. In relation to costs, Chelex is the most economical whereas the QIAamp and organic are relatively expensive [46]. The Chelex 100 method does not require any additional transfer steps after the initial transfer of sample to an Eppendorf tube, which significantly reduces the possibility of handling error. Vandenberg *et al*. concluded that while organic extraction provides DNA of sufficient quantity and quality for STR typing, it is time consuming, requires many transfer steps, necessitates working with hazardous chemicals and the cost of consumables is high. In contrast, resin-based methods are rapid, simple, safe, and inexpensive, and were found to be the most effective in yielding DNA suitable for amplification from relatively small volumes of blood [47]. Scherczinger *et al*. also investigated the utility of the QIAGEN QIAamp DNA isolation procedure. They found that it was ideal for preparation of blood samples for DNA data-basing and stated that DNA isolated by this method was of high molecular weight, with a yield that was two- to four-fold greater than the organic extraction [47]. This method eliminated the multiple precipitation/concentration steps associated with the organic extraction. The authors concurred with Vandenberg *et al.* in concluding that the risks associated with sample handling error, aerosol cross-contamination and exposure to infectious agents are minimized when using the Qiagen isolation procedure [47].

Studies Relating to Serological Testing

Several studies relating to presumptive testing are reported in the literature. A variety of tests are available to forensic scientists for the presumptive identification of blood, all of which involve the peroxide-mediated oxidation of an organic compound catalyzed by the iron found in hemoglobin [48]. Three of the most commonly used presumptive blood tests utilized in forensic science are phenolphthalein, leucomalachite green, and luminol. Studies have been conducted to determine the sensitivity of these tests; however, the results are extremely inconsistent [49-50].

Anti-human hemoglobin antibody-based systems provide information on both the presence of hemoglobin and human specificity [51]. Several studies are reported in the literature demonstrating the specificity of techniques to detect human hemoglobin [52-53]. Similar studies report the sensitivity and specificity of techniques directed at the identification of semen [55-56] Numerous studies have also been conducted on methods for the identification of saliva in the crime laboratory [56-60].

Stability of Whole Blood

Blood collected from crime scenes are typically small, placing a premium on the efficiency of processing. Each time a specimen is to be processed, as small, an amount as possible is consumed, and the remainder of the specimen is placed in long-term storage, where it may remain unanalyzed for months, years, or even decades. Therefore, it is critical that the environment be as hospitable to long-term blood component preservation as possible. Refrigeration provides the advantage of preserving cell structure and leaving organelles generally intact, thereby better preserving genetic material. However, refrigeration has the disadvantage that samples will degrade measurably over the course of only a few days [61]. Beyond that, specimens must be placed in frozen storage in order to remain viable for analysis

over the long-term. While freezing has the advantage of preserving individual cell components, it has the disadvantage in that – since the primary component is water – it leads to cytoplasmic expansion and formation of ice crystals. Once cell and nuclear membranes are ruptured, DNA and RNA are released into the free solution, leading to hydrolysis, degradation, and reduced recoverability downstream. Freezing also has several pragmatic disadvantages: frozen storage is expensive to maintain; samples inevitably accumulate over time, leading to ever-increasing frozen storage space requirements; and, any failure of the freezer system can potentially lead to complete loss of all samples. The concept of room-temperature storage of biological specimens is therefore attractive for several reasons, assuming the downstream recovery rate of genetic material is preserved to a degree comparable to that of frozen storage.

Factors Compromising Whole Blood Stability

When discussing whole-blood stability, it is important to remember that ultimately the forensic scientist is concerned with the long-term preservation of bodily fluids for purposes of identification and DNA profiling. Both of these issues are reliant upon the integrity of the plasma component, which in turn provides a stable environment for the RBC and WBC. Studies [62-66] have identified five primary plasma components whose concentrations can be correlated with whole-blood stability: C-reactive proteins, retinol, ferretin, folic acid, and fatty acids. In general, naturally occurring levels of these components will sustain plasma integrity for a maximum of 28 hours, after which plasma and then cellular integrity begins to decline [61]. Any potential method of room-temperature storage would ideally preserve near in-vitro levels of the critical plasma components, or would selectively preserve WBC and RBC cytoplasm, nuclei and cell membrane components.

CHAPTER 2: METHODS

Preliminary studies to construct experimental design

Several preliminary studies were performed in preparation of this research. The first examined the volume of blood, semen, and saliva necessary to saturate the Puritan® cotton-tipped applicators without spillage. The optimal volume was determined to be 100 µL of the respective body fluids, and this volume was used in all of the experiments. The second study examined the maximum volume of SampleMatrix™ that could be applied to cover the stained swabs without spillage. The optimal volume was determined to be 40 µL of the two SampleMatrix™ formulations SM1 and SM2. For the studies that examined SampleMatrix™ as a post-collection coating agent, the 40 µL volume was applied either immediately after the biological fluid was applied to the swab (treatment referred to as wet) or after the biological fluid was allowed to dry in a Biosafety cabinet for 24 hours (treatment referred to as dry). After the preparation of the swabs, all samples were allowed to dry for 24 hours in a Biosafety cabinet prior to storage.

The third study evaluated the DNA extraction efficiency of the QIAGEN QIAamp® DNA Macro Kit versus the QIAGEN QIAamp® DNA Micro Kit for the isolation of nuclear DNA from forensic casework samples. Based on the preliminary findings, the QIAamp DNA Micro Kit was selected as one of three extraction methods used in this research. Unless otherwise specified, the entire swab of each sample used for DNA analysis.

Many samples were stored under room environmental conditions in this study. During the testing period, the room temperature ranged from 19.6°C to 27.1°, and the relative humidity of the room ranged from 3% to 78%. The daily range in relative humidity was a great as 3% to 78%. Samples were stored at room conditions without a desiccant.

All samples generated in this research were quantified for human nuclear DNA, with the exception of those subject to conventional serological testing. A description of the DNA quantification method can be found at the end of this chapter. A select number of the DNA samples were taken to STR analysis, and a description of the typing procedure can be found at the end of this chapter.

The California State University of Los Angeles, Institutional Review Board, granted approval for the use of human subjects in this research. Volunteers were solicited to participate in this study by posting fliers at the Hertzberg-Davis Forensic Science Center. Applicants were informed that participation was voluntary and that all forms of personal identification were considered confidential. For each study, the blood, semen or saliva samples were obtained from the same person (unless otherwise specified), who was reportedly at least 18 years of age and of good health. A licensed phlebotomist drew the blood samples. For semen samples, the donor was required to be non-vasectomized, and was instructed to self-collect 4-5 mL of seminal fluid in a collection vial no earlier than the evening prior to sample preparation. The donor was instructed that a 3-day abstinence period should be observed prior to sample collection. For the saliva samples, the donor was instructed to self-collect ~20 mL of saliva into a specimen cup over the course of a day. After receipt, all of the biological samples were stored at 4 °C until sample preparation. Additionally, negative controls were analyzed in parallel with the biological samples for all of the experiments.

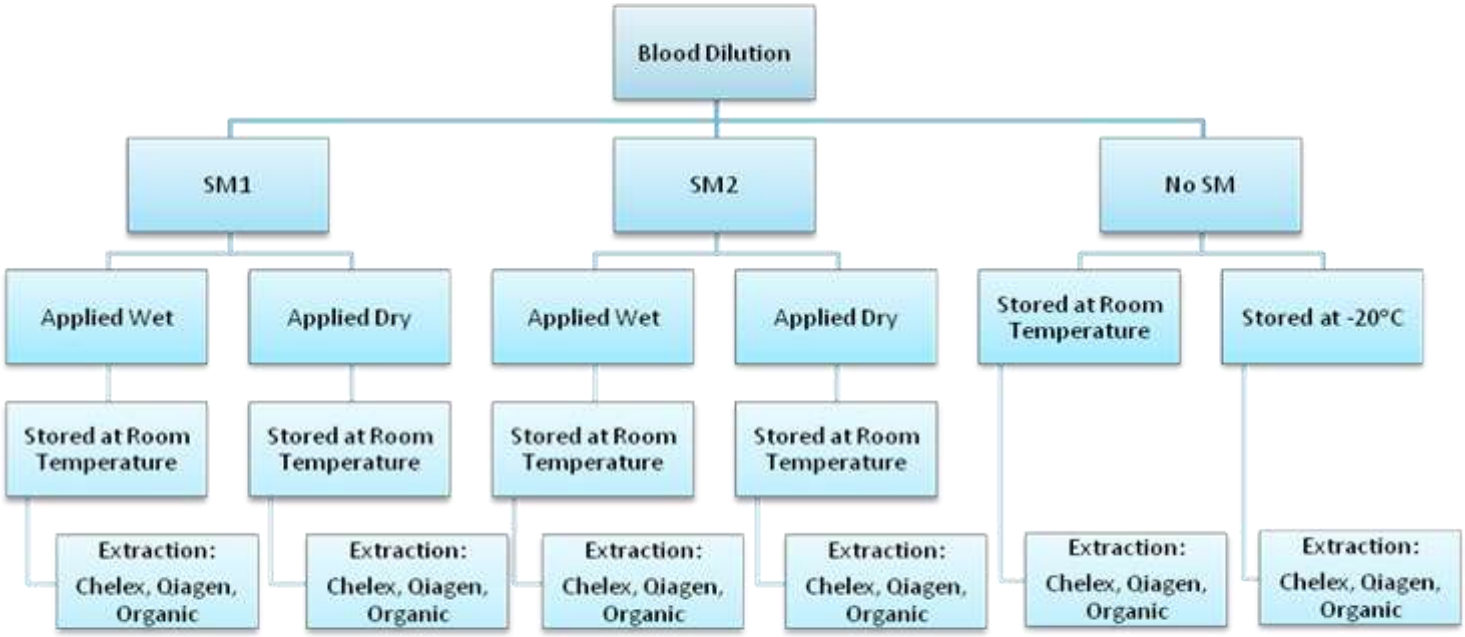
SampleMatrix™ SM1 and SM2 as post-collection coating agents for bloodstained swabs

Experimental Design

This study evaluated the stability of blood samples using SampleMatrix™ as a room temperature storage medium. Multiple variables were examined including: sample dilution, storage medium, storage condition, and extraction chemistry. A blood dilution series was prepared as follows: neat, 1/100, 1/200, 1/400, and 1/800. Additionally, two different formulations of SampleMatrix™ (SM1 and SM2) were compared as post-collection coating agents for stability of the samples stored at room temperature. 180 samples were examined. The samples were stored either at room temperature or at -20°C for a period of six months, and were extracted by three methods: a Chelex 100 procedure, an organic procedure, and the QIAGEN QIAamp® DNA Micro Kit. All of the samples were prepared in duplicate and negative control samples were prepared and analyzed concurrently with the experimental samples.

Experimental Variables

Dilution	Neat, 1/100, 1/200, 1/400 and 1/800
Extraction Method	QIAamp DNA Micro Kit, Chelex 100, Organic
Coating Agent	SampleMatrix™ 1 (SM1), SampleMatrix™ 2 (SM2), No SampleMatrix™ (No SM)
Sample Dry or Wet	Coating agent added when blood sample is wet; Coating agent added after blood sample has dried
Storage Condition	Room temperature; -20°C



Experimental Design

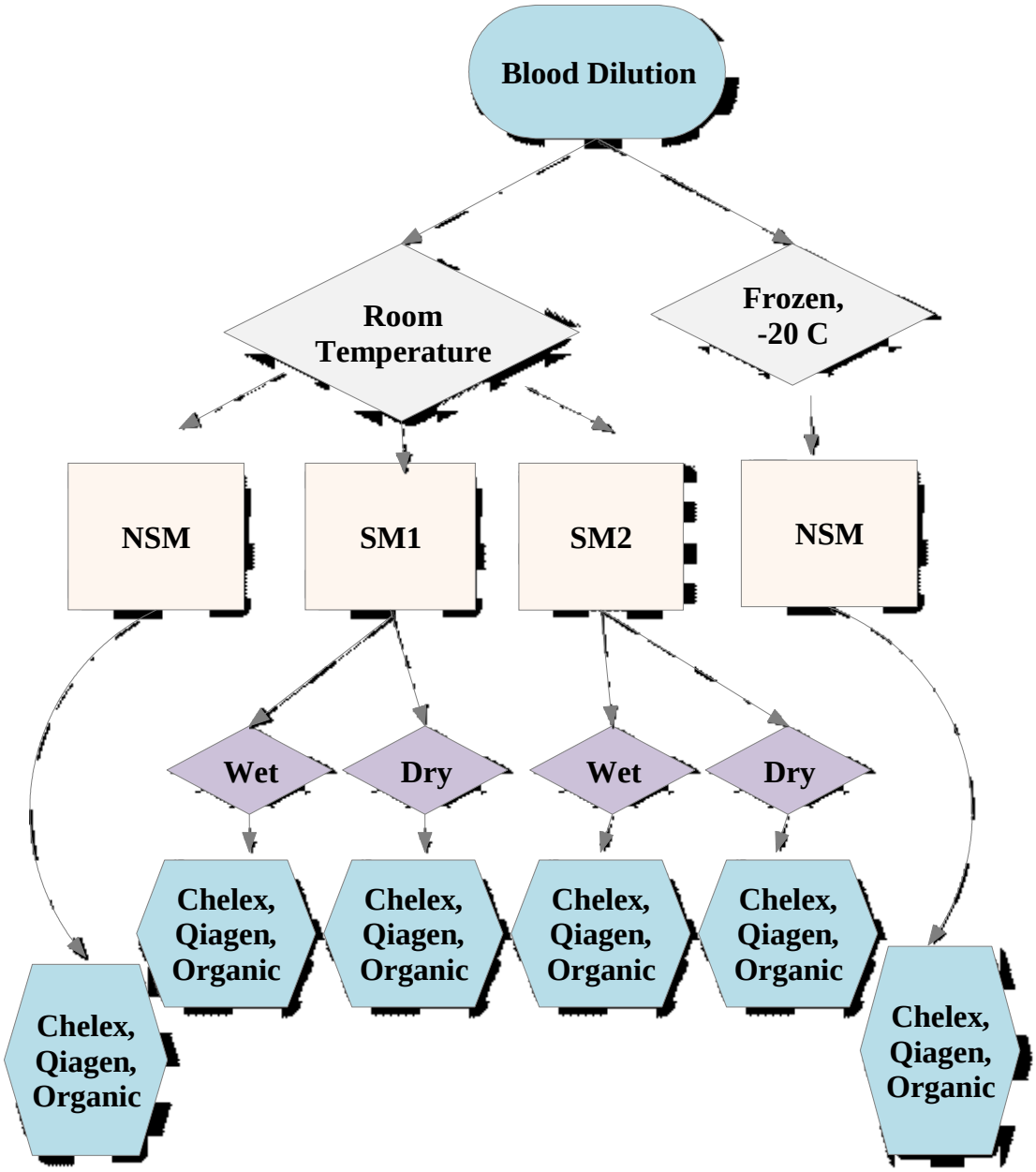
SampleMatrix™ SM1 and SM2 as post-collection coating agents for bloodstained swabs stored for 17 to 24 months

Experiment Design

This study evaluated the stability of the SampleMatrix™-treated bloodstains as compared to other storage methods. There were multiple controlled variables, including storage medium, storage condition, sample dilution, and extraction chemistry. A blood dilution series was prepared as follows: Neat, 1/100, 1/200, 1/400, and 1/800. Negative controls were also prepared. In addition, two different SampleMatrix™ formulations (SM1 and SM2) were compared for their effectiveness in providing room temperature stability of DNA. The samples were stored either at room temperature or at -20°C for a period of 17 to 24 months, and were extracted for DNA analysis by three methods: a Chelex 100 procedure, an organic procedure, and the QIAGEN QIAamp® DNA Micro Kit. The samples were prepared and analyzed in duplicate or quadruplicate and negative samples were analyzed concurrently with the experimental samples. Specifically, all unprotected samples stored at room temperature and at -20°C were performed in quadruplicate, and all SampleMatrix™ (SM1 and SM2) applications were performed in duplicate. 180 samples were examined in this study.

Experimental Variables

Blood Dilution	Neat, 1/100, 1,200, 1/400, and 1,800
Coating Agent	SampleMatrix™ 1 (SM1), SampleMatrix™ 2 (SM2), No SampleMatrix™ (No SM)
Storage Condition	Room Temperature; -20°C
Sample Wet or Dry	Coating agent added when blood sample is wet; Coating agent added after blood sample has dried
Extraction Method	Chelex, Qiagen, Organic

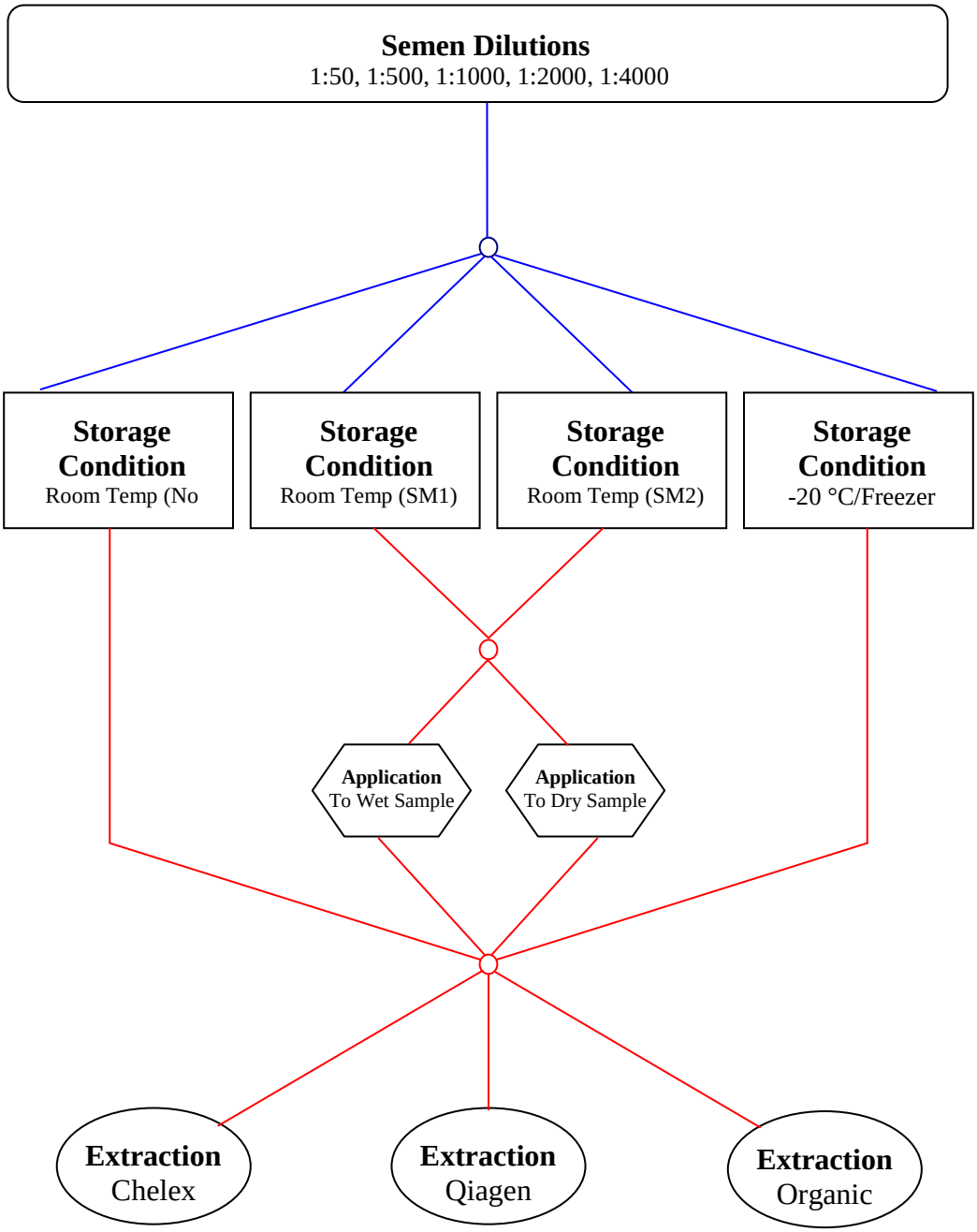


Experimental Design

SampleMatrix™ SM1 and SM2 as post-collection coating agents for semen-stained swabs

Experimental Design

This study examined the stability of DNA in semen samples of different dilutions, which were subject to different storage conditions (-20 °C and room temperature storage) and different coating treatments (with and without the SampleMatrix™ 1 and SampleMatrix™ 2). The effect of adding SampleMatrix™ to a wet semen swab sample versus its addition to a dry semen swab sample was also examined. The semen concentrations used in this study were 1:50, 1:500, 1:1000, 1:2,000, and 1:4000. Each sample condition was performed in duplicate for 180 samples in this study. After a six-month storage period, the samples were extracted for DNA analysis by three different methods: the QIAGEN QIAamp Micro Kit; a Chelex 100 procedure and an organic procedure. The experimental design for this study is illustrated below.



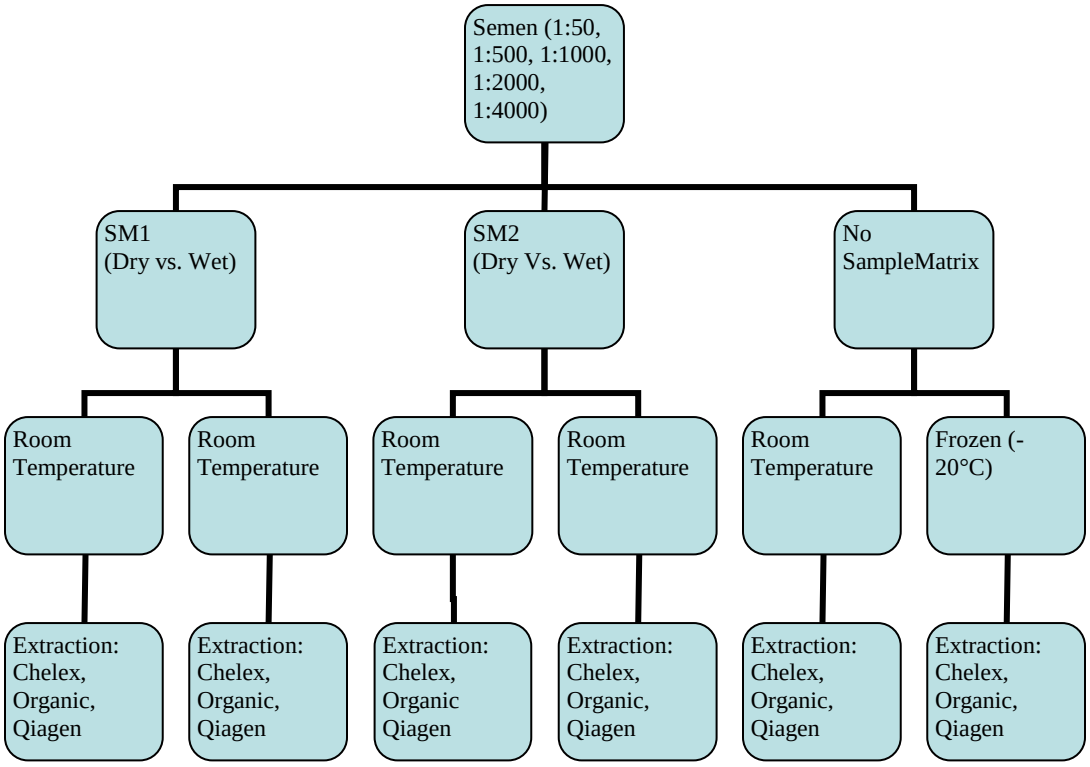
SampleMatrix™ SM1 and SM2 as post-collection coating agents for semen-stained swabs stored for 17 months

Experiment Design

The objective of this study was to determine the stability of semen samples protected by SampleMatrix stored for over 17 months. Numerous parameters were examined in this study including sample concentration, storage condition, SampleMatrix™ formulation, SampleMatrix™ application, and extraction method. 240 samples were analyzed in this study.

Experimental Variables

Dilution	1:50, 1:500, 1:1000, 1:2,000, 1:4000
Storage Condition	Room Temperature or Frozen (-20°C)
Coating Agent	SampleMatrix™ 1 (SM1), SampleMatrix 2 SM2), No SampleMatrix™
Coating Method	SampleMatrix™ applied to wet semen sample or to dry semen sample



Experimental Design

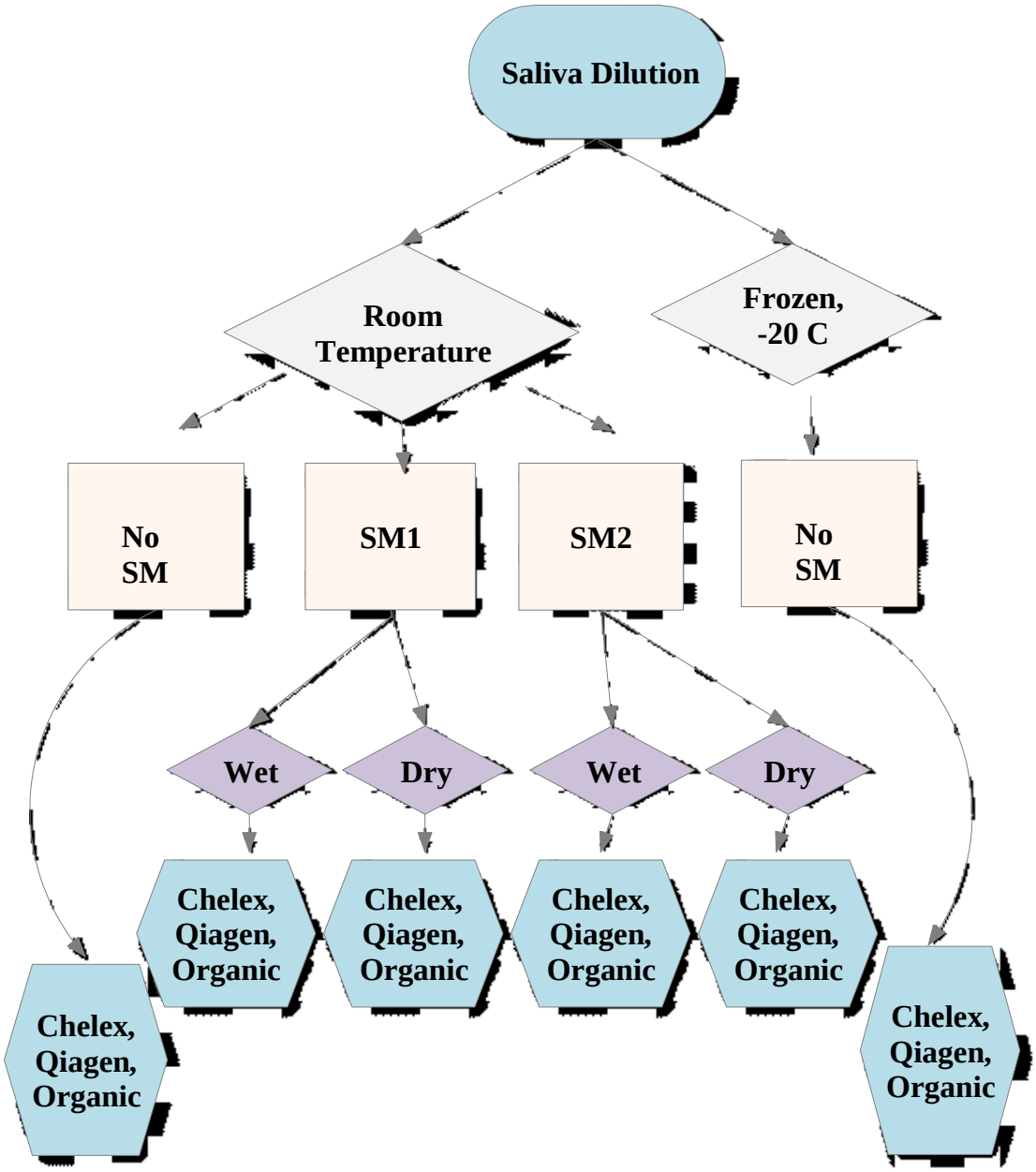
SampleMatrix™ SM1 and SM2 as post-collection coating agents for saliva-stained swabs

Experiment Design

This study examined the stability of SampleMatrix™–treated saliva stains as compared to saliva stains stored under other conditions for a 6-month period. Numerous variables were examined in this study including sample concentration, storage condition, SampleMatrix™ formulation, SampleMatrix™ application, and extraction method. The samples were stored either at room temperature or at -20°C and were extracted with three different methods: the QIAGEN QIAamp Micro Kit; a Chelex 100 procedure; and an organic procedure. The non-SampleMatrix™ treated samples (NSM) that were stored at room temperature and at -20°C were performed in quadruplicate. The SampleMatrix™ treated samples were prepared in duplicate.

Experimental Variables

Saliva Dilution	Neat, 1/10, 1/50, 1/100, and 1/200
Coating Agent	SampleMatrix™ 1 (SM1), SampleMatrix™ 2 (SM2), No SampleMatrix™ (No SM)
Storage Condition	Room Temperature; -20°C
Application	SampleMatrix™ added to wet sample; SampleMatrix™ added to dry sample
Extraction Method	Chelex, Qiagen, Organic



Experimental Design

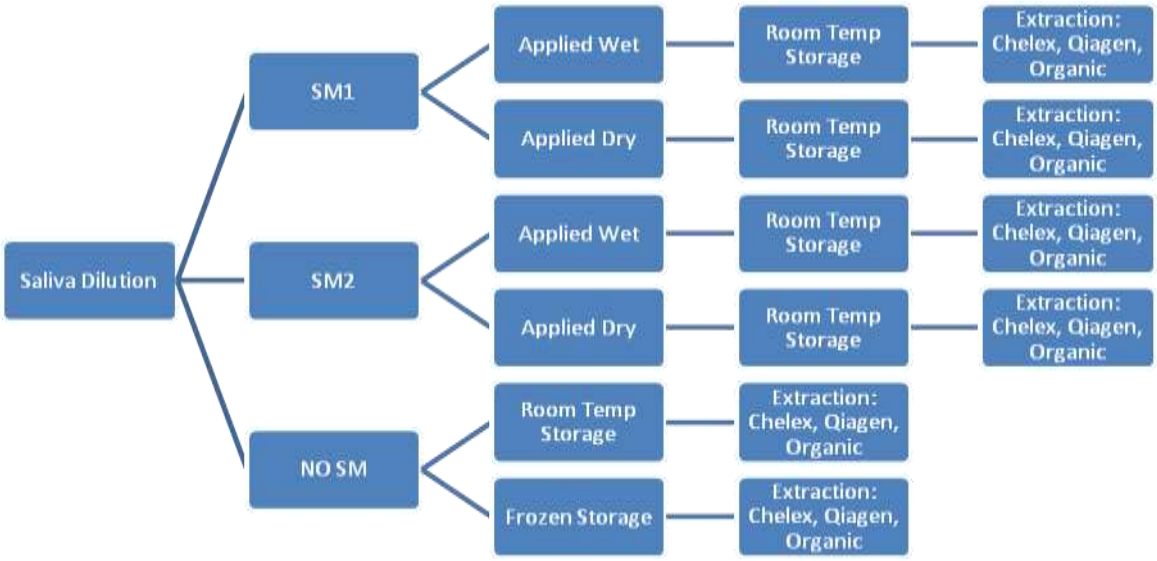
SampleMatrix™ SM1 and SM2 as post-collection coating agents for saliva-stained swabs stored for 17 to 24 months

Experimental Design

This study examined the stability of SampleMatrix™–treated saliva swabs as compared to saliva swabs stored under other conditions for a 17 to 24 month period. A saliva dilution series was prepared as follows: neat, 1:10, 1:50, 1:100 and 1:200. Two different formulations of SampleMatrix™ (SM1 and SM2) were additionally compared. All samples were prepared in either quadruplicate or duplicate. The samples were stored at -20ºC or at room temperature and were extracted with the QIAGEN QIAamp Micro Kit, a Chelex 100 procedure and an organic procedure. Two hundred and sixty eight samples were analyzed in this study.

Experimental Variables

Dilution	Neat, 1:10, 1:50, 1:100, 1:200
Extraction Method	Chelex, Qiagen, Organic
Coating Agent	SampleMatrix™ 1 (SM1), SampleMatrix™ 2 (SM2), No SampleMatrix™ (No SM)
Application	SampleMatrix™ added to wet sample; SampleMatrix™ added to dry sample
Storage Condition	Room temperature; -20°C

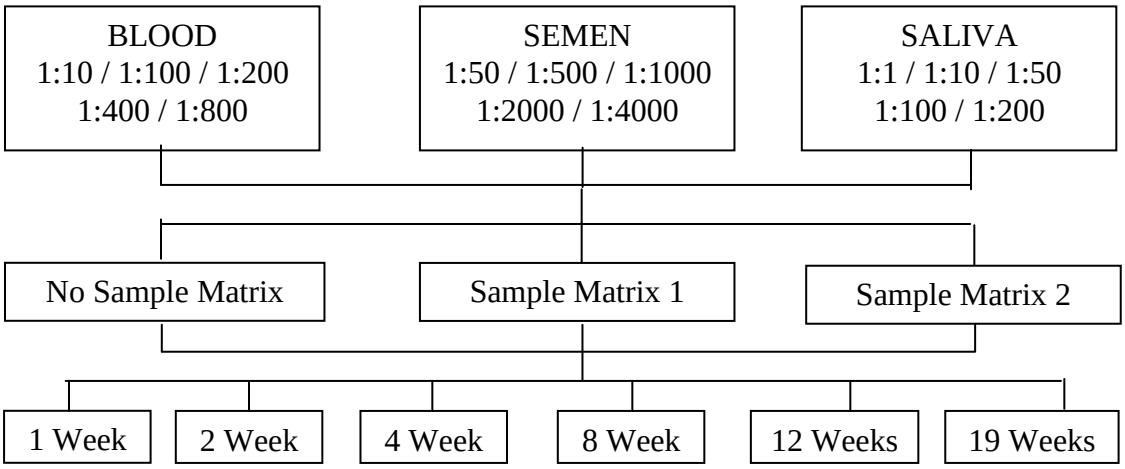


Experimental Design

SampleMatrix™ SM1 and SM2 as post-collection coating agents for blood-, saliva-, and semen-stained swabs under accelerated aging conditions

Experiment Design

This study examined the stability of biological samples treated with and without SampleMatrix™ and subject to accelerated aging conditions. Several independent variables were investigated, including: biological fluid (blood, semen, and saliva), sample concentration, SampleMatrix™ formulation, and the duration of exposure to elevated temperature. All of the samples were prepared in triplicate. Samples were placed in a 50 °C oven (Thermocenter - Salvis Lab) for one week to 19 weeks.



Experimental Design

SampleMatrix™ SM1 and SM2 as swab wetting agents for the collection of bloodstains

Sample Preparation

Brick, wood, glass, carpet, and cotton substrates were chosen for this study because they are common substrates encountered in forensic casework. The brick substrate was cleaned with 10% bleach followed with a distilled water rinse. The surface of the wood substrate was shaved with a planer, cleaned with 10% bleach, and rinsed with distilled water. Two different types of wood were used: cedar and pine. The glass substrate was cleaned with 10% bleach and rinsed down with distilled water. A section of beige colored carpet and 250 thread count white cotton sheets were purchased new. 100 µL of a blood sample (neat or diluted) was deposited on to each substrate with a pipette. Once the sample diffused on the substrate, the stain was outlined with a permanent marker to demarcate the area to be swabbed; this was particularly important for visualizing the more dilute stains. All bloodstains were allowed to air dry at room temperature for 24 hours. The following swabbing technique was employed for stain collections: 40 µL of SM1, SM2, or Nano pure water was applied to the cotton swab, which was then rolled 25 times and dabbed 50 times on each stain. The swabs were allowed to air dry under a laminar airflow hood, and then placed in labeled envelopes. The swabs using water as the wetting agent were stored in a freezer (-20 °C), whereas the swabs containing SM1 and SM2 as the wetting agent were stored at room temperature. 450 samples were collected. The samples were stored for a

period of 6 months, and subsequently extracted with the QIAGEN QIAamp Micro Kit; a Chelex 100 procedure; and an organic procedure.

Experimental Variables

Dilution	Neat, 1/100, 1/200, 1/400 and 1/800
Extraction Method	Qiagen, Chelex, Organic
Wetting Agent	SampleMatrix™ 1 (SM1), SampleMatrix™ 2 (SM2), H2O
Substrates	Cement, Cotton, Carpet, Glass, Wood

SampleMatrix™ SM1 and SM2 as swab wetting agents for the collection of semen-stains

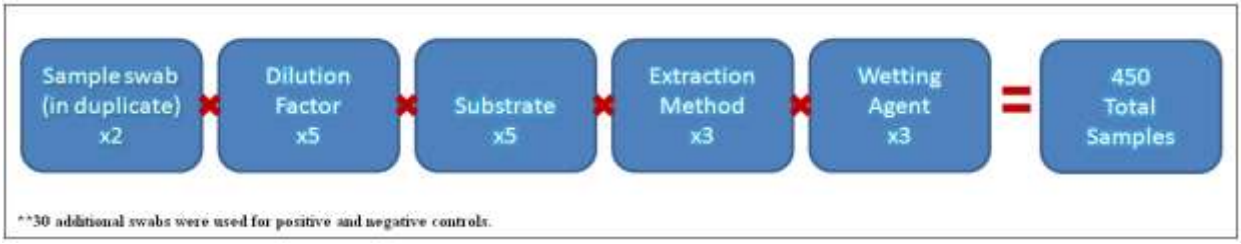
Sample Preparation

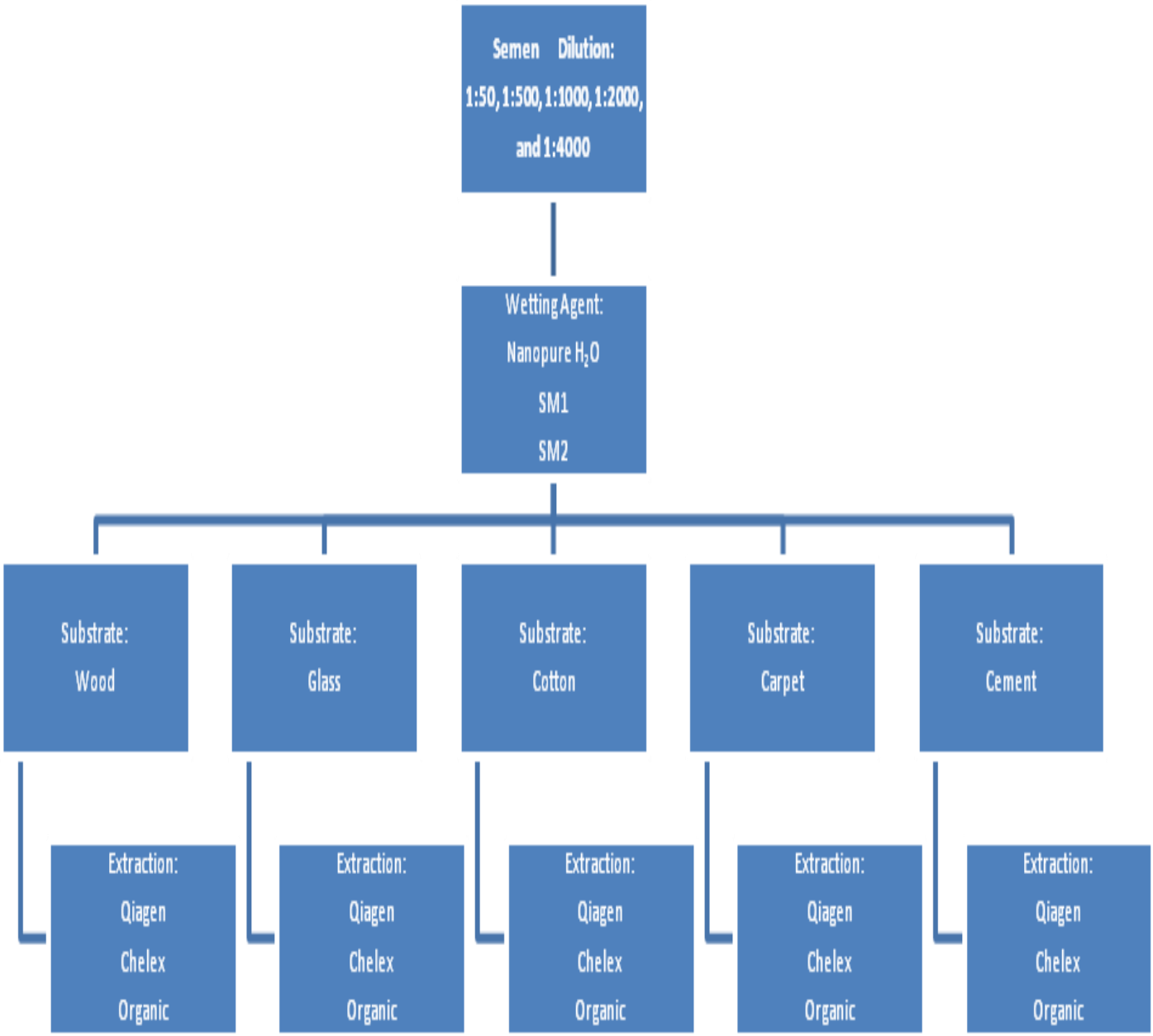
Five substrates were selected for analysis in this study: brick, wood, glass, carpet, and cotton bed sheets. The substrates were selected to represent a variety of materials (texture and porosity) that may be encountered at crime scenes. A cleaning pre-treatment was performed on each of the five selected substrates in order to remove possible foreign DNA. Each brick substrate utilized was cleaned with 10% bleach using a household scrub followed by rinsing with distilled water. The top layer of wood was removed using a carpenter's plane, followed by a 10% bleach, and distilled water wash. Two different types of wood were used: cedar and pine. The glass pane substrates were wiped down with bleach and cleaned with distilled water. The beige colored carpet and the white cotton bet sheets (250-thread count) were both purchased new and were not pre-treated prior to analysis.

100 µL of a semen sample (neat or diluted) was pipetted in duplicate onto each of the five substrates. The wet stains were then outlined with a permanent marker to demarcate the area to be swabbed and allowed to dry overnight.

For stain collection, clean Puritan brand cotton swabs were wetted with 40 µL of one of the three different wetting agents: Nano pure water, Sample Matrix™ 1 (SM1) or Sample Matrix™ 2 (SM2). Collection of the dried sample from each of the substrates was standardized. Each wet swab was rolled (on its side) over the entirety of the deposited semen sample 25 times. The tip of the cotton swab was then applied over the entire semen stain 50 times. This process was repeated for each of the five substrates (for each extraction method per dilution, in duplicate). Following sample collection, the cotton swabs were dried overnight and then packaged in white envelopes for storage at room temperature for a period of 6 months. The swabs were subsequently extracted with the QIAGEN QIAamp Micro Kit; a Chelex 100 procedure, and an organic procedure.

Experimental Parameters





Experimental Design

SampleMatrix™ SM1 and SM2 as swab wetting agents for the collection of saliva-stains

Sample Preparation

Brick, wood, glass, carpet, and cotton substrates were chosen for this study. The brick substrate was cleaned with 10% bleach followed with a distilled water rinse. The top surface of the wood substrate was shaved with a planer, cleaned with 10% bleach, and rinsed with distilled water. The glass substrate was cleaned with 10% bleach and rinsed down with distilled water. A section of beige colored carpet and 250 thread count white cotton sheets were purchased new. 100 µL of the saliva sample was deposited on to each substrate with a pipette. Once the sample diffused on the substrate, the stain was marked with a permanent marker to demarcate the area to be swabbed. All saliva stains were allowed to air dry for 24 hours. The following technique was then employed to collect the stains by swabbing: 40 µL of SM1, SM2, or Nano pure water was applied to the cotton swab, which was then rolled 25 times and dabbed 50 times on each stain. The swabs were allowed to air dry under a laminar airflow hood, and then placed in envelopes. The swabs using water as the wetting agent were stored in a freezer (-20 °C), whereas the swabs wetted with SM1 and SM2 were stored at room temperature. The same procedure was performed for the negative controls. All samples were performed in duplicate. The samples were stored for 6 months, and then extracted with the QIAGEN QIAamp Micro Kit; a Chelex 100 procedure; and an organic procedure.

Experimental Variables

Dilution	Neat, 1/10, 1/50, 1/100 and 1/200
Extraction Method	Qiagen, Chelex, Organic
Wetting Agent	SampleMatrix™ 1 (SM1), SampleMatrix™ 2 (SM2), H2O
Substrates	Cement, Cotton, Carpet, Glass, Wood

Effects of SampleMatrix™ SM1 and SM2 on conventional serological testing of blood

Preliminary Studies

Preliminary studies were conducted to establish the experimental design of this study. The first of these studies involved determining the most effective method of adding the presumptive test reagents to the blood samples. Two approaches were considered: directly adding the reagents to a cutting of the swab versus using the "taco method," in which a disk of Whatman® Qualitative Filter Paper was folded in half, saturated with Nano pure water, and applied to the swab to cause a transfer of a portion of the blood sample onto the filter paper. The reagents were then applied directly onto the filter paper. The direct method proved to be much more sensitive than the "taco method"; the former method rendered positive results through the 1:1,000 diluted samples whereas the latter method only rendered positives results with the neat blood samples for each presumptive test. In the course of performing this preliminary study, it was also observed that the SM2 SampleMatrix™ formulation produced a false positive for both the phenolphthalein and leucomalachite green presumptive tests. Specifically, a color change indicative of a positive result was observed before the addition of the hydrogen peroxide in both tests. This finding was presented to the R & D scientists at Biomatrica, Inc., who indicated that the likely cause of the false positive reaction was the dye. According to the scientists, the SM2 formulation contained phenol red as a dye whereas the SM1 formulation contained Red 40. Given that the dye plays no functional role in the protective properties of SampleMatrix™, Biomatrica, Inc. subsequently removed the dye from both formulations. The new colorless formulations were retested before the study samples were prepared to ensure that the problem had been rectified. This testing confirmed that false positives were not observed with the colorless formulations of SampleMatrix™.

Experimental Design

The focus of this study was to determine the effectiveness of SampleMatrix™ as a means to store blood samples at room temperature. The following variables were controlled within the course of the study: sample dilution, storage conditions, and storage time. Blood samples of varying dilutions were created by serial dilution. The dilutions included neat, 1:100, 1:200, 1:400, 1:800, 1:1,000, 1:10,000, and 1:100,000. Samples were stored under one of the following conditions: at room temperature with no SampleMatrix™ added, at room temperature coated with SampleMatrix™, and at -20° C. Two different formulations of SampleMatrix™ (SM1 and SM2) were tested in order to evaluate which is most effective in preserving blood samples at room temperature. Samples were also stored for varying periods prior to testing; this ranged from immediate application of blood or SampleMatrix™ (where applicable) to two months. Further, negative control samples consisting of saline, SM1, and SM2 were prepared and evaluated along with the experimental samples. All of the samples for each independent variable were tested in triplicate and separate negative controls were included for each storage condition and storage period.

Sample Preparation

Various blood dilutions were prepared in physiological saline (0.085 g NaCl in 100mL of Nano pure water). The blood dilution series used in this study was obtained from a single human

blood source. The subsequent blood dilutions were thoroughly mixed prior to preparing the experimental samples.

Swab Preparation

672 samples were prepared using Puritan® cotton-tipped applicators (swabs) in accordance with the following variables: blood dilution, storage condition, and storage time.

Experimental Parameters

Blood Dilution	Neat, 1:100, 1:200, 1:400, 1:800, 1:1000, 1:10000, 1:100000
Storage Condition	RT (room temperature), (SM1), (SM2), -20 (-20°C)
Storage Time	Immediate, 1 day, 5 days, 1 week, 2 weeks, 1 month, 2 months

A 50µL aliquot of the appropriate blood dilution was applied to the tip of a corresponding labeled cotton swab. For samples that required SampleMatrix™, 50 µL of either SM1 or SM2 was applied to the swab immediately following the application of the blood. All samples were allowed to dry for approximately 30 minutes prior to storage in paper envelopes. The samples labeled RT, SM1, and SM2 were all stored at room temperature for their respective storage time intervals. The samples labeled -20 were stored at -20°C for their respective storage time intervals. The immediate samples were tested following the 30-minute drying time.

Presumptive Tests

Three presumptive tests used in a forensic capacity to identify blood stains were employed in this study: phenolphthalein, leucomalachite green (LMG), and Luminol. Each of these tests was conducted on one-half of each sample swab. In order to split the sample into two fractions, the tip of the sample swab was cut in half using a sterile scalpel blade and removed from the wooden applicator by cutting around the base of the cotton swab. The presumptive test reagents were added directly to one of the resulting sample swab fractions.

Phenolphthalein Color Test

The cotton swab samples were placed in a series of separate, pre-labeled wells. Two drops of prepared phenolphthalein reagent were placed on each sample and allowed to set at room temperature for thirty seconds to monitor for a false positive reaction. If no color change occurred, two drops of 3% hydrogen peroxide were added. A positive reaction indicative of the presence of blood was noted when a pink color was observed within one minute of applying hydrogen peroxide.

Leucomalachite Green Color Test

The cotton swab samples were placed in a series of separate, pre-labeled wells. Two drops of prepared leucomalachite green reagent were placed on each sample and allowed to dry at room temperature for thirty seconds to monitor for a false positive reaction. If no color change occurred, two drops of 3% hydrogen peroxide were added. A dark green color change observed within one minute of hydrogen peroxide application, signifying the oxidation of the colorless

leucomalachite green to malachite green catalyzed by hemoglobin, was indicative of the presence of blood.

Luminol Test

The cotton swab samples were placed in a series of separate, pre-labeled wells. Two drops of prepared luminol reagent were placed on each sample and allowed to dry at room temperature for thirty seconds to monitor for a false positive reaction. If no luminescence was observed, then two drops of 3% hydrogen peroxide were added. Luminescence observed within one minute of hydrogen peroxide application was indicative of the presence of blood. In this hemoglobin-catalyzed reaction, luminol (3-aminophthalhydrazide) is oxidized to 3-aminophthalate, whose electrons are in an excited state. Blue light is emitted as energy that is released once the excited electrons of the 3-aminophthalate return to the ground state. The luminol test was conducted in a dark room in order to visualize the reaction more effectively. The lighting was subdued after both steps of the reaction to observe any false positives as well as the results.

Species of Origin Test

As with the presumptive tests, one-half of each sample swab was used for the species of origin test (50 µL of blood initially applied). The cotton swab samples were placed in pre-labeled collection tubes that held 1.5 mL of extraction buffer provided by the SERATEC® HemDirect Hemoglobin Assay kit. The tubes were placed on an orbital shaker and left to agitate at room temperature for two hours. Following agitation, three drops of each sample were placed in the sample well of the assay. The test contains two monoclonal murine anti-human hemoglobin antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane as a line. The upstream control region contains immobilized polyclonal goat ant-rabbit antibodies that are also fixed on the membrane as a line. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad that contains the dried and gold-labeled second monoclonal murine anti-human hemoglobin antibody that will bind the hemoglobin present in the sample. Additionally, the pad contains gold-labeled rabbit antibodies. As the sample migrates across the membrane via capillary action, the colored gold-labeled rabbit antibodies will bind to the anti-rabbit-antibody at the control region, resulting in the formation of the red control line. If the sample contains human hemoglobin, the human hemoglobin-gold-labeled anti-human hemoglobin-antibody complex will bind to the immobilized monoclonal antibody of the test region, resulting in the formation of a red line. Thus, in the present study, a positive result was indicated by the presence of two red colored lines, one at the control region and the other at the test result region. A single red colored line at the control region denoted a negative result. The SERATEC® Hem Direct Hemoglobin Assay results were interpreted after five minutes and negative results were confirmed after ten minutes per the manufacturer's recommendations.

Effects of SampleMatrix™ on conventional serological testing of semen

Sample Collection and Preparation

Semen samples were collected from two reportedly healthy, adult male volunteers. Cotton swabs were prepared in triplicate for each semen dilution for a given time period. The concentrations selected were: neat, 1:100, 1:200, 1:400, 1:800, 1:1000, and 1:10,000; however, preliminary studies indicated that the 1:10,000 dilution did not react with the AP reagent and was therefore eliminated from the study. The times evaluated were one day, three days, one week, two weeks, one month, and two months. Four different sets of swabs were prepared for each of these dilutions and times. The four sets were semen stored at room temperature; semen stored at -20°C, semen coated with SM1 SampleMatrix™ formulation and stored at room temperature; and semen coated with SM2 SampleMatrix™ formulation and stored at room temperature. A 50 µL aliquot of the semen sample (neat or diluted) was applied to a cotton swab, allowed to air dry, and then placed in a -20°C freezer or stored at room temperature in a laboratory hood for the specified period of time. A 50 µL aliquot of each semen dilution was also applied to a cotton swab that was followed by the application of 50 µL of either SM1 or SM2 SampleMatrix™ formulation. These swabs were also allowed to air dry and stored at room environmental conditions for the respective time.

Sample Analysis Using Acid Phosphatase Test

Seminal acid phosphatase (SAP) is an enzyme that is secreted by epithelial cells that line the prostate gland. This enzyme catalyzes the hydrolysis of certain organic phosphates, such as α-Naphthyl phosphate. The enzyme cleaves the naphthyl group, facilitating the interaction between the naphthyl group and an azo dye; in this study, Brentamine Fast Blue B. The coupling of the dye with the naphthyl produces a colored product that forms the basis for a positive reaction for the presumptive detection of seminal fluid.

In the present study, each swab was analyzed using the acid phosphatase test for each time period and storage condition. One quarter of each swab was removed and placed in a tray that contained individual wells for each quarter-swab. The tray was labeled to indicate dilution, time, and storage condition for each well. The acid phosphatase solutions were added directly into each well.

Solution A was prepared by dissolving five mg of Fast Blue B in five mL of acetate buffer. Solution B was prepared by dissolving five mg of α-naphthyl phosphate in five mL of acetate buffer. Two drops of solution A were added to a well and any observed color change was noted. If no color change was evident, two drops of solution B were added to the same well. The observation of a purple color in the wells within 15 seconds of application of solution B was recorded as a positive reaction. Positive controls (known fresh semen stains), negative controls (Nano pure water, SM1, and SM2), and the experimental samples were each tested simultaneously for acid phosphatase activity for each time. The results were recorded in note form and were documented photographically.

Sample Analysis Using SERATEC® PSA SEMIQUANT Test Cassettes

The SERATEC® PSA SEMIQUANT test is an immunochromatographic PSA membrane test. These tests are based on antigen-antibody reactions, along with conjugated dye particles, to recognize the presence of PSA in a solution. Each assay contains mobile monoclonal anti-human PSA antibodies with attached dye particles. When human PSA is introduced into the assay, the

antibody-dye conjugates bind the PSA and migrate to a reaction zone on the cassette. Attached to the reaction zone are polyclonal antihuman PSA antibodies, which also bind the mobile antibody-antigen complexes. When these complexes become concentrated enough, a color band appears in the result window of the kit indicating the presence of PSA and, therefore, a positive result. The unbound monoclonal anti-human antibodies continue to a control zone where they bind to immobilized anti-Ig antibodies. These concentrated complexes also form a color band, which indicates a control band and confirms that the test is working properly.

The SERATEC® PSA Semiquant test for the detection of prostate-specific antigen was performed in duplicate. The protocol recommended by the manufacturer was followed with the exception that the entire swab was used in the analysis rather than 1/3 of the swab. Whole swabs were incubated in centrifuge tubes using 250 µL PSA Buffer Solution (pH = 8.2) for a two hour period. After the two-hour incubation period, the samples were centrifuged for one minute. A 200 µL aliquot of the supernatant was dispensed into the test well of each labeled cassette. The results were interpreted and photographed after 10 minutes had elapsed. A positive reaction was indicated when three-color bands were visible in the results window. These three bands correspond to the control band, quantification band, and test band. A negative result was recorded when only the control and quantification band were visible.

Microscopic Staining and Evaluation

One of the triplicate sets of swabs for the AP tests was used to prepare the microscope slides for the morphological evaluation. Half of the swab was placed in a centrifuge tube with 500 µL of deionized water and agitated for 30 minutes. The swab was then transferred to a spin basket and centrifuged for five minutes. A 10µL aliquot of the pellet was then pipetted onto a microscope slide and allowed to air dry before staining. Only neat semen swabs were used for the microscopic evaluation for the following times: one day, three days, one week, two weeks, and one month. The Hematoxylin-Eosin staining technique was used to observe the microscope slides. Each slide was viewed under 400x magnification and the quantity of sperm cells was scored as follows:

Few	less than five spermatozoa found
+	hard to find
++	some in same fields, easy to find
+++	many or some in most fields
++++	many in every field.

Effects of SampleMatrix™ on conventional serological testing of saliva

Sample Collection and Preparation

To evaluate the effectiveness of Sample Matrix™ to protect saliva samples, the amylase activity of samples were measured by two methods were selected to detect radial diffusion and the SALIgAE® commercial kit test. Furthermore, the integrity of epithelial cell structure was evaluated microscopically as a measure of stabilization. Saliva specimens were collected in 35mL polypropylene tubes, and the samples were prepared by applying the saliva directly to the swabs at the time of collection. Saliva was pipetted in 50µL aliquots onto the tips of the Puritan swabs. For the swabs protected with Sample Matrix™, the saliva was allowed to dry on the swab for 30 minutes before the application of 50µL Sample Matrix™ to the swab.

350 swabs were prepared, which represented various concentrations of whole saliva stored under different conditions for different periods. Specifically, the storage periods examined were: immediate, one day, three day, one week, two weeks, four weeks and two months; the saliva concentrations examined were neat, 1:10, 1:50, 1:100, and 1:200; and the storage conditions were room temperature (RT) swabs protected with Sample Matrix™ (SM1 and SM2 formulations), room temperature swabs unprotected (control), and frozen (-20ºC) swabs unprotected. Negative and positive controls were also prepared for each time. All of the swabs were prepared in triplicate and stored for a period of up to two months. Each sample was prepared and handled the same way prior to storage.

Sample Preparation

The entire swab was excised and extracted with water for the Radial Diffusion SALIgAE® tests whereas a ¼ cutting of the swab was used for the evaluation of Epithelial Cell Morphology. 120µL of ultrapure water was used to extract samples for the radial diffusion and microscopic tests; 50µL of ultrapure water was used to extract samples for the SALIgAE® test. The samples were extracted for 30 minutes at room temperature with continuous agitation and periodic vortexing. The swabs were then placed into a spin basket (filter cup), centrifuged for five minutes, and the supernatant was recovered.

Radial Diffusion Test

All of the agarose plates were prepared simultaneously. A gel was prepared in a Petri dish (Falcon 100 x 15 mm) by combining 0.1g general-purpose agarose (APEX), 0.01g soluble starch (SERI), and 10mL buffer solution (SERI B116). The buffer solution was prepared using a pre-portioned powdered amylase diffusion buffer mixed with 500 mL of distilled water. The reagent mixture was warmed to a rolling boil in a microwave (approximately 20 seconds) and poured into the Petri dish to solidify. Once the agarose solidified, 1.5 mm diameter wells were formed in the gel with a Pasteur pipette attached to an aspirator. 20µL of each sample extract was placed into a well, and the plate was then incubated in a 37°C oven for 20m hours. The petri dish was removed from incubation and stained with ten mL of a saturated iodine solution (0.05M KI/I2). A clear circle around the well indicated amylase activity, measurements, and photographs were obtained for each reaction. Sigma α amylase [BAN 240L] from Bacillus amyloliquefaciens was used as a positive control.

SALIgAE® Test Kit

According to the technical information sheet provided by Abacus Diagnostics Inc., SALIgAE® is designed for the identification of saliva in forensic casework. The test offers higher sensitivity and specificity, is capable of detecting trace levels of saliva, and can be performed within ten minutes. A positive reaction in this test results in the formation of a yellow colored solution. A SALIgAE® test kit consists of the following: ten test vials and testing instructions. Each kit was allowed to equilibrate at room temperature for 30 minutes. Testing for this procedure was performed in duplicate. 50 µL of the sample extract was then pipetted into the test vial. The results were read immediately with the addition of the extract and at 10 minutes. The results were recorded based on the following scale: 0= no color change; 1= very weakly visible; 2= weakly visible; 3= visible; and 4= strong color change.

Epithelial Cell Morphology

A 10 µL aliquot of the extraction pellet was pipetted onto a microscope slide. The slides were then dried, stained with hematoxylin and 5% eosin, and mounted with a coverslip using Cytoseal 60. The slides were examined using a Leica DM EP polarizing microscope (400 x). The epithelial cell concentration and condition were then scored with the scale below. A cell was considered "lysed" if the cell membrane was disrupted.

Condition of Cells	Amount of Cells Visible per Field of View
I=Intact	A=Abundant
L=Lysed	F=Few
N=No Cells	

Effects of SM3 on the preservation of blood cell morphology

Experimental Design

The study was designed to test the effectiveness of the SM3 formulation to stabilize red blood cell and white blood cell morphology with room temperature storage. Whole blood samples were subjected to one of the two storage conditions (treated or untreated) and then sampled at discreet time intervals. Samples were prepared in duplicate. 25 µL of fresh blood (≤ 4 days) was pipetted into the wells of a micro titer plate. For the experimental samples, 50 µL of the SM3 formulation was immediately added to the liquid blood samples and mixed. The samples were then allowed to dry and subsequently stored at room temperature for 2-230 days. After storage, the samples were rehydrated with 50 µL of physiological saline, for 7-180 minutes, and then prepared as wet mounts or hematoxylin and eosin stained slides. The slides were examined with an Olympus BH-2 microscope at 200 x and 400 x magnifications under transmitted light or phase contrast.

DNA Extraction Methods

Chelex-100 Extraction Procedure

Each cotton swab sample was dissected into thirds using a clean disposable surgical blade. Swabs were further shredded to increase surface area. The swab was placed into a pre-labeled 2 mL microcentrofuge tube. The surgical blade was washed with ethanol and rinsed with Nano pure water, followed by two wipes with a clean Kim wipe™ paper tissue after each dissection was performed. 1 mL of Nano pure water was pipetted into the 2 mL microcentrofuge tube containing the swab and mixed gently on an orbital shaker for 30 minutes. After incubation, the swab was placed into a spin basket and centrifuged for 3 minutes at 15,000 x g. The supernatant was removed and discarded, leaving the remainder of the pellet in 20-30 µL of supernatant. This volume was brought up to a final volume of 200 µL by adding 5% Chelex to the tube. The wet swab was removed from the spin basket and returned to the tube for further incubation in a water bath at 56 ºC for 30 minutes. The sample was then vortexed at high speed for 5-10 seconds, and incubated in a boiling water bath for 8 minutes. The sample was vortexed again at high speed for 5-10 seconds. The wet swab was removed from the tube and placed into a spin basket, where it was spun in a micro centrifuge for 3 minutes at 10,000 – 15,000 x g. The volume of the supernatant was measured, and the sample was then stored at 4°C.

QIAGEN QIAamp Micro Kit Procedure

A modification of the forensic casework samples procedure (QIAamp DNA Micro Handbook) was employed in the present study. The entire cotton swab was cut into small pieces and placed in a pre-labeled 1.5 mL micro-centrifuge tube. 300 µL ATL buffer and 20 µL proteinase-K were added into each sample tube. 20 µL 1.0M DTT was also added to the tube for the semen samples. The samples were pulse-vortexed for approximately 10 seconds and placed into a 56 °C water bath for 1 hour, pulse vortexing every 10 mins. The samples were briefly centrifuged after incubation to remove the condensate from the caps. 300 µL AL buffer and 1ng of carrier-RNA (dissolved in 1 µL AE buffer) were added to each sample tube and incubated in a 70 ºC water-bath for 10 mins, again pulse-vortexing every 3 minutes. The samples were centrifuged at 14000 rpm for 1 min and the extract was transferred to a QIAamp column. In addition, the cotton swabs were placed into a spin-basket, which was placed in the original

micro-centrifuge tube. The centrifuge tube containing the spin-basket was centrifuged at 14000 rpm for 2 minutes and the flow through was combined with the original aliquot in the respective QIAamp column. The columns were centrifuged at 8,000 rpm for 1minute and the collection tubes with the flow through were discarded and replaced with a new collection tube. 500 µL AW1 buffer was added into each column and centrifuged at 8,000 rpm for 1 min. The collection tubes with the flow through were again discarded and replaced with a new collection tube. 500 µL AW2 buffer was added into each column and centrifuged at 8,000 rpm for 1min and the collection tubes with flow through were replaced with a new collection tube. The spin columns were then centrifuged at 14,000 rpm for 3 minutes to dry the membrane and placed into a clean 1.5 mL micro-centrifuge tube. 50 µL AE buffer was added to the columns and allowed to incubate at room temperature for 5 minutes prior to centrifugation at 14,000 rpm for 1min. The DNA extract collected in the micro-centrifuge tubes was stored in a refrigerator (4 ºC) in preparation for qPCR analysis.

Organic Extraction Procedure

The organic procedure included: 1) a cell lysis step, 2) a phenol/chloroform/isoamyl alcohol (PCI) extraction step, and 3) an Amicon® Ultra-4 wash, concentration, and recovery step. Specifically, the cotton swab cuttings were placed into a 2 mL microcentrofuge tube with 400 µL of stain extraction buffer [10 mM Tris, 10 mM EDTA, 100 mM NaCl, 39 mM dithiothreitol, 2% SDS, 20 µL of 10 mg/mL Proteinase K, and pH 8.0] for a minimum of 2 hours at 56°C. Afterwards, the cuttings were removed from the solution and placed in a spin basket, which was subsequently inserted into the original tube. The sample was then pulse-vortexed and centrifuged for 5 minutes at 14,000 rpm. The spin basket and swab were discarded and 500 µL of PCI was then added to the supernatant, pulse-vortexed, and centrifuged for 5 minutes at 14,000 rpm. The upper, aqueous layer of the sample was removed and placed into a new 2 mL micro centrifuge tube. 200 µL of stain extraction buffer was then added to the original tube with the PCI solution as a back-extraction step. The sample was pulse-vortexed and centrifuged for 5 minutes at 14,000 rpm. The upper aqueous layer was removed and pooled with the aqueous layer recovered previously. An additional PCI extraction was performed on the pooled sample. The resultant aqueous layer was then recovered and subject to an Amicon® Ultra-4 Centrifugal Filter Device washing, concentration, and recovery step. This was accomplished by adding 2 mL of TE^{-4} Buffer to the top chamber of the Amicon® Ultra-4 Centrifugal Filter Device, followed by the entire pooled aqueous layer (DNA extract) from the organic extraction. The device was centrifuged for 15 minutes at 3,000 x g and 25 ºC. The filtrate was discarded from the bottom chamber of the Amicon® Ultra-4 Centrifugal Filter Device. The wash was repeated twice, each time adding 2 mL of TE^{-4} Buffer to the upper chamber of the Amicon® Ultra-4 Centrifugal Filter Device and centrifuging, as described above. After the final spin, a gel loading pipette tip was used to retrieve the DNA sample from the Amicon® Ultra-4 Centrifugal Filter Device. If no sample was visible in the pipette tip, 25 µL of TE^{-4} Buffer was added to the upper chamber to reconstitute the DNA and pulse-vortexed briefly to facilitate recovery. Samples were then stored at 4 ºC.

DNA Extract Volume

The total volume of each DNA extract from the three extraction methods was measured by a pipette and recorded in microliters. This volume was multiplied by the DNA concentration

(ng/µL) of the sample, as determined by qPCR, to give the total amount of DNA recovered from the sample.

DNA Quantification

Samples were quantified using the Quantifiler Human DNA Quantification Kit (Applied Biosystems Lot #0708079) 7300 Real Time PCR Instrument (Applied Biosystems) ABI 7300 Real Time PCR System. A DNA reference series was prepared in duplicate using human DNA standards (200 ng/µL) provided in the ABI Quantifiler kit. The standards were diluted using TE buffer, pH 8. The concentration of the standards ranged as follows: 50.0, 16.7, 5.560, 1.850, 0.620, 0.210, 0.068 and 0.023 in ng/µL. Two tubes of master mix were prepared by mixing 557.5 µL of Human Primer Mix and 627.5 µL of PCR Reaction Mix. 23 µL of the master mix was pipetted into each well of a 96 well optical reaction plate. 2 µL of each standard, a TE blank and each sample was dispensed into their respective well. The plate was sealed with an optical adhesive cover and centrifuged for 30 seconds at 3000 rpm to remove any air bubbles in the wells. The sample plate was placed into the ABI 7300 system that was pre-programmed with the following: Stage 1 - 1 Rep of 95.0 for 10 minutes; Stage 2 - 40 Reps of 95.0 for 15 seconds, then 60.0 for 1 minute.

STR Amplification and Typing

The selected DNA samples were amplified once using the AmpF*l*STR® Profiler Plus™ PCR Amplification Kit or the AmpF*l*STR® Identifiler™ PCR Amplification Kit (PE Biosystems, Foster City, CA). The Profiler Plus kit co-amplifies the gender marker amelogenin and the STR loci: TH01, CSF1PO, D16S539, TPOX, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820. The Identifiler kit amplifies the above loci plus two additional loci: D2S1338 and D19S433A. A target amount of 1.5 ng DNA was used in this study. DNA samples that were below the target amount were concentrated using a Millipore Amicon® Ultra-4 centrifugal filter device, a Millipore Microcon centrifugal filter device, or were amplified neat. The DNA samples with a concentration above the target were diluted with TE-4 as needed. Samples were amplified on a GeneAmp® PCR System 9700 following the kit-manufacturer's recommendations.

AmpF*l*STR® amplification products were analyzed once by capillary electrophoresis and laser induced fluorescence using the ABI PRISM 310 Genetic Analyzer. Briefly, 1.5 µL of amplicon and 1 µL of GeneScan-500 [ROX] Internal Lane Size Standard were added to 24 µL of deionized formamide, denatured at 95°C for 3 minutes, then snap-cooled on ice for 2 minutes. The PCR products were then injected for 5 seconds at 15kV, and electrophoresed for 24 minutes at 15kV and 60°C. The electrophoretic capillary was 50µm by 47 cm, and filled with Performance Optimized Polymer-4 (POP4) and 1X Genetic Analyzer Buffer. Allelic peaks were sized and typed using the GeneScan™ Version 3.1 and Genotyper™ Version 2.5 software, with a peak detection threshold of 75 relative fluorescence units (RFU).

CHAPTER 3: RESULTS OF CONVENTIONAL SEROLOGY STUDIES

I. BLOOD ANALYSIS (N > 2000)

The objective of this study was to evaluate the effect of SampleMatrix™ on presumptive blood tests and species of origin test. The effect of each storage condition on the sensitivity to phenolphthalein, leucomalachite green, luminol, and the SERATAC® HemDirect Hemoglobin Assay was evaluated. Additionally, in assessing the results obtained, the compatibility of both formulations of SampleMatrix™ with the presumptive tests and species of origin test as well as the relative performance of each formulation were determined. The reported results for each test were based on triplicate sampling and are representative of the results obtained across all three trials. Separate negative controls were analyzed tested simultaneously for each dilution and storage period.

Phenolphthalein Color Test

The results obtained for the phenolphthalein test were consistent with all samples for times spanning immediate testing to one week, regardless of their storage condition; each displayed the same degree of color change for their respective dilution (Tables 2-5). Also, all of the 1:10,000 and 1:100,000 dilutions gave negative results regardless of their storage condition. The same result also held true for the samples that were stored for two weeks with the exception of the 1:1,000 sample that was coated with SM2, which did not exhibit a color change, while the remaining samples showed a slight color change (Table 6). However, the results for the one and two-month old samples showed a clear advantage for samples stored under freezing temperatures. In focusing on the one-month storage period, the samples stored at -20°C displayed a more intense color change than any of the room temperature samples for dilutions of 1:400, 1:800, and 1:1,000 (Table 7). The samples stored at room temperature without SampleMatrix™ added and those coated with SM1 produced similar results, whereas the sample coated with SM2 did not exhibit a color change for the 1:1,000 diluted sample. For the two-month-old samples, the samples stored at -20°C again exhibited a more color change for all dilutions beginning with the 1:200 diluted samples (Table 8). The samples stored at room temperature without addition of SampleMatrix™ showed a considerable decrease in sensitivity relative to the one-month samples, with both the 1:800 and 1:1,000 samples failing to produce a color change. The samples coated with either SampleMatrix™ formulation did not exhibit discernable differences with respect to the one-month samples. No false positives were identified.

Color Change Intensity Scale.

++++	intense color change
+++	moderate color change
++	weak color change
+	slight color change
0	no color change

Table 2: Results of Phenolphthalein Color Test: Immediate Testing

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	+++	++	+	0	0	0
SM1	++++	++++	++++	+++	++	++	0	0	0
SM2	++++	++++	++++	+++	++	++	0	0	0

Table 3: Results of Phenolphthalein Color Test: Samples Stored for 1 Day

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	+++	++	++	0	0	0
SM1	++++	++++	++++	+++	++	++	0	0	0
SM2	++++	++++	++++	+++	++	++	0	0	0
-20	++++	++++	++++	+++	++	++	0	0	0

Table 4: Results of Phenolphthalein Color Test: Samples Stored for 5 Days

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	+++	++	++	0	0	0
SM1	++++	++++	++++	+++	++	++	0	0	0
SM2	++++	++++	++++	+++	++	++	0	0	0
-20	++++	++++	++++	+++	++	++	0	0	0

Table 5: Results of Phenolphthalein Color Test: Samples Stored for 1 Week

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,0000	Neg
RT	++++	++++	++++	+++	++	++	0	0	0
SM1	++++	++++	++++	+++	++	++	0	0	0
SM2	++++	++++	++++	+++	++	++	0	0	0
-20	++++	++++	++++	+++	++	++	0	0	0

Table 6: Results of Phenolphthalein Color Test: Samples Stored for 2 Weeks

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	+++	++	+	0	0	0
SM1	++++	++++	++++	+++	++	+	0	0	0
SM2	++++	++++	++++	+++	++	0	0	0	0
-20	++++	++++	++++	+++	++	+	0	0	0

Table 7: Results of Phenolphthalein Color Test: Samples Stored for 1 Month.

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	++	+	+	0	0	0
SM1	++++	++++	++++	++	+	+	0	0	0
SM2	++++	++++	++++	++	+	0	0	0	0
-20	++++	++++	++++	+++	++	++	0	0	0

Table 8: Results of Phenolphthalein Color Test: Samples Stored for 2 Months.

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++	+	0	0	0	0	0
SM1	++++	++++	++	+	+	+	0	0	0
SM2	++++	++++	++	+	+	0	0	0	0
-20	++++	++++	+++	++	++	++	0	0	0

Leucomalachite Green Color Test

Beginning with the five-day-old samples, those stored at -20°C showed a higher degree of color change at lower dilutions (Table 11). The samples stored at room temperature without SampleMatrix™ added and those coated with SM2 displayed no color change beyond the 1:400 dilution for samples stored for two weeks, one month, and two months (Tables 13-15). Samples coated with SM1 performed slightly better, exhibiting a weak color change for the 1:800 and 1:1,000 dilutions associated with the two-week-old samples; however, the samples stored for one and two months followed the same trend as the room temperature and SM2 protected samples. The samples stored at -20°C exhibited improved sensitivity, displaying a color change for all samples ranging from neat to the 1:1,000 dilutions up to the two-month storage period. No false positives were identified.

Table 9: Results of Leucomalachite Green Color Test: Immediate Testing

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+++	++	+	+	0	0	0
SM1	++++	++++	+++	++	++	++	0	0	0
SM2	++++	++++	+++	++	++	++	0	0	0

Table 10: Results of Leucomalachite Green Color Test: Samples Stored for 1 Day.

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+++	+++	++	+	0	0	0
SM1	++++	++++	+++	+++	++	+	0	0	0
SM2	++++	++++	+++	+++	++	+	0	0	0
-20	++++	++++	+++	+++	++	+	0	0	0

Table 11: Results of Leucomalachite Green Color Test: Samples Stored for 5 Days

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+++	+++	++	+	0	0	0
SM1	++++	++++	+++	+++	++	+	0	0	0
SM2	++++	++++	+++	+++	++	+	0	0	0
-20	++++	++++	+++	+++	++	++	0	0	0

Table 12: Results of Leucomalachite Green Color Test: Samples Stored for 1 Week

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+++	+++	++	+	0	0	0
SM1	++++	++++	+++	+++	++	+	0	0	0
SM2	++++	++++	+++	+++	++	+	0	0	0
-20	++++	++++	+++	+++	++	++	0	0	0

Table 13: Results of Leucomalachite Green Color Test: Samples Stored for 2 Weeks

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	+++	+++	+	0	0	0	0	0
SM1	++++	+++	+++	+	+	+	0	0	0
SM2	++++	+++	+++	+	0	0	0	0	0
-20	++++	+++	+++	++	++	+	0	0	0

Table 14: Results of Leucomalachite Green Color Test: Samples Stored for 1 Month

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	+++	+++	+	0	0	0	0	0
SM1	++++	+++	+++	+	0	0	0	0	0
SM2	++++	+++	+++	+	0	0	0	0	0
-20	++++	+++	+++	++	+	+	0	0	0

Table 15: Results of Leucomalachite Green Color Test: Samples Stored for 2 Months

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++	++	+	0	0	0	0	0
SM1	++++	++	++	+	0	0	0	0	0
SM2	++++	++	++	+	0	0	0	0	0
-20	++++	+++	+++	++	+	+	0	0	0

Luminol Test

The most distinct divergence exhibited between the samples stored at -20°C and those stored at room temperature was observed with the luminol test. A discernable difference in chemiluminescence sensitivity for the samples stored at -20°C was observed as early as one day (Table 17). This trend continued across most times, excluding one week. However, the results obtained for the unprotected samples stored at room temperature compared to those coated with either SampleMatrix™ formulation appeared to be somewhat inconsistent. For instance, all of the one-week samples stored at room temperature displayed a more intense color change relative to the equivalent five-day samples (Tables 18-19). In addition, none of the samples stored at room temperature exhibited a color change for the 1:800 dilutions at two weeks, yet did show a weak color change for the corresponding dilution at one month (Tables 20-21). However, at two months, only the samples stored at -20°C displayed a color change for sample dilutions exceeding 1:200 (Table 22). No false positives were identified.

Table 16: Results of Luminol Test: Immediate Testing

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	+++	++	+	0	0	0
SM1	++++	++++	++++	+++	++	+	0	0	0
SM2	++++	++++	++++	+++	++	+	+	0	0

Table 17: Results of Luminol Test: Samples Stored for 1 Day

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	++++	+++	++	+	0	0	0
SM1	++++	++++	++++	+++	++	+	0	0	0
SM2	++++	++++	++++	+++	++	+	0	0	0
-20	++++	++++	++++	+++	+++	++	0	0	0

Table 18: Results of Luminol Test: Samples Stored for 5 Days

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+++	++	+	+	0	0	0
SM1	++++	++++	+++	++	+	+	0	0	0
SM2	++++	++++	+++	++	++	+	0	0	0
-20	++++	++++	++++	+++	+++	++	0	0	0

Table 19: Results of Luminol Test: Samples Stored for 1 Week

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+++	+++	++	+	0	0	0
SM1	++++	++++	+++	+++	++	++	0	0	0
SM2	++++	++++	+++	+++	+++	++	0	0	0
-20	++++	++++	+++	+++	+++	++	0	0	0

Table 20: Results of Luminol Test: Samples Stored for 2 Weeks

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++	+	+	0	0	0	0	0
SM1	++++	++++	++	++	0	0	0	0	0
SM2	++++	++++	++	++	0	0	0	0	0
-20	++++	++++	+++	+++	+++	++	0	0	0

Table 21: Results of Luminol Test: Samples Stored for 1 Month

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+	+	+	0	0	0	0
SM1	++++	++++	++	++	+	0	0	0	0
SM2	++++	++++	++	++	+	0	0	0	0
-20	++++	++++	+++	+++	++	+	0	0	0

Table 22: Results of Luminol Test: Samples Stored for 2 Months

	Neat	1:100	1:200	1:400	1:800	1:1,000	1:1,0000	1:1,00000	Neg
RT	++++	++++	+	0	0	0	0	0	0
SM1	++++	++++	+	0	0	0	0	0	0
SM2	++++	++++	+	0	0	0	0	0	0
-20	++++	++++	++++	++	+	+	0	0	0

Species of Origin Test

Based on the results obtained for the preceding presumptive blood tests, only the neat, 1:400, and 1:800 samples stored for one week, one month, and two months were tested using the SERATEC® HemDirect Hemoglobin Assay. The results of the test were either recorded as positive (appearance of a red line at the control region and the test region) or negative (appearance of a red line at the control region only). The results obtained were consistent over the two-month testing period (Table 23). All neat samples, regardless of storage condition, rendered a positive result. For the 1:400 diluted samples, only those stored at -20°C tested positive. None of the 1:800 diluted samples was positive. No false positives were identified.

Table 23: Results of the SERATEC® HemDirect Hemoglobin Assay

		Storage Time		
Storage Condition	Dilution	1 Week	1 Month	2 Months
RT	Neat	Positive	Positive	Positive
RT	1:400	Negative	Negative	Negative
RT	1:800	Negative	Negative	Negative
RT	- Control	Negative	Negative	Negative
SM1	Neat	Positive	Positive	Positive
SM1	1:400	Negative	Negative	Negative
SM1	1:800	Negative	Negative	Negative
SM1	- Control	Negative	Negative	Negative
SM2	Neat	Positive	Positive	Positive
SM2	1:400	Negative	Negative	Negative
SM2	1:800	Negative	Negative	Negative
SM2	- Control	Negative	Negative	Negative
-20	Neat	Positive	Positive	Positive
-20	1:400	Positive	Positive	Positive
-20	1:800	Negative	Negative	Negative
-20	- Control	Negative	Negative	Negative

II. SEMEN (N > 1000)

The objective of this study was to evaluate the effect of SampleMatrix™ on AP detection, PSA detection, and spermatozoa morphology tests. The effect of the storage conditions on the sensitivity to the AP test, the SERATEC® PSA SEMIQUANT assay, and the ability to find intact spermatozoa in a microscopic examination was evaluated. All of the tests utilized in the study required a visual observation by the researcher. The results were based on a color change reaction for the AP test, the development of a color band in the PSA test, and the location and observation of spermatozoa on a microscope slide. The reported results for each test were based on duplicate sampling and are representative of the results obtained across both trials. Separate negative controls were analyzed tested simultaneously for each dilution and storage period.

Prostatic Acid Phosphatase Results

The acid phosphatase test results were rated as intense (++++), bright (+++), soft (++), faint (+), or negative (-) based on the intensity of the color reaction obtained. The two-month samples protected with SM2 were mistakenly overlooked; therefore, no data was collected for that particular time. Generally, it appears that the samples protected with SampleMatrix™ exhibit an increased sensitivity to the acid phosphatase test. Overall, the room temperature and the frozen samples resulted in a greater number of negative reactions relative to the semen samples protected with SampleMatrix.™ Negative results for the AP reaction were as high as 39% for the frozen samples and 28% for the room temperature samples. In comparison, negative results for the AP reaction when testing samples protected with SM1 and SM2 were noted in 17% and 7% of the samples, respectively. No false positives were identified. Without taking into account dilution factor and the storage time, the SampleMatrix™ protected samples exhibited a higher sensitivity for the AP test because they produced more reactions that are positive. This can be seen by comparing the overall data for each storage condition in Figures 5-8 below:

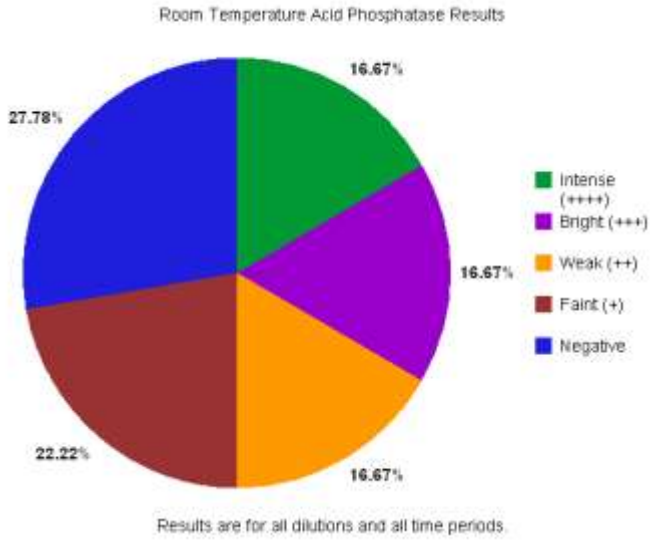


Fig. 5: Summary of Acid Phosphatase Test Results for all room temperature samples

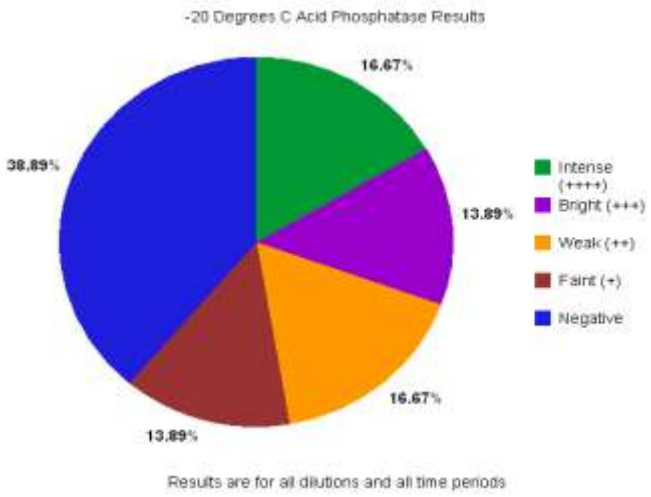


Fig. 6: Summary of Acid Phosphatase Test Results for all frozen samples

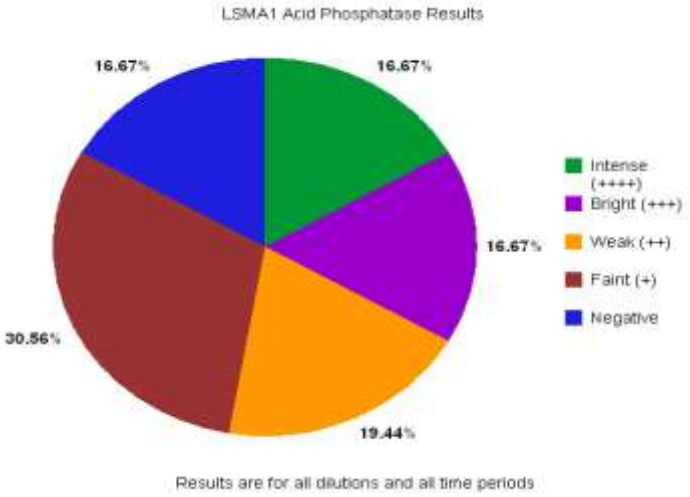


Fig. 7: Summary of Acid Phosphatase Test Results for all SM1 samples

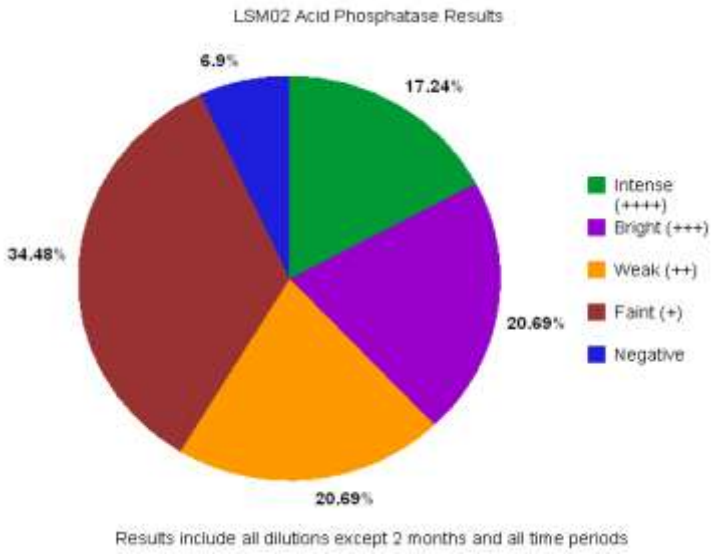


Fig. 8: Summary of Acid Phosphatase Test Results for all SM2 samples

The results obtained for the neat semen and 1:100 semen dilution were consistent for all samples regardless of storage method and for all times; each displayed the same intensity of color change. This can be seen in the Figures 9-10. Figure 9 also shows that after only one day of storage, the frozen samples were the first to exhibit a negative result.

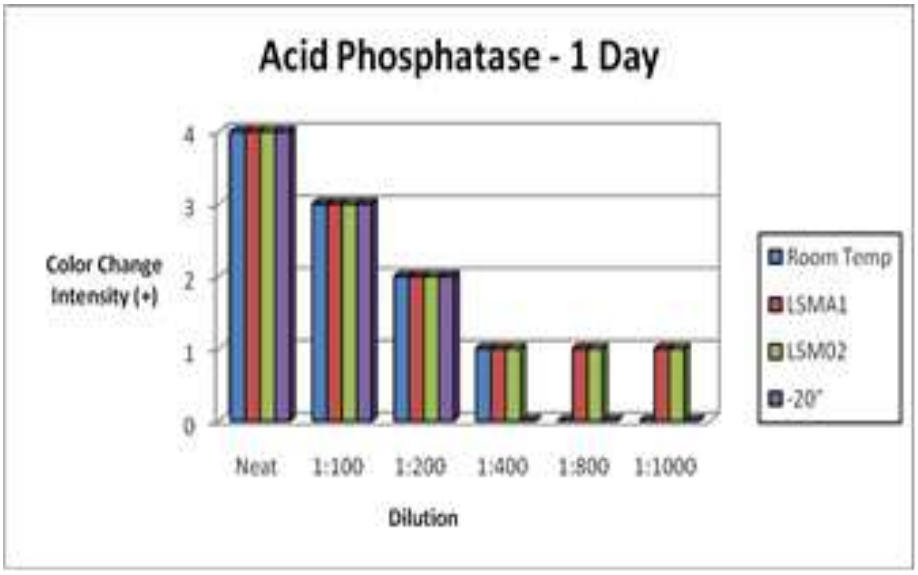


Fig. 9: Results of Acid Phosphatase Color Test: Samples Stored for 1 Day

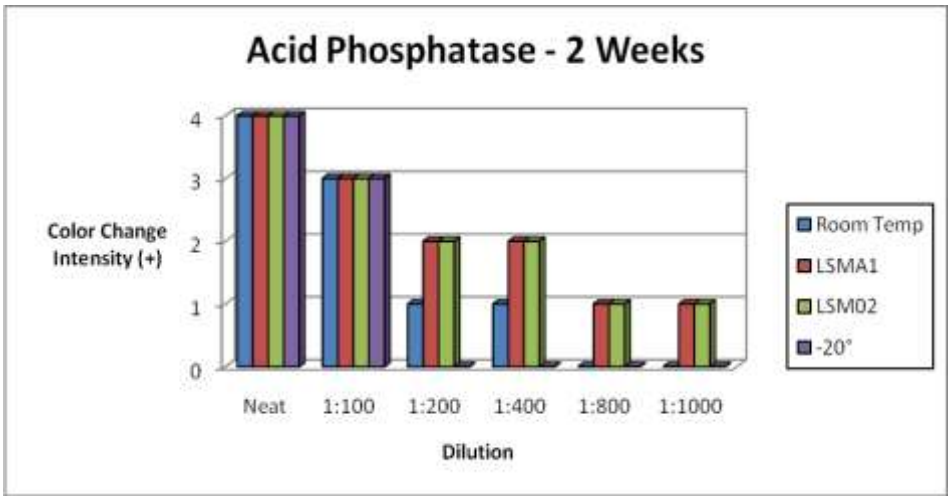


Fig. 10: Results of Acid Phosphatase Color Test: Samples Stored for 2 Weeks

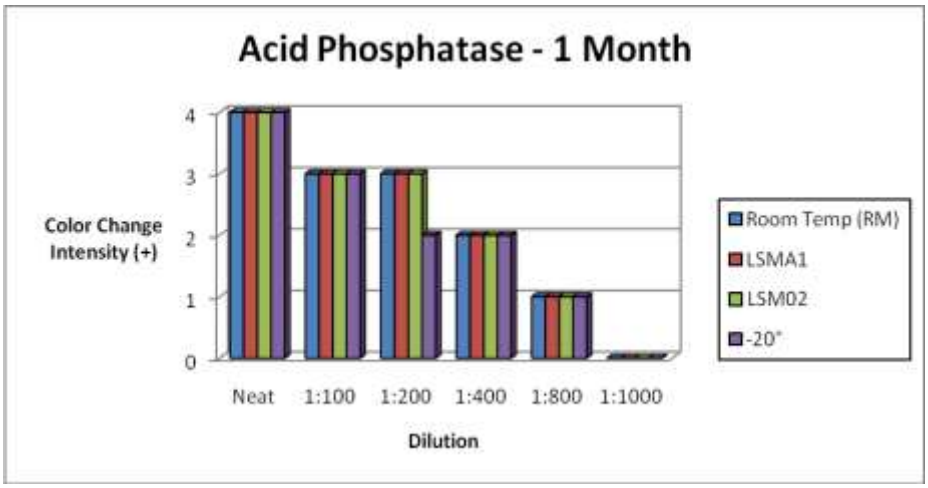


Fig. 11: Results of Acid Phosphatase Color Test: Samples Stored for 1 Month

At semen dilutions greater than 1:100, the results begin to vary depending on time and storage method. The lowest dilution to result in a negative reaction was a 1:200 semen dilution stored in frozen conditions for two weeks (Figure 11). Figure 11 also shows that the only samples that gave a positive AP reaction at the highest dilution of (1:1,000) were those protected by either SM1 or SM2. When evaluating the AP results for the longest storage period (one month) there is no apparent difference in the intensity of the color change observed. All of the semen samples yielded indistinguishable color intensity values for all of the dilutions tested. It should also be noted that for this time, none of the 1:1,000 semen dilutions gave a positive reaction. In terms of the storage method, the samples protected with SM1 or SM2 showed the most promising results in terms of the acid phosphatase test. The SM2 samples gave positive reactions up until the 1:1,000 semen dilutions and the SampleMatrix™ protected samples were the only ones that showed positive reactions at the 1:1,000 dilutions for the one-day and two-week samples. However, the one-month samples gave equivalent results for each storage method when controlling for dilution. In this same respect, the samples that were stored at room

temperature unprotected or frozen for one day and two weeks, exhibited the lowest color intensity in detecting acid phosphatase. The Acid Phosphatase study shows that the storage method apparently affects the results of the test. However, one must also consider the subjective nature of interpreting color reactions; the variation in sample preparation; and minor variations in reagent preparation that may affect the sensitivity of the test. These factors may account for the stronger results encountered for the samples stored for extended times. It may also explain the inconsistent results encountered with the more dilute samples. Further, ideally entire swabs should be tested rather than cuttings that were approximately equivalent in size as employed in the present study.

Prostate-Specific Antigen Results

The results were rated as T>q, t<Q, T=Q, or negative. The letter "T" represents the line in the test kit results window corresponding to the sample concentration of PSA. The letter "Q" represents the line in the results window corresponding to the internal quantitative standard of the test kit, which is equivalent to a PSA concentration of 4ng/mL. Therefore, the results indicate whether the sample concentration is approximately greater than, less than, or equivalent to the internal standard concentration. The absence of a visible test line in the result window was recorded as a negative reaction. In contrast to the AP results, the frozen samples demonstrated a higher sensitivity to the detection of PSA while the SampleMatrix^{TM} protected samples showed a reduced activity. Upon closer inspection, it appears that there may have been an error in preparing the two month frozen samples. The activity of the frozen samples steadily declined as the storage period increased. For the two-month results, the frozen samples unexpectedly gave a positive reaction for each dilution. This confounding result led to the exclusion of the two-month results for making the most accurate comparison among all the different variables. Although the two-month period was excluded from the study, the frozen semen samples consistently showed high PSA activity across all of the remaining time intervals. There was one instance where the SM2 protected samples gave a positive PSA result for the 1:1,000 semen dilution stored for one week; however, the frozen semen samples consistently gave positive reactions at the higher semen dilutions.

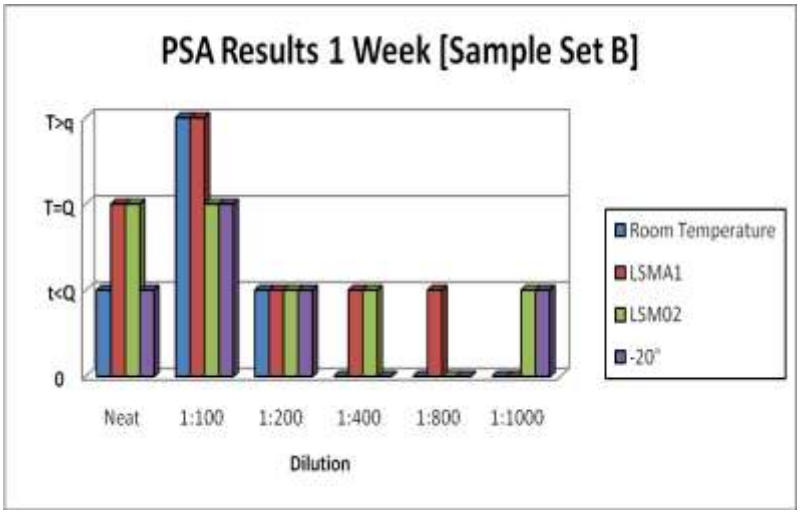


Fig. 12: PSA Results at 1 Week storage time for sample set B

The lowest dilution to produce a negative PSA result was a 1:200 semen dilution stored either at room temperature unprotected or protected with SM2 for one month. At the one month time, it is also evident that both the frozen and SM1 samples produce more positive results for the highest dilution at the longer storage times (Figures 13-14).

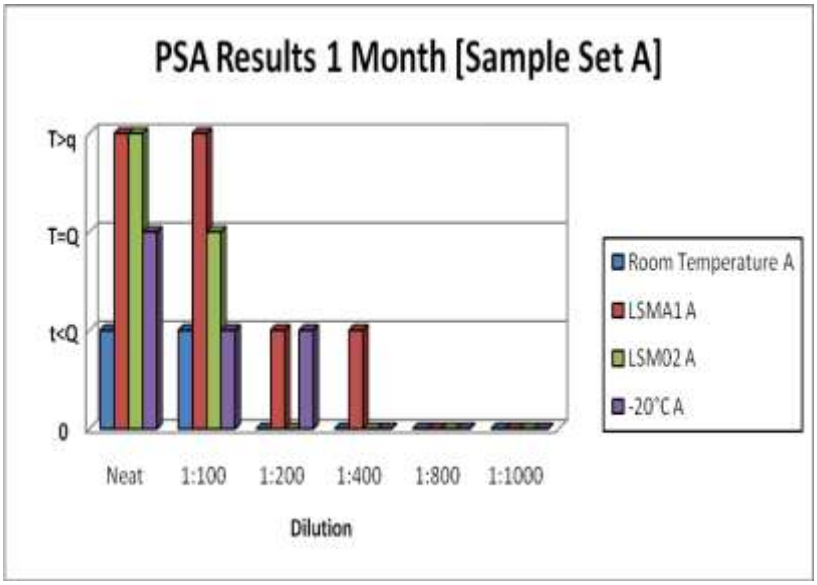


Fig. 13: PSA Results at 1 Month storage time for samples (set A)

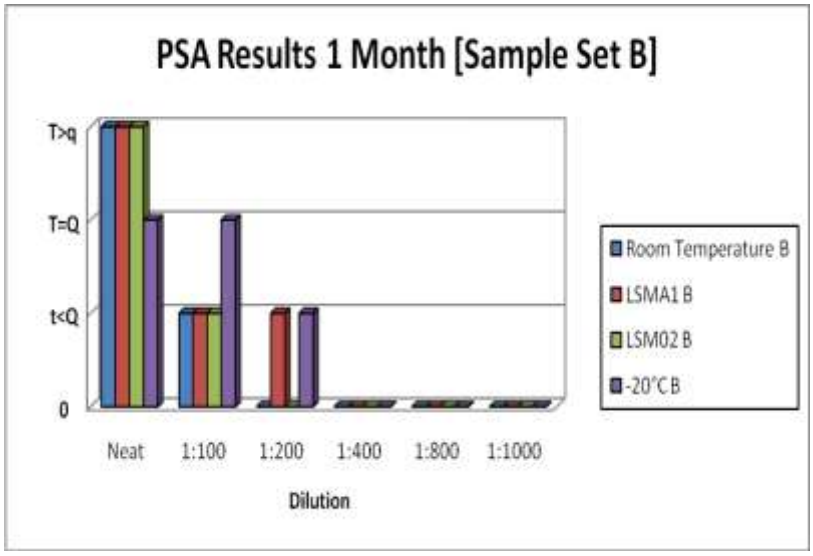


Fig. 14: PSA Results at 1 Month storage time for samples (set B)

Microscopic Staining and Evaluation Results

The microscopic evaluation results were scored using a rating system to quantify the intact spermatozoa present on the microscope slide. Only neat samples were viewed for the times one day, three days, one week, two weeks, and one month. The results were consistent across the four storage conditions: room temperature, SM1, SM2 and -20°C. The only apparent difference was that it was slightly easier to find spermatozoa for the SampleMatrix™ protected samples. This was because the spermatozoa appeared as a cluster of cells; therefore, they were easier to identify compared to isolated spermatozoa. It should be noted that these clusters were evident only in some of the SampleMatrix™ protected samples. The rating of the samples is illustrated in Table 24.

Storage Condition	One Day	Three Days	One Week	Two Weeks	One Month
Room Temperature	++++	++++	+++	+++	+
SM1	++++	++++	+++	+++	++
SM2	++++	++++	+++	+++	++
-20°C	++++	++++	+++	++++	+++

Table 24: Microscopic morphological examination of neat semen samples

III. SALIVA (N = 350)

The swabs with varying dilutions were stored on swabs in a hood to prevent contamination. The swabs were also separated according to time; the room temperature, SM1 and SM2 swabs were stored under a biological hood while the frozen swabs were stored in a freezer. The reported results for each test were based on duplicate sampling and are representative of the results obtained across both trials. Separate negative controls were analyzed tested simultaneously for each dilution and storage period.

Radial Diffusion/ α-Amylase Testing

The purpose of the amylase study was to determine if the level of amylase activity remained constant or declined as a function of time when comparing various storage conditions. The results were achieved by measuring the diameter (mm) of each diffusion ring after a one-day incubation using a Combo Circle Template. Negative controls were performed with all times to ensure there were no false positive reactions. Most negative control samples did test negative but exceptions were observed with the four-week samples protected with SM2 and SM1, the two-month samples protected with SM2 and the immediate samples protected with SM1. The reported results for each test were based on triplicate sampling and are representative of the mean result obtained across all three trials. The data are summarized in Fig. 15-18.

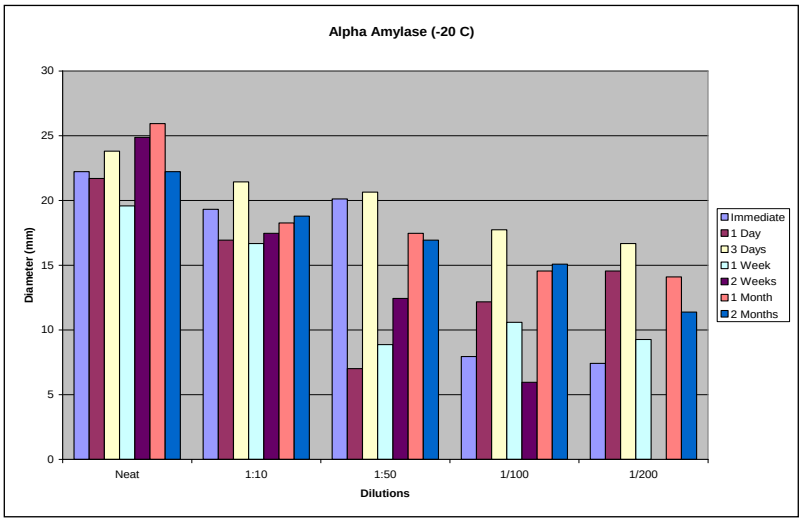


Fig. 15: *Alpha Amylase Radial Diffusion Test Results-Frozen Storage Conditions*

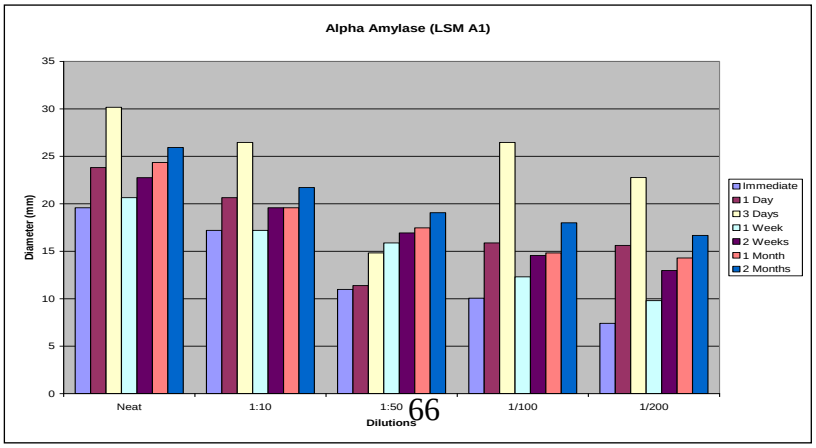


Fig. 16: *Alpha Amylase Radial Diffusion Test Results (SM1)*

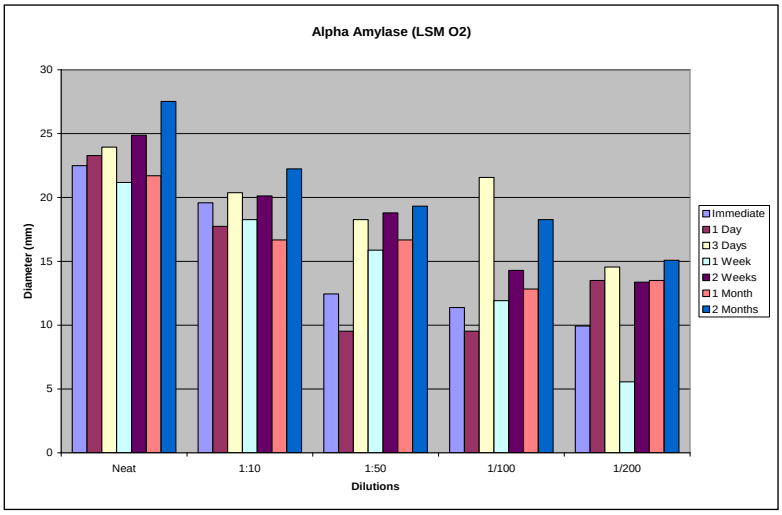


Fig. 17: *Alpha Amylase Radial Diffusion Test Results (SM2)*

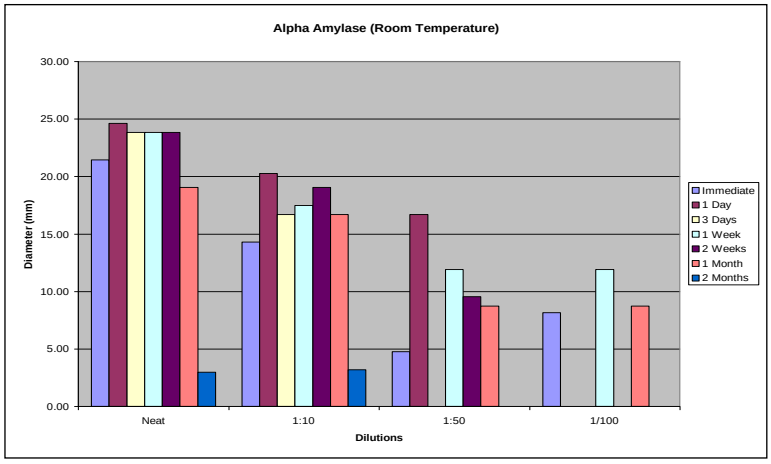


Fig. 18: *Alpha Amylase Radial Diffusion Test Results-Room Temperature Storage Frozen (-20ºC)*

Diameter readings were higher with the neat samples and declined in size with sample dilution. However, there were exceptions in this trend. In the immediate samples, the 1:50 dilution gave a stronger reading than the 1:10 dilution but had a lower reading compared to the 1:200 dilution for the three-day samples. The one-week sample also showed this trend but, as expected, the neat gave the largest diameter and 1:200 dilution corresponded to the smallest diameter in the one month and two month samples. For two week samples, no visible diffusion rings were observed in two out of three samples tested for in the 1:100 dilution and no visible diffusion was evident for all three analyses of the 1:200 dilutions. The neat and 1:10 dilutions exhibited the highest overall values across all of the study variables with no obvious variation in

diameter size. The 1:50 and 1:100 dilutions showed some variation with the 1:200 dilution demonstrating no visible rings for particular times. The results indicate that the three-day samples correspond to the highest average diameter. In terms of sample dilution, the neat samples invariably correspond to the largest diameter reading.

SM1

Dilutions for this storage condition showed the expected trend with the more concentrated samples having larger diameters while the more diluted samples showed smaller diameter readings. With the exception of 1:50 dilution, all samples showed a gradual reduction in diameter as the sample dilution increased. Throughout the course of the study, there was no significant variation in diameter reading for specific dilutions. The three-day 1:100 and 1:200 dilutions gave similar or almost equivalent values to the neat and 1:10 samples. The three day samples showed the highest amylase activity overall with the exception of the 1:50, which gave the lowest diameter value of all five dilutions. For each dilution, the immediate samples gave the weakest reading of all seven-time periods. Considering the average diameter value for each time, three day gave the highest value while, as expected, the neat sample gave the highest diameter values.

SM2

As expected, there was a positive correlation trend, with the more concentrated samples resulting in larger diameter values. The three-day samples were the only exception to this trend in that a larger diameter reading was recorded for the 1:100 dilution compared with the 1:50 dilution. Across all times, the two-month samples gave the largest diameter reading followed closely by the three-day samples. As expected the neat extract produced the highest diameter values.

Room Temperature

Room temperature samples also exhibited the same trend, with the more concentrated samples resulting in larger diameter values in comparison to the more dilute samples. Across each time, there is a visible decline in diameter values. Typically, the room temperature control samples were the most likely, of all storage conditions, to exhibit no reaction. Swabs stored for one day showed the largest average diameter size, while, in terms of dilution, the 1:200 swabs were negative for all the periods tested (immediate to two-month samples).

Comparing storage conditions, saliva swabs protected with SM2 gave the highest diameter readings across all dilutions and times, followed by the saliva swabs protected with SM1. The unprotected room temperature samples gave a higher number of negative results for the radial diffusion test during the course of the study; only the 1:200 dilution samples were positive in three of the seven times tested. This is in contrast to the saliva swabs protected with SM2 and SM1 where all times and all dilutions gave positive results.

SALIgAE® Testing

The purpose of this test was to detect saliva over a ten-minute period by visualizing a color change in the reagents provided in the SALIgAE® kits. The results were achieved by incubating a swab cutting for 30 minutes, per the manufacturer's recommendations. The samples were then centrifuged for one minute and a 50µL extract was pipetted in the vial containing the SALIgAE® reagent. Any color change was noted immediately after the extract was pipetted into

the vial, after two minutes (if any change was visible), and a final observation was made after ten minutes. Negative controls were performed in parallel with each time to ensure that there were no false positive reactions. The results are summarized in Figures 19-22. The color results were rated subjectively as having no color change, very weakly visible, weakly visible, visible and strong color change. The reported results for each test were based on duplicate sampling and are representative of the results obtained across both trials.

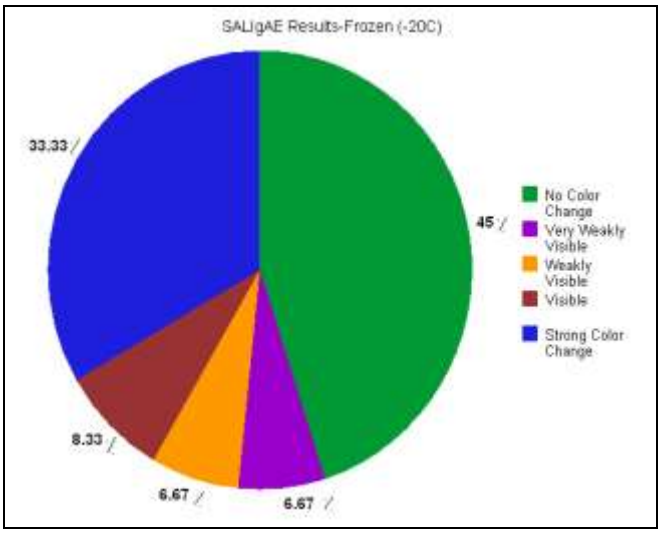


Fig. 19: *SALIgAE® Test Results-Frozen Storage Conditions*

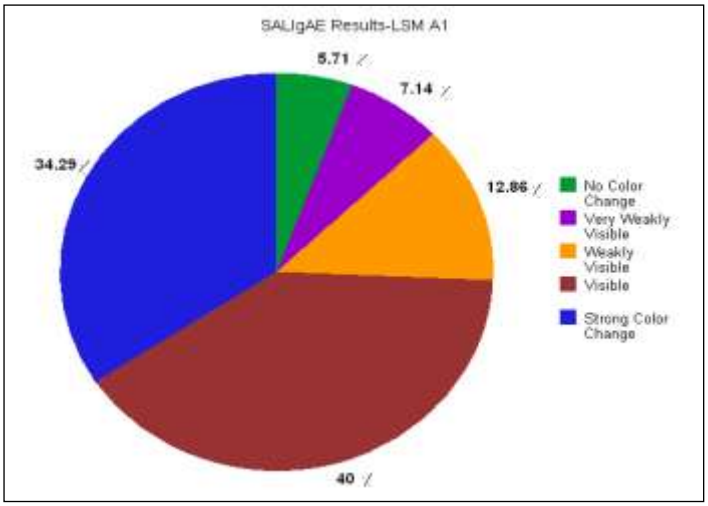


Fig. 20: *SALIgAE® Test Results SM1*

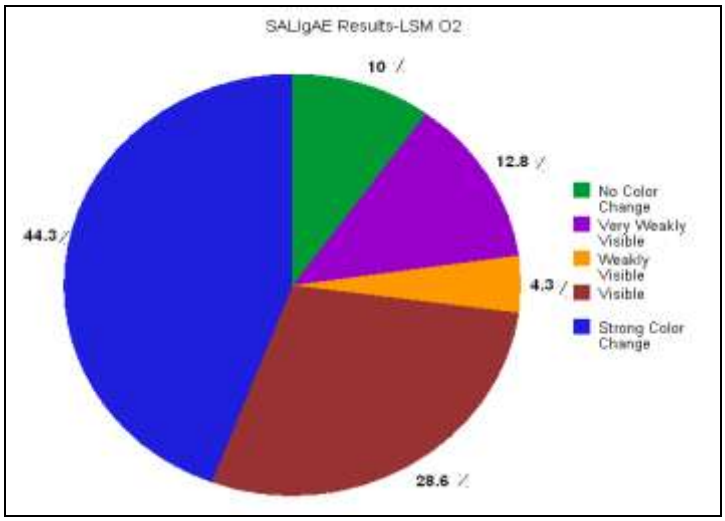


Fig. 21: *SALIgAE® Test Results SM2*

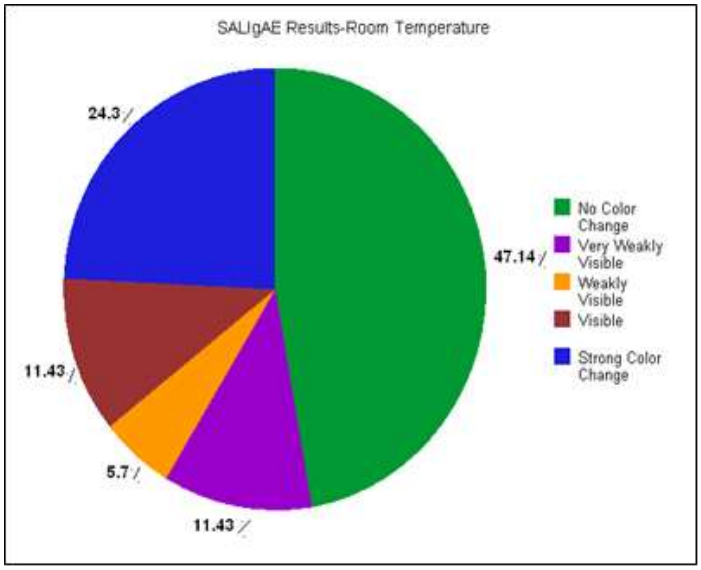


Fig. 22: *SALIgAE® Test Results-Room Temperature Storage*

45% of the frozen samples gave no visible color change; 33% gave a strong color change; 8% were very weakly visible and 7% were weakly visible, while an additional 7% resulted in a visible color change. A majority of the samples protected with SM1 gave a visible or strong color change (94.3%). However, a strong color change was present in 34% of the samples, while no color change was present in the smallest proportion (5%) followed by very weakly visible (7%) and weakly visible (6%) results. A majority of the samples protected with SM2 (44.3%) had a strong color change with the least abundant samples being weakly visible (4.3%). Visible color changes were visible in 28.6% samples, very weakly visible and no color change samples were present in 12.8% and 10% of the samples, respectively. Ambient storage showed the greater number of samples (55%) that exhibited no color change, while 24% of the samples exhibited a strong color change. Very weakly visible and visible reactions were obtained in equal amounts (11%), while weakly visible reactions were observed for 6% of the samples.

With respect to time, the swabs stored for two weeks gave the highest percentage of samples falling within the "strong color change" (45%), followed closely behind by immediate and four week samples, with 40% of the samples falling into the four week category. Samples stored for two months had the highest percentage (37%) fall within the "no color change" category. This was followed by the one week and three day samples, where approximately 31% and 32.5% of the samples fall into this same category, respectively. Saliva samples stored for one day and four weeks both had the lowest percentage (17.5%) fall under the "no color change" category. Considering the dilution factor, it is clear to see that the immediate samples gave the best results, with a majority (98.15%) of the samples showing a "strong color change." In sharp contrast, only a minority of corresponding samples stored for two months (3.77%) gave a "strong color change" while the majority (58.49%) exhibited "no color change". The 1:10 dilution samples gave positive results at all dilutions. Overall, 50% of the saliva samples resulted in a color development to some degree.

Microscopic Evaluation

Epithelial Morphology

The purpose of viewing the epithelial cells microscopically was to evaluate the effectiveness Sample Matrix™ had in terms of protecting the cellular components of saliva. All of the swabs were extracted and prepared as a smear for microscopic observation in order to evaluate the cell integrity over time and for each dilution. The reported results for each test were based on duplicate sampling and are representative of the mean obtained across both trials. The data are summarized in Figures 23-26 and take into account all dilutions for each storage condition. Frozen samples gave the best results in terms of epithelial cell morphology. 69% of the cells remained "intact" throughout the course of the study; this data takes into account all dilutions. For samples stored at room temperature with SM1, 55% were "lysed" and 45% of the cells "intact." In comparison, for the SM2 protected samples 65% exhibited "lysed" cells, with 34% "intact." Finally, the unprotected room temperature samples exhibited the greatest percentage of cell lysis (80%) while only 20% of the samples were "intact."

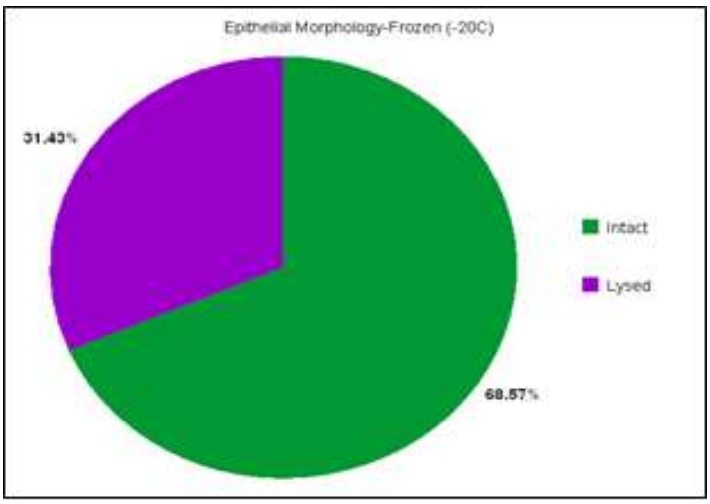


Fig. 23: *Epithelial Morphology Results-Frozen Storage Conditions*

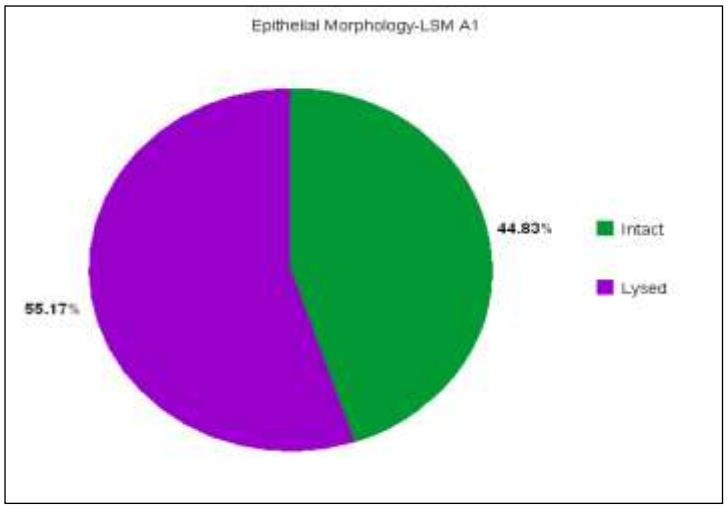


Fig. 24: *Epithelial Morphology Results SM1*

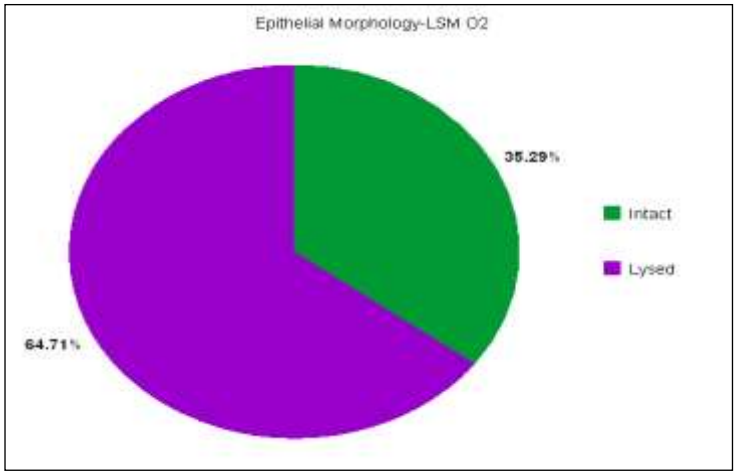


Fig. 25: *Epithelial Morphology Test Results SM2*

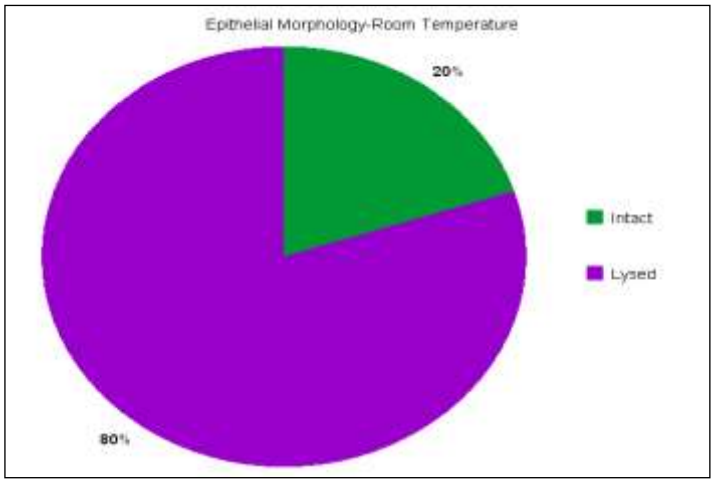


Fig. 26: *Epithelial Morphology Results-Room Temperature Storage*

Epithelial Cell Concentration

In addition to evaluating the integrity of the epithelial cells, the slides were observed microscopically to determine cell abundance over a given period. Although there was a correlation in that the number of cells declined with increased storage times, the results summarized in Figures 27-30 show the relative abundance of cells (not exact counts) that were present over a period of two months at all dilutions for each storage condition.

Almost 60% of the frozen samples collectively exhibited either “abundant” (11.4%), “few” (42.9%) or “abundant/few” (2.9%) cells while 17.1% and 25.7% exhibited “no cells” or “few/no cells,” respectively. The samples protected with SM1 and stored at room temperature gave almost equivalent results across all of the following three categories: “abundant” (20%), “few” (28.6%), “no cells” (31.4%). In comparison, the samples protected with SM2 and at room temperature showed similar patterns as the samples stored with SM1 in that a large percentage fell into the “no cell” category (28.5%). SM2 exhibited fewer cells in the “abundant” category (14.3%), while only a few fell into the “abundant/few” category (2.9%). The unprotected room temperature samples gave the least promising results, with almost half the samples (45.8%) falling into the “no cells” visible category. This was expected considering these samples were not protected during the course of the study.

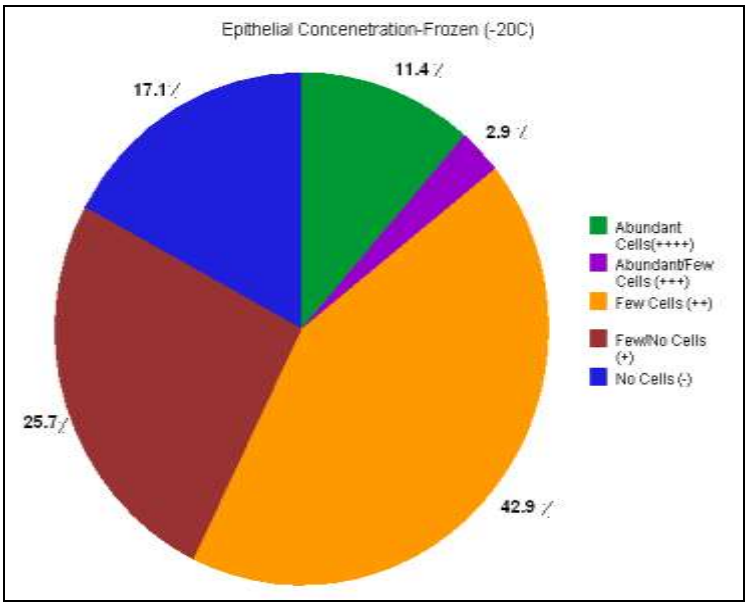


Fig. 27: *Epithelial Concentration Results-Frozen Storage Conditions*

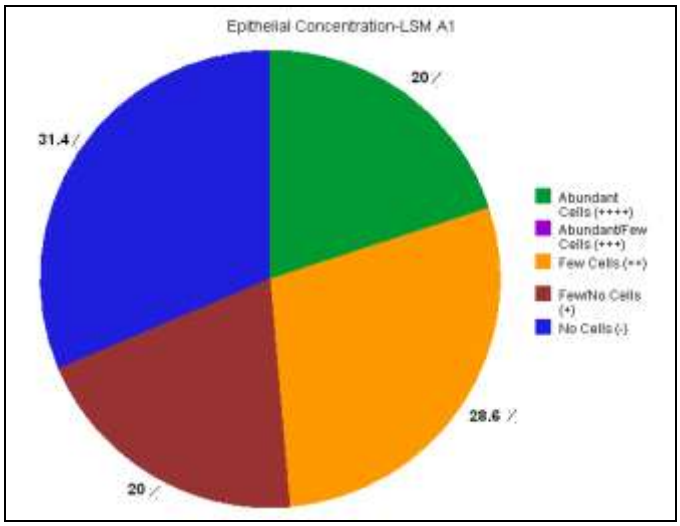


Fig. 28: *Epithelial Concentration Results SM1*

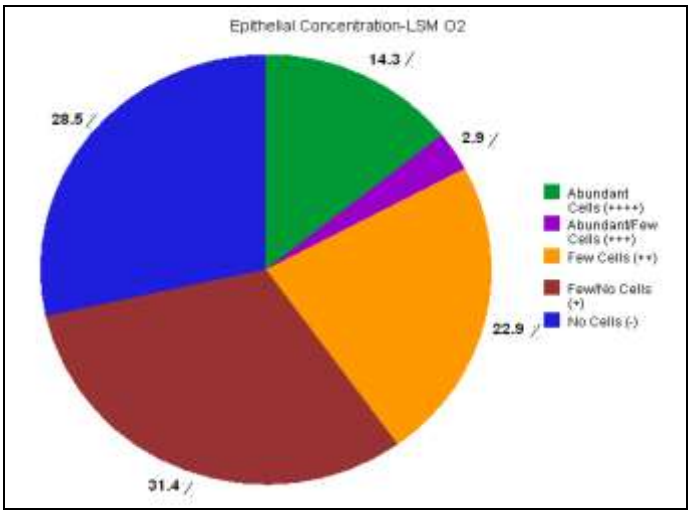


Fig. 29: *Epithelial Concentration Test Results SM2*

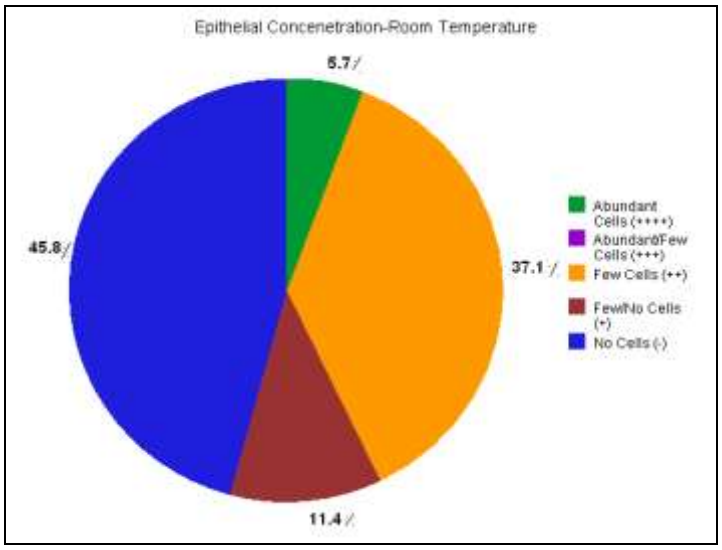


Fig. 30: *Epithelial Concentration Results-Room Temperature Storage*

CHAPTER 4: RESULTS OF WETTING AGENT STUDIES

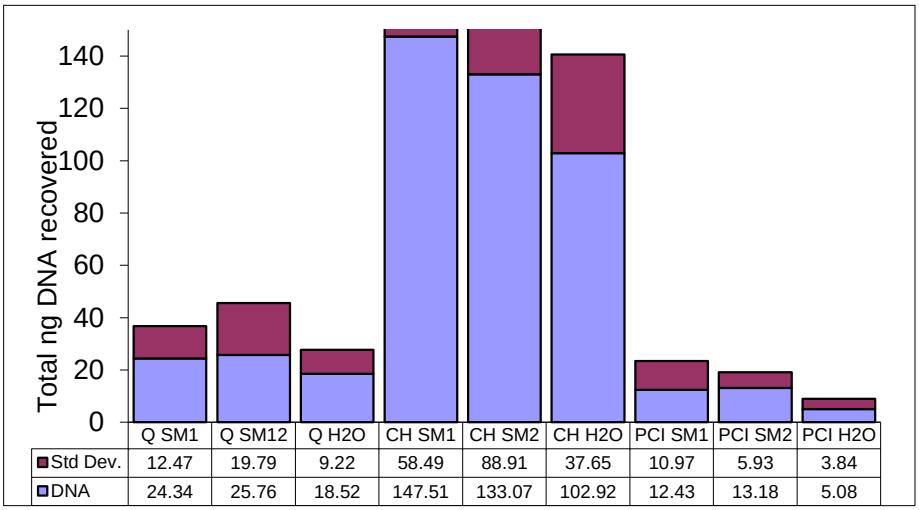
I. SIX MONTH STUDIES

The objective of these studies was to evaluate the total mean recovery of DNA from stains (blood, saliva, or semen) that were swabbed from different substrates and subsequently stored for six-month period. The independent variables include extraction chemistry, stain dilution factor, and the two SampleMatrix™ formulations (SM1 and SM2) that were compared to the conventional approach of using water as a wetting agent. The reported results for each biological fluid is based on duplicate sampling and are representative of the mean obtained across both trials. Separate negative controls were analyzed tested simultaneously for each dilution and storage period.

A. BLOOD (N = 450)

Extraction Method Compatibility

A comparison was made of the total mean DNA (ng) recovered from three wetting agents (SM1, SM2 & H_2O) with respect to three extractions methods (Figure 31). All three extraction mean methods had a higher total DNA recovery with SM1 and SM2 when compared to H_2O; however, this difference in the mean recovery is not statistically significant (ANOVA and t-test analysis). Samples recovered using the SM1 wetting agent and extracted with Chelex show a 43% higher DNA recovery when compared to H_2O as the wetting agent.



	Q SM1	Q SM12	Q H2O	CH SM1	CH SM2	CH H2O	PCI SM1	PCI SM2	PCI H2O
Std Dev.	12.47	19.79	9.22	58.49	88.91	37.65	10.97	5.93	3.84
DNA	24.34	25.76	18.52	147.51	133.07	102.92	12.43	13.18	5.08

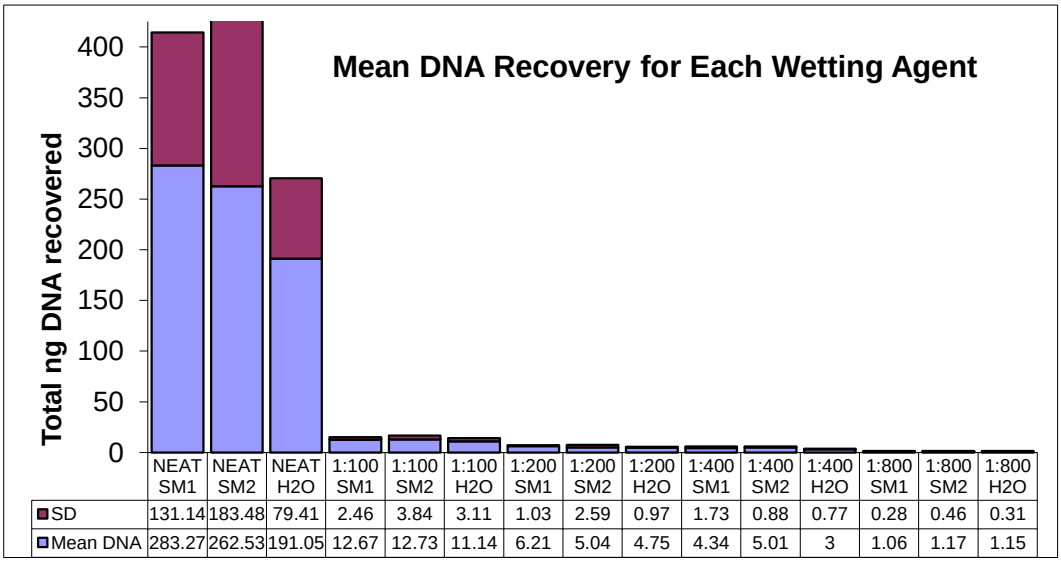
Fig. 31: Total Mean DNA Recovery Comparison of Three Wetting Agents to the Three Extraction Methods: Qiagen, Chelex, and Organic

The Recovery of DNA Based on Blood Dilution

Figure 53 shows an overall comparison of the total DNA recovered by each wetting agent to five serial blood dilutions. The SampleMatrix™ wetting agents gave a higher total DNA (ng)

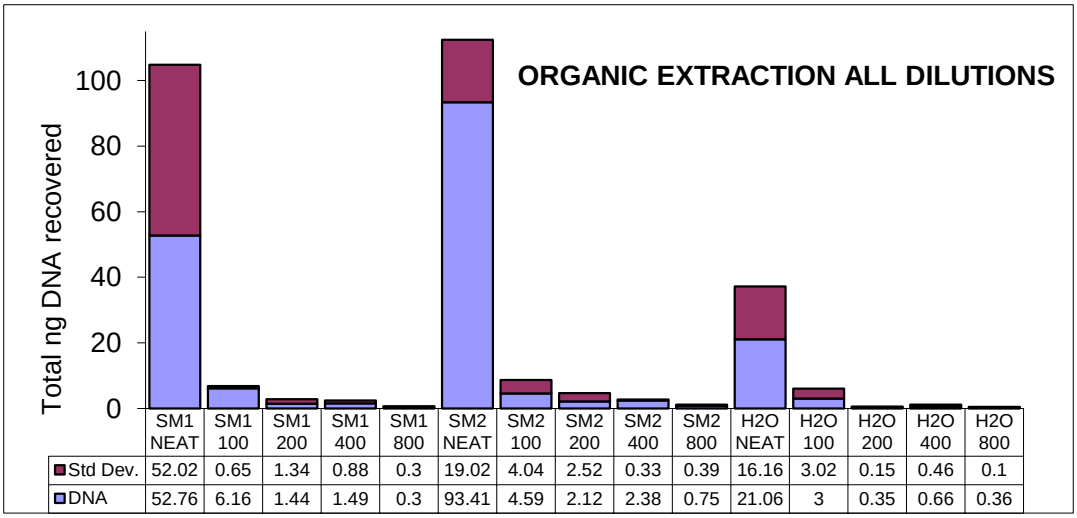
recovery compared to the H_2O wetting agent. SM2 ranked slightly higher than SM1 (> 1ng) at three dilutions (1:100, 1:400, and 1:800). The total mean DNA recovered from all five dilutions was higher with SM1, followed by SM2, with H_2O as the wetting agent ranking third. SM2 gave the largest total average standard deviation while SM1 has the lowest standard deviation at three dilutions (1:100, 1:200, and 1:800).

Figures 32-35 compare each of the wetting agents to the five serial blood dilutions for each extraction method. SampleMatrix™ formulations SM1 and SM2 show a greater mean DNA recovery with four of the five dilutions, the only exception is the 1:800 dilution for Qiagen (SM1= 0.45 ng and H_2O = 0.46 ng). For the 1:800 dilutions, SM2 ranked highest in DNA recovery with the organic and chelex extraction methods. Tables 5-10 show higher DNA recovery from all SampleMatrix™ wetting agents when compared to water. Additionally, a ratio comparison of SM1/H_2O, SM2/H_2O, and SM1/SM2 demonstrates the effectiveness in the mean recovery of DNA at each serial blood dilution for each extraction method. The ratios show higher DNA recovery with SampleMatrix™ as compared with water. While SampleMatrix™ shows a higher mean DNA recovery for most dilutions when compared to water, this difference in the mean recovery is not statistically significant (ANOVA and t-test analysis).



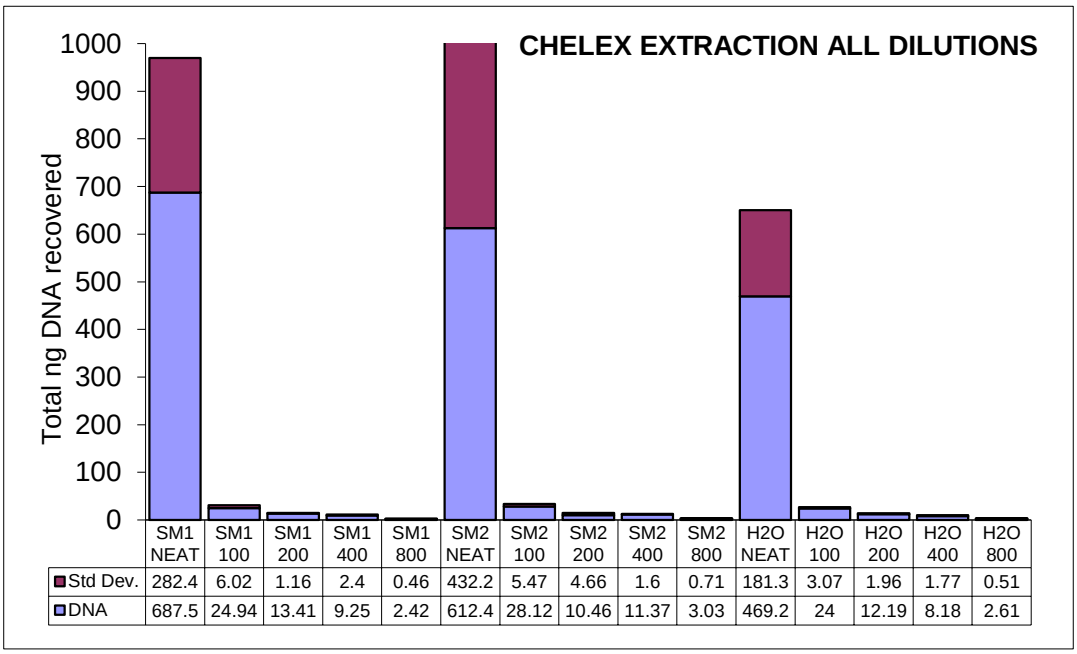
	NEAT SM1	NEAT SM2	NEAT H2O	1:100 SM1	1:100 SM2	1:100 H2O	1:200 SM1	1:200 SM2	1:200 H2O	1:400 SM1	1:400 SM2	1:400 H2O	1:800 SM1	1:800 SM2	1:800 H2O
SD	131.14	183.48	79.41	2.46	3.84	3.11	1.03	2.59	0.97	1.73	0.88	0.77	0.28	0.46	0.31
Mean DNA	283.27	262.53	191.05	12.67	12.73	11.14	6.21	5.04	4.75	4.34	5.01	3	1.06	1.17	1.15

Fig.32: Overall Total DNA (ng) Recovery for Three Wetting Agents Based on Blood Dilution



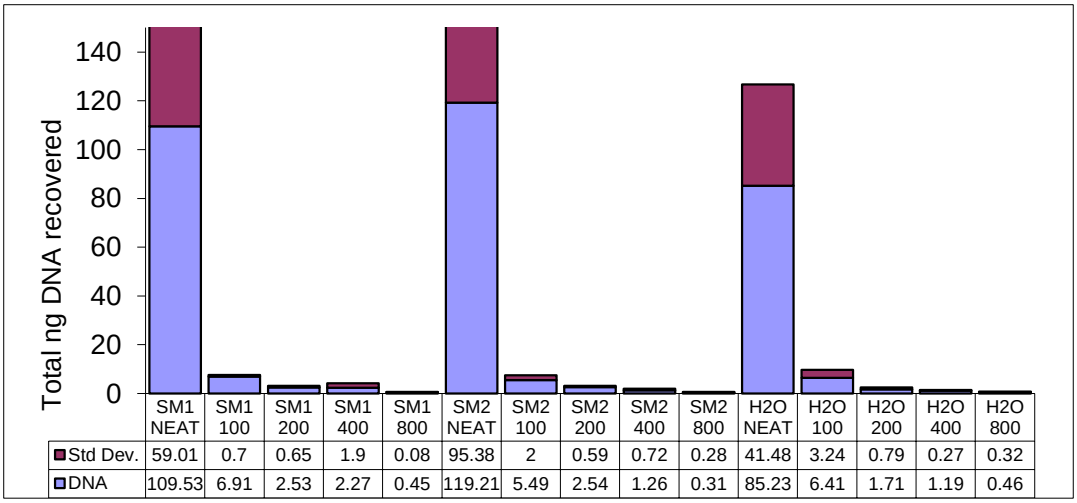
	SM1 NEAT	SM1 100	SM1 200	SM1 400	SM1 800	SM2 NEAT	SM2 100	SM2 200	SM2 400	SM2 800	H2O NEAT	H2O 100	H2O 200	H2O 400	H2O 800
Std Dev.	52.02	0.65	1.34	0.88	0.3	19.02	4.04	2.52	0.33	0.39	16.16	3.02	0.15	0.46	0.1
DNA	52.76	6.16	1.44	1.49	0.3	93.41	4.59	2.12	2.38	0.75	21.06	3	0.35	0.66	0.36

Fig. 33: Total DNA Recovery by Blood Dilution for Organic Extraction Method



	SM1 NEAT	SM1 100	SM1 200	SM1 400	SM1 800	SM2 NEAT	SM2 100	SM2 200	SM2 400	SM2 800	H2O NEAT	H2O 100	H2O 200	H2O 400	H2O 800
Std Dev.	282.4	6.02	1.16	2.4	0.46	432.2	5.47	4.66	1.6	0.71	181.3	3.07	1.96	1.77	0.51
DNA	687.5	24.94	13.41	9.25	2.42	612.4	28.12	10.46	11.37	3.03	469.2	24	12.19	8.18	2.61

Fig. 34: Total Mean DNA Recovery of Each Wetting Agent by Blood Dilution for Chelex Extraction



	SM1 NEAT	SM1 100	SM1 200	SM1 400	SM1 800	SM2 NEAT	SM2 100	SM2 200	SM2 400	SM2 800	H2O NEAT	H2O 100	H2O 200	H2O 400	H2O 800
Std Dev.	59.01	0.7	0.65	1.9	0.08	95.38	2	0.59	0.72	0.28	41.48	3.24	0.79	0.27	0.32
DNA	109.53	6.91	2.53	2.27	0.45	119.21	5.49	2.54	1.26	0.31	85.23	6.41	1.71	1.19	0.46

Fig. 35: Total Mean DNA Recovery for Each Wetting Agent by Blood Dilution for Qiagen Extraction Method

The Recovery of DNA from Five Different Substrates

Figure 36 shows an overall comparison by total DNA (ng) recovery. SampleMatrix™ formulations show higher DNA recovery for each substrate in comparison to water. Figures 37-38 illustrate a comparison by total DNA (ng) recovery of all three wetting agents to five different substrates for each extraction method. An interesting observation is that chelex extraction method gave the highest DNA recovery at all five substrates with all three wetting agents, SM1 ranking the highest in total DNA recovery (Figure 39). As predicted, the glass substrate gave the highest DNA recovery with all three wetting agents across all three-extraction methods. Cement gave the lowest DNA recovery with all three wetting agents for Qiagen and Organic extraction methods. Overall, both SampleMatrix™ formulations gave the highest DNA recovery for three of the five substrates across all of the three extraction methods. This difference in the mean recovery is not statistically significant (ANOVA and t-test analysis).

Figures 40-42 compare all three wetting agents for each dilution with each substrate when employing the chelex extraction method. Neat blood exhibited very high recovery with all three wetting agents, SM1 ranked higher for three substrates (cement, cotton and wood), followed by SM2 ranking first at two substrates (carpet and glass) and water ranking the lowest. The differences observed in the mean recovery are not statistically significant (ANOVA and t-test analysis).

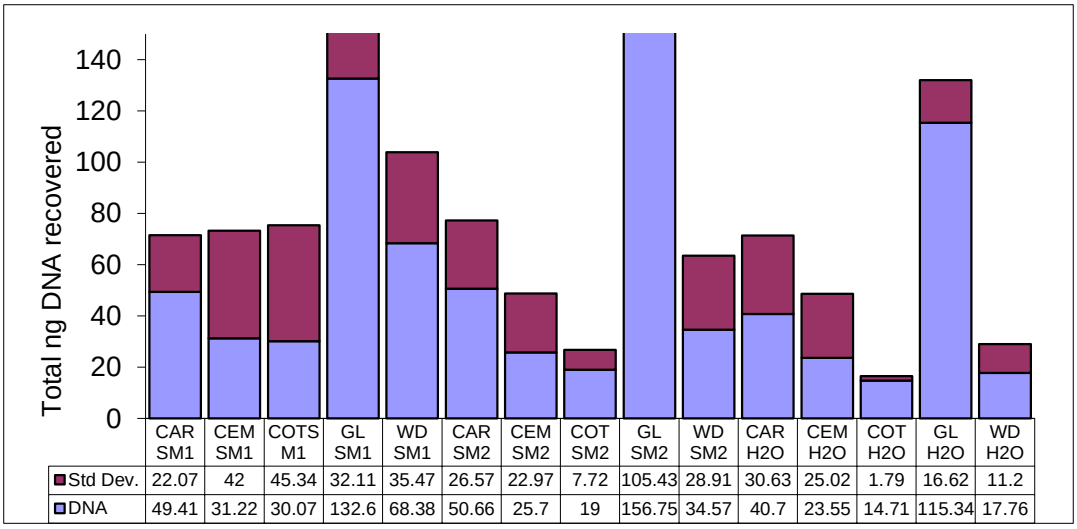


Fig. 36: Total DNA (ng) Recovery of Each Wetting Agent Based on Substrate

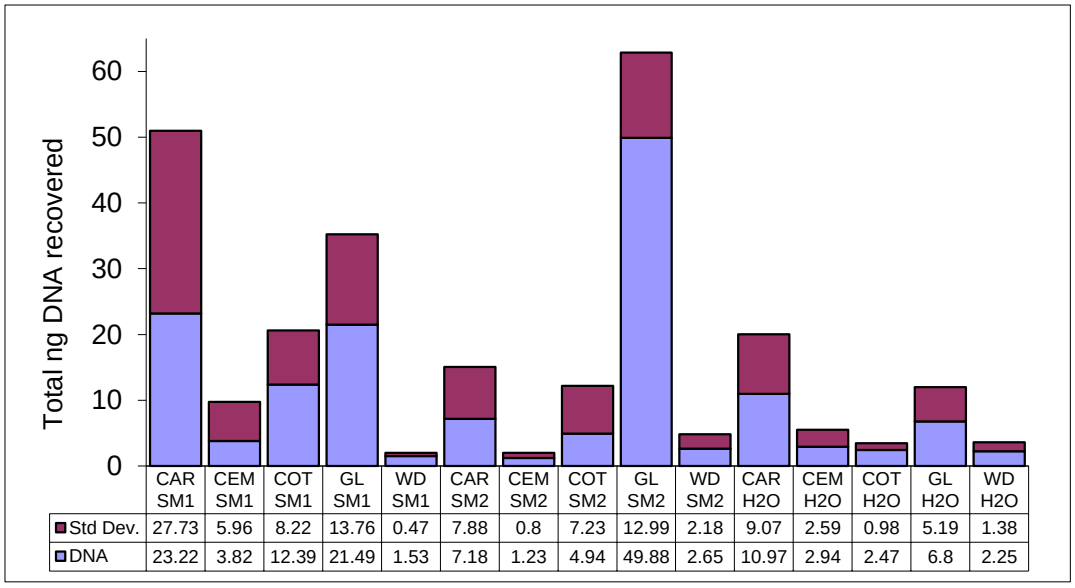


Fig. 37: Total Mean DNA (ng) Recovery of Each Wetting Agent for Different Substrates Using Organic Extraction Method

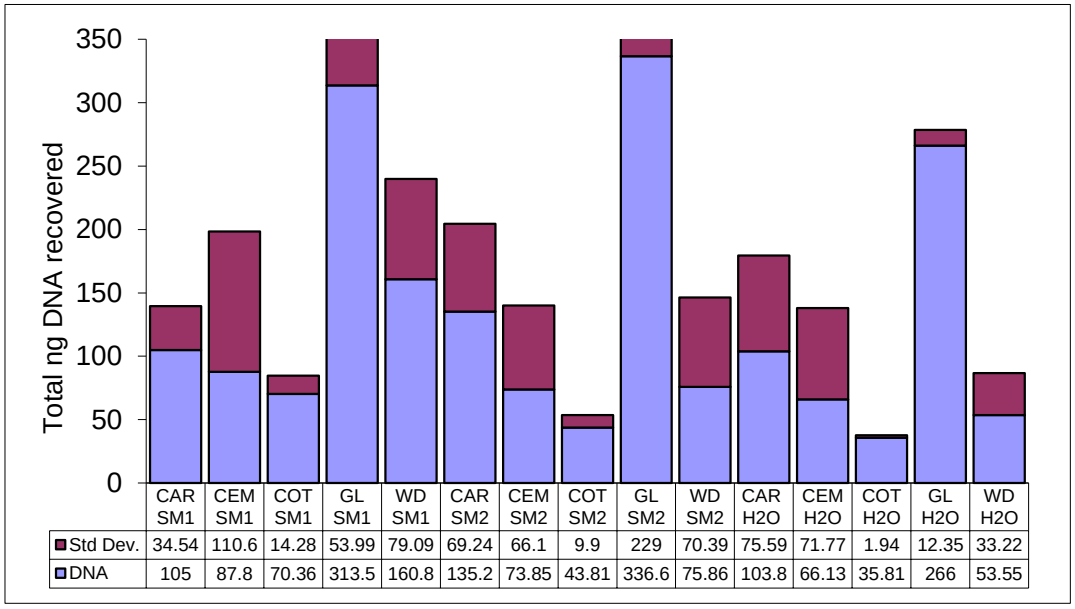


Fig. 38: Total Mean DNA (ng) Recovery of Each Wetting Agent for Different Substrates Using Chelex Extraction Method

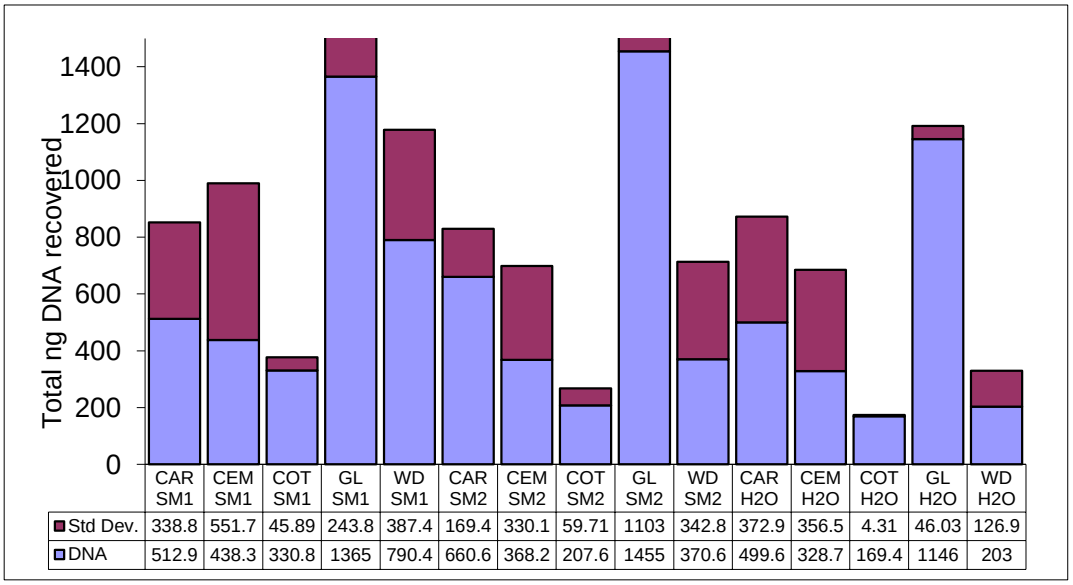
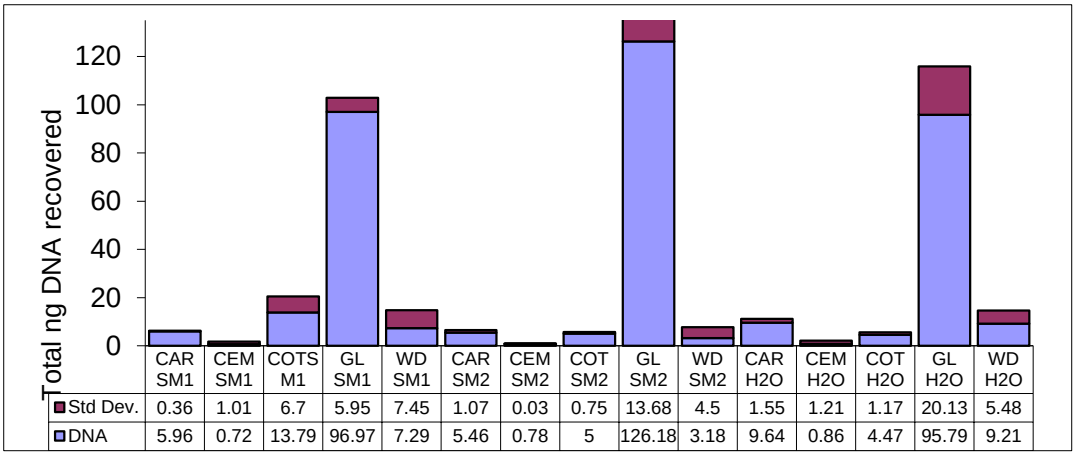
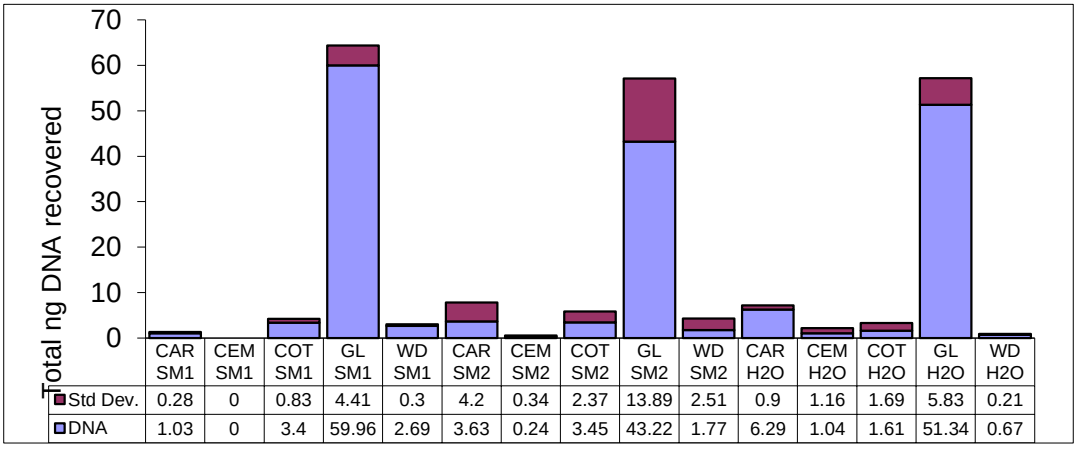


Fig. 39: Comparison of Wetting Agents by Total DNA Recovery for Neat Blood



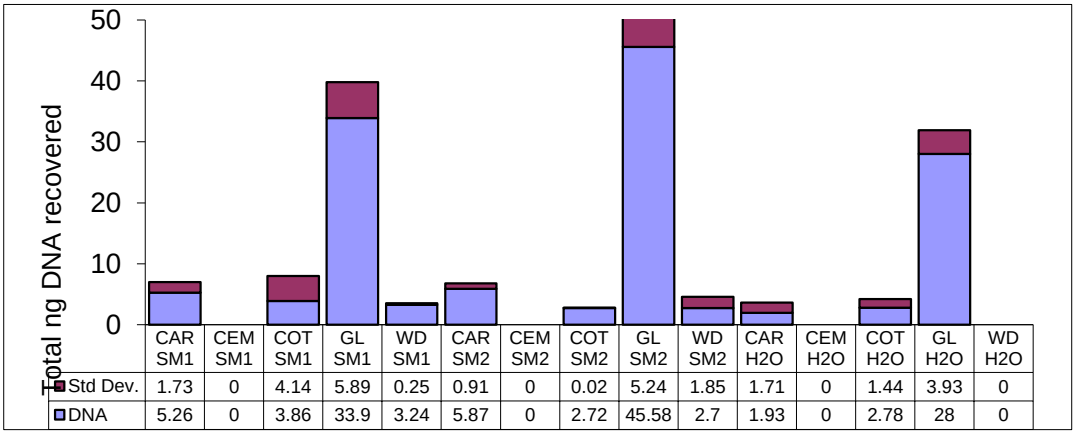
	CAR SM1	CEM SM1	COTS M1	GL SM1	WD SM1	CAR SM2	CEM SM2	COT SM2	GL SM2	WD SM2	CAR H2O	CEM H2O	COT H2O	GL H2O	WD H2O
Std Dev.	0.36	1.01	6.7	5.95	7.45	1.07	0.03	0.75	13.68	4.5	1.55	1.21	1.17	20.13	5.48
DNA	5.96	0.72	13.79	96.97	7.29	5.46	0.78	5	126.18	3.18	9.64	0.86	4.47	95.79	9.21

Fig. 40: Comparison of Wetting Agents by Total DNA Recovery for 1:100 Blood



	CAR SM1	CEM SM1	COT SM1	GL SM1	WD SM1	CAR SM2	CEM SM2	COT SM2	GL SM2	WD SM2	CAR H2O	CEM H2O	COT H2O	GL H2O	WD H2O
Std Dev.	0.28	0	0.83	4.41	0.3	4.2	0.34	2.37	13.89	2.51	0.9	1.16	1.69	5.83	0.21
DNA	1.03	0	3.4	59.96	2.69	3.63	0.24	3.45	43.22	1.77	6.29	1.04	1.61	51.34	0.67

Fig. 41: Comparison of Wetting Agents by Total DNA Recovery for 1:200 Blood



	CAR SM1	CEM SM1	COT SM1	GL SM1	WD SM1	CAR SM2	CEM SM2	COT SM2	GL SM2	WD SM2	CAR H2O	CEM H2O	COT H2O	GL H2O	WD H2O
Std Dev.	1.73	0	4.14	5.89	0.25	0.91	0	0.02	5.24	1.85	1.71	0	1.44	3.93	0
DNA	5.26	0	3.86	33.9	3.24	5.87	0	2.72	45.58	2.7	1.93	0	2.78	28	0

Fig. 42: Comparison of Wetting Agents by Total DNA Recovery for 1:400 Blood

Comparison of SampleMatrix™ Formulations

A ratio was calculated of DNA recovery for each SampleMatrix™ formulation relate to total mean DNA for each extraction method (Figure 43). SM1 and SM2 show very similar values at each extraction method, with SM2 scoring slightly higher than SM1 for Qiagen and Organic extraction, with a difference of only 0.03ng and 0.02ng, respectively. Both formulations yielded high DNA recovery with chelex, but SM1 yielded a slightly higher DNA recovery (0.27ng difference). The difference in the mean recovery is not statistically significant (ANOVA and t-test analysis).

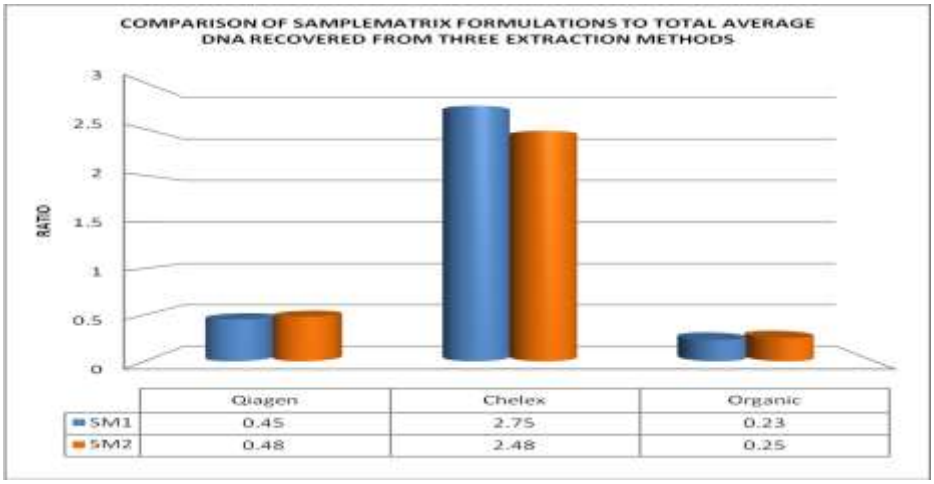


Fig. 43: Ratio of Samples with SM1 and SM2 Using Three Extraction Methods to Total Mean DNA (ng) Recovered

ANOVA Statistical Analysis - SampleMatrix™ as Wetting Agent for Blood (6M Storage)

The ANOVA analysis was performed on data that combined all of the blood dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

CEMENT QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	2.0250	5.65306	1.78765	-2.0190	6.0690	.00	18.01
SM2	10	2.0240	5.27547	1.66825	-1.7498	5.7978	.00	16.80
H2O	10	1.5720	3.41886	1.08114	-.8737	4.0177	.00	10.02
Total	30	1.8737	4.71480	.86080	.1131	3.6342	.00	18.01

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.365	2	.683	.029	.972
Within Groups	643.286	27	23.825		
Total	644.651	29			

CEMENT CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	87.8030	260.67020	82.43116	-98.6692	274.2752	.00	828.44
SM2	10	73.8030	190.23052	60.15617	-62.2797	209.8857	.00	601.65
H2O	10	66.1300	182.41546	57.68483	-64.3622	196.6222	.00	580.80
Total	30	75.9120	206.70847	37.73963	-1.2742	153.0982	.00	828.44

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2415.313	2	1207.656	.026	.974
Within Groups	1236708.059	27	45804.002		
Total	1239123.372	29			

COTTON QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	7.4600	19.15916	6.05866	-6.2456	21.1656	.00	61.44
SM2	10	8.2390	18.24532	5.76968	-4.8129	21.2909	.00	57.64
H2O	10	5.8400	11.10383	3.51134	-2.1032	13.7832	.00	32.17
Total	30	7.1797	16.01648	2.92420	1.1990	13.1603	.00	61.44

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.955	2	14.977	.055	.947
Within Groups	7409.343	27	274.420		
Total	7439.297	29			

COTTON CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	70.3650	138.79392	43.89049	-28.9222	169.6522	.00	373.00
SM2	10	43.8130	87.66064	27.72073	-18.8956	106.5216	.00	240.00
H2O	10	35.8080	70.44450	22.27651	-14.5850	86.2010	.00	172.45
Total	30	49.9953	100.64265	18.37475	12.4148	87.5759	.00	373.00

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6544.250	2	3272.125	.308	.738
Within Groups	287195.089	27	10636.855		
Total	293739.339	29			

CARPET QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	19.9790	41.44267	13.10532	-9.6673	49.6253	.09	110.57
SM2	10	9.6250	19.45680	6.15278	-4.2936	23.5436	.00	53.59
H2O	10	7.3550	18.72509	5.92139	-6.0401	20.7501	.00	59.93
Total	30	12.3197	28.11673	5.13339	1.8207	22.8186	.00	110.57

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	905.745	2	452.873	.555	.580
Within Groups	22020.216	27	815.564		
Total	22925.961	29			

CARPET CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	105.0340	222.27726	70.29024	-53.9736	264.0416	.00	632.70
SM2	10	135.1720	299.08424	94.57874	-78.7800	349.1240	.00	900.16
H2O	10	103.7870	242.79184	76.77752	-69.8958	277.4698	.71	763.04
Total	30	114.6643	248.20557	45.31593	21.9828	207.3458	.00	900.16

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6316.241	2	3158.120	.048	.953
Within Groups	1780257.967	27	65935.480		
Total	1786574.208	29			

CARPET PCI COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	23.2160	65.65875	20.76312	-23.7534	70.1854	.00	209.58
SM2	10	7.1840	17.09363	5.40548	-5.0440	19.4120	.19	55.62
H2O	10	10.9720	25.67111	8.11792	-7.3920	29.3360	.02	81.66
Total	30	13.7907	41.00661	7.48675	-1.5215	29.1028	.00	209.58

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1404.298	2	702.149	.400	.674
Within Groups	47360.430	27	1754.090		
Total	48764.728	29			

GLASS QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	62.8190	114.78346	36.29772	-19.2921	144.9301	1.20	355.97
SM2	10	83.7280	196.87825	62.25837	-57.1102	224.5662	.15	633.82
H2O	10	73.1880	142.95135	45.20519	-29.0732	175.4492	.82	431.02
Total	30	73.2450	150.11816	27.40770	17.1900	129.3000	.15	633.82

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2185.980	2	1092.990	.045	.956
Within Groups	651342.401	27	24123.793		
Total	653528.381	29			

GLASS CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	313.4980	561.01113	177.40730	-87.8252	714.8212	9.96	1537.50
SM2	10	336.6440	695.82474	220.03910	-161.1190	834.4070	11.82	2235.00
H2O	10	266.0240	464.81250	146.98662	-66.4828	598.5308	9.43	1178.10
Total	30	305.3887	562.03228	102.61259	95.5224	515.2550	9.43	2235.00

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25922.341	2	12961.171	.038	.962
Within Groups	9134605.904	27	338318.737		
Total	9160528.246	29			

WOOD QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	29.4030	67.37001	21.30427	-18.7906	77.5966	.39	210.30
SM2	10	25.1930	56.21051	17.77532	-15.0176	65.4036	.29	171.43
H2O	10	4.6540	8.46754	2.67767	-1.4033	10.7113	.18	27.75
Total	30	19.7500	50.32217	9.18753	.9594	38.5406	.18	210.30

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3506.959	2	1753.479	.677	.517
Within Groups	69930.353	27	2590.013		
Total	73437.312	29			

T TEST Statistical Analysis (Independent Samples) - SampleMatrix™ as a Wetting Agent for Blood (6M STORAGE)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations as compared to the unprotected control for each substrate and the specific extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison.

NEAT BLOOD QIAGEN CEMENT

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	9.9725	8.60545	4.30272
	NO SM	2	7.7250	3.24562	2.29500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	118.009	.000	.340	4	.751	2.24750	6.60533	16.09183	20.58683
	Equal variances not assumed			.461	3.983	.669	2.24750	4.87652	11.31508	15.81008

NEAT BLOOD CHELEX CEMENT

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	403.2650	373.40685	186.70343
	NO SM	2	328.7250	356.48788	252.07500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.011	.920	1.285	4	.268	36.85500	28.67725	-42.76582	116.47582
	Equal variances not assumed			1.239	1.904	.346	36.85500	29.74694	-97.52730	171.23730

NEAT BLOOD QIAGEN CARPET

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	71.8200	32.34991	16.17496
	NO SM	2	34.9650	35.30584	4.96500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.011	.920	1.285	4	.268	36.85500	28.67725	-42.76582	116.47582
	Equal variances not assumed			1.239	1.904	.346	36.85500	29.74694	-97.52730	171.23730

NEAT BLOOD CHELEX CARPET

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	586.7650	234.71710	117.35855
	NO SM	2	499.5600	372.61699	263.48000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.036	.366	.365	4	.733	87.20500	238.79376	-575.79276	750.20276
	Equal variances not assumed			.302	1.418	.801	87.20500	288.43498	-1796.86178	1971.2718

NEAT BLOOD PCI CARPET

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	71.5550	94.54361	47.27181
	NO SM	2	51.5000	42.65268	30.16000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.037	.366	.274	4	.798	20.055	73.27355	-183.38498	223.49498
	Equal variances not assumed			.358	3.967	.739	20.055	56.07360	-136.13731	176.24731

NEAT BLOOD CHELEX GLASS

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1409.930	654.49826	327.24913
	NO SM	2	1145.550	46.03265	32.55000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.78	.171	.538	4	.619	264.38	491.27823	-1099.62703	1628.387
	Equal variances not assumed			.804	3.059	.479	264.38	328.86395	-770.92998	1299.690

NEAT BLOOD PCI GLASS

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	140.1225	92.05784	46.02892
	NO SM	2	18.7300	12.31780	8.71,000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	4.222	.109	1.753	4	.154	121.39250	69.24909	-70.87381	313.65881
	Equal variances not assumed			2.591	3.206	.076	121.39250	46.84576	-22.40785	265.19285

NEAT BLOOD QIAGEN COTTON

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	36.6200	26.78514	13.39257
	NO SM	2	26.0750	8.61963	6.09500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	29.589	.006	.516	4	.633	10.54500	20.43264	-46.18511	67.27511
	Equal variances not assumed			.717	3.873	.514	10.54500	14.71428	-30.84227	51.93227

NEAT BLOOD CHELEX COTTON

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	269.1625	83.37927	41.68963
	NO SM	2	169.4050	4.30628	3.04500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.225	.147	1.595	4	.186	99.75750	62.56225	-73.94314	273.45814
	Equal variances not assumed			2.387	3.032	.096	99.75750	41.80069	-32.48420	231.99920

NEAT BLOOD PCI COTTON

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNA MT	SM RT	4	39.2050	35.59656	17.79828
	NO SM	2	9.9900	3.26683	2.31,000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.614	.273	1.093	4	.336	29.21500	26.73487	-45.01290	103.44290
	Equal variances not assumed			1.628	3.099	.199	29.21500	17.94756	-26.88087	85.31087

NEAT BLOOD QIAGEN WOOD

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	131.8825	69.93249	34.96625
	NO SM	2	18.0650	13.69666	9.68500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	17.146	.014	2.156	4	.097	113.81750	52.78362	-32.73333	260.36833
	Equal variances not assumed			3.137	3.418	.043	113.81750	36.28274	5.93559	221.69941

NEAT BLOOD CHELEX WOOD

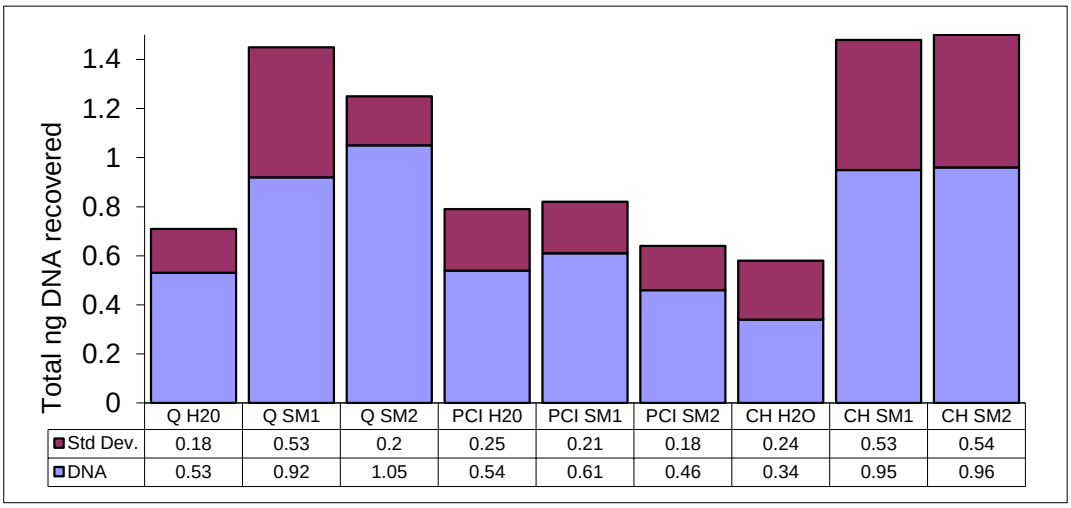
	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	580.5400	384.63375	192.31687
	NO SM	2	202.9600	126.88324	89.72000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.854	.408	1.286	4	.268	377.58	293.66075	-437.75294	1192.91294
	Equal variances not assumed			1.779	3.895	.152	377.58	212.21559	-217.97166	973.13166

B. SEMEN STUDIES (N = 450)

Extraction Chemistry Compatibility

The mean total DNA recovered (ng) was calculated for each extraction method and each wetting agent (Fig. 44). For the Qiagen extraction, SM1 resulted in ~74% increase in recovered DNA relative to water, whereas SM2 gave ~98% increase in recovery. For the organic extraction, there was increase in recovery relative to water when using SM1; however, SM2 experienced a 15% decrease in recovery compared to water. When employing the Chelex extraction, there was a 279% increase in DNA recovery for both SM1 and SM2 in comparison to water. Based on statistical analyses, the differences in the mean recovery are not statistically significant (t-test analysis).



	Q H20	Q SM1	Q SM2	PCI H20	PCI SM1	PCI SM2	CH H2O	CH SM1	CH SM2
Std Dev.	0.18	0.53	0.2	0.25	0.21	0.18	0.24	0.53	0.54
DNA	0.53	0.92	1.05	0.54	0.61	0.46	0.34	0.95	0.96

Fig. 44: Comparison of Each Extraction Method to Wetting Agent

The total DNA recovered was examined as a function of wetting agent and dilution for the three extraction methods. The results show recovery at all dilution levels for each substrate (Figs. 45-47). At least one of the SampleMatrix™ formulations resulted in a higher DNA recovery with each extraction method; however, these differences in the mean recovery are not statistically significant (t-test analysis). The results do suggest that SampleMatrix™ is compatible with the chemistry of each extraction; this holds true regardless of dilution factor or the particular substrate sampled.

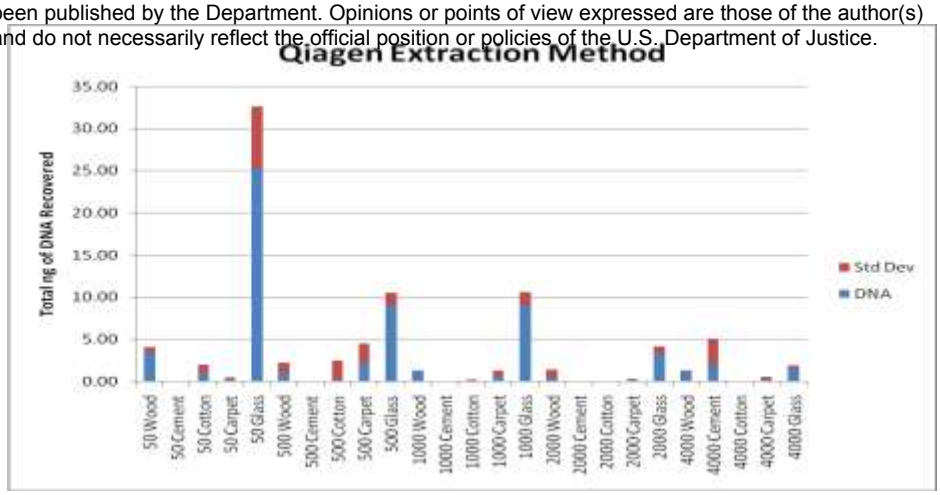


Fig. 45: Overall Recovery of the Qiagen Extraction Method *v* Dilution

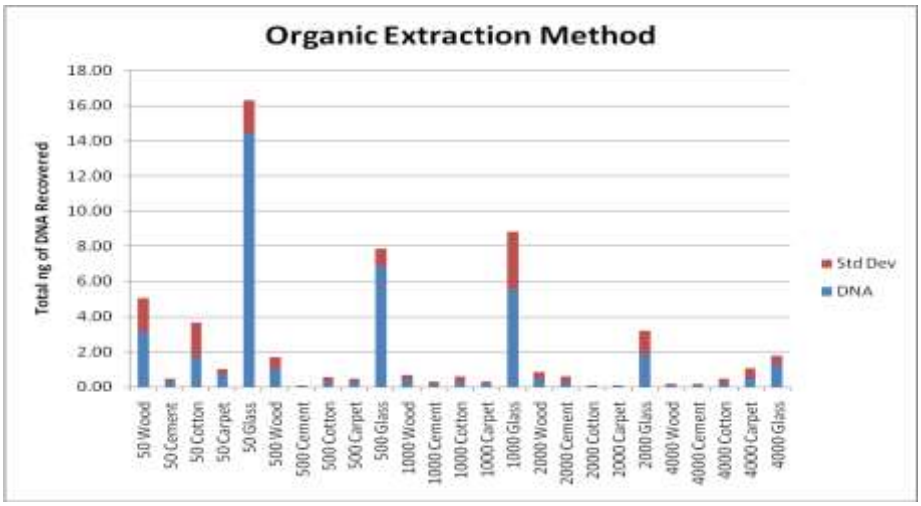


Fig. 46: Overall Recovery of the Organic Extraction Method *v*. Dilution

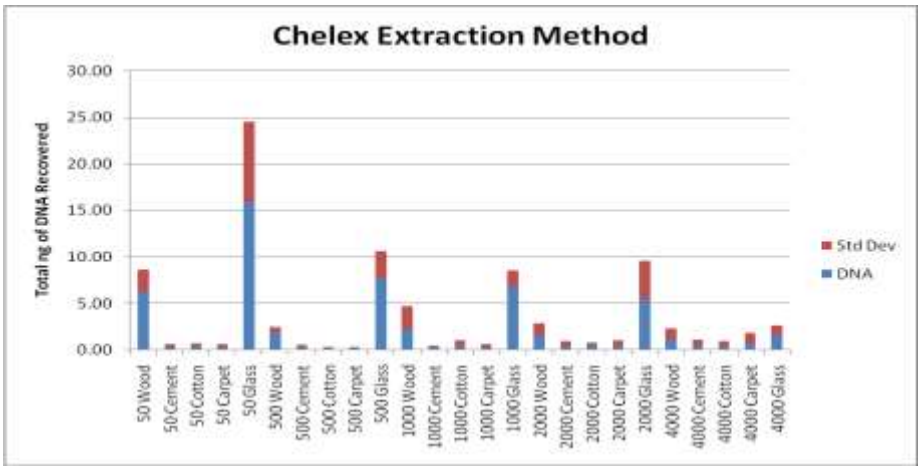
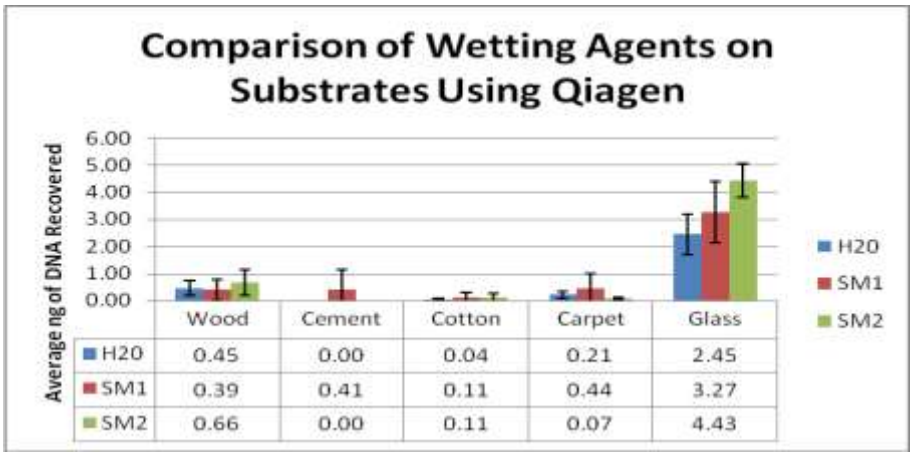


Fig. 47: Overall Recovery of the Chelex Extraction Method *v*. Dilution

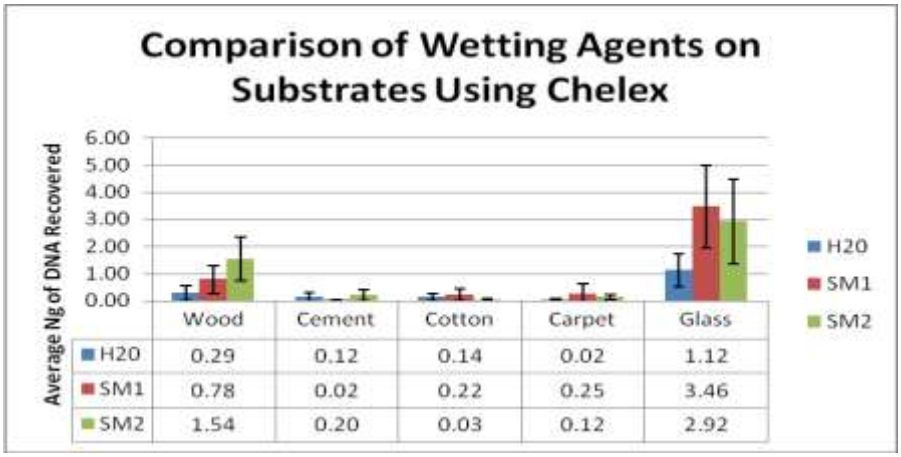
The Effect of Substrates on SampleMatrix™

Glass substrates allowed for the highest recovery of DNA. In contrast, cement resulted in the lowest recovery of DNA. The other substrates, carpet, wood, and cotton provided varying values of DNA recovery. DNA was successfully recovered from all substrates with each wetting agent. Both SampleMatrix™ formulations outperformed water in recovering DNA in four of the five substrates, which included wood, cement, carpet, and glass. Water outperformed SM2 in terms of DNA recovery from the cotton bed sheet by 53%, but SM1 greatly outperformed water by 131%. The differences in the mean recovery are not statistically significant (t-test analysis). For the Qiagen extraction (Fig 48), SM1 and SM2 show a greater DNA recovery than water for the majority of the substrates. For the Chelex extraction (Fig. 49), SM1 and SM2 as wetting agents show greater DNA recovery than water on average for 80% of all substrates tested. A ratio of SampleMatrix™ versus water showed that overall, SampleMatrix™ SM1 and SM2 outperformed water 80% of the time. Water was more efficient than SM1 for cement and more efficient than SM2 for the cotton substrate. For the organic extraction (Fig. 50), SM1 shows greater DNA recovery than water on average for 60% of the substrates and SM2 recovered more DNA than water for 40% of the substrates. Again, the differences in the mean recovery are not statistically significant (t-test analysis).



	Wood	Cement	Cotton	Carpet	Glass
H20	0.45	0.00	0.04	0.21	2.45
SM1	0.39	0.41	0.11	0.44	3.27
SM2	0.66	0.00	0.11	0.07	4.43

Fig. 48: Effects of Substrate on the SampleMatrix™ Formulations for the Qiagen Extraction



	Wood	Cement	Cotton	Carpet	Glass
H20	0.29	0.12	0.14	0.02	1.12
SM1	0.78	0.02	0.22	0.25	3.46
SM2	1.54	0.20	0.03	0.12	2.92

Fig. 49: Effects of Substrate on the SampleMatrix™ Formulations for the Chelex Extraction

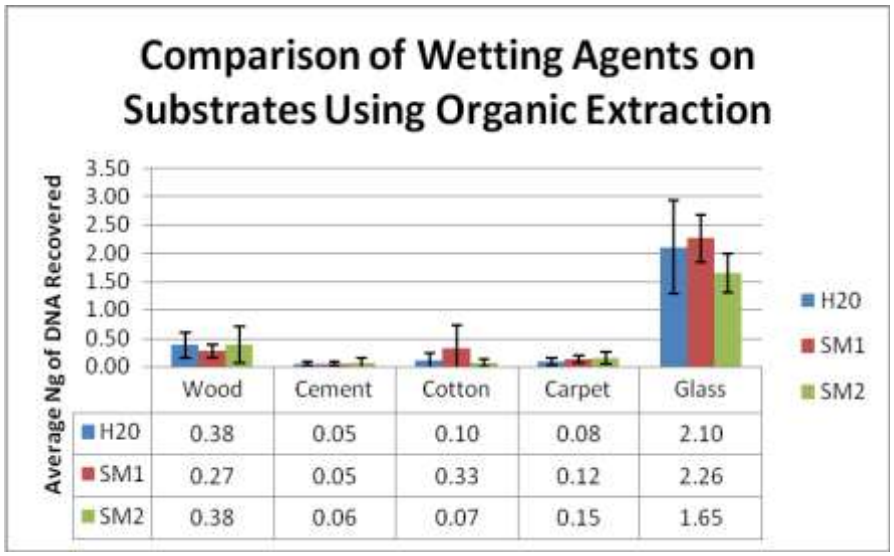


Fig. 50: Effects of Substrate on the SampleMatrix™ Formulations for the Organic Extraction

The Effects of Sample Dilutions on SampleMatrix™

An overall comparison of DNA recovery as a function of dilution shows a consistent trend of SampleMatrix™ exhibiting higher DNA yields at all dilutions [Figure 51].

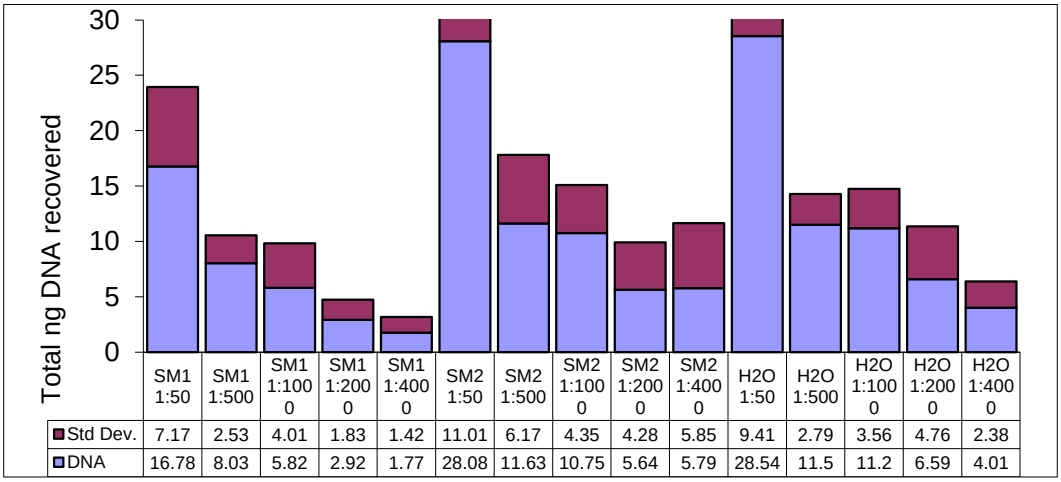


Fig. 51: The Overall DNA Recovery of Wetting Agents by Dilution

Overall, for the Qiagen extraction, SM1 and SM2 recovered greater amounts of DNA than water at all dilutions (Fig 52). The difference in the mean recovery is not statistically significant (t-test analysis).

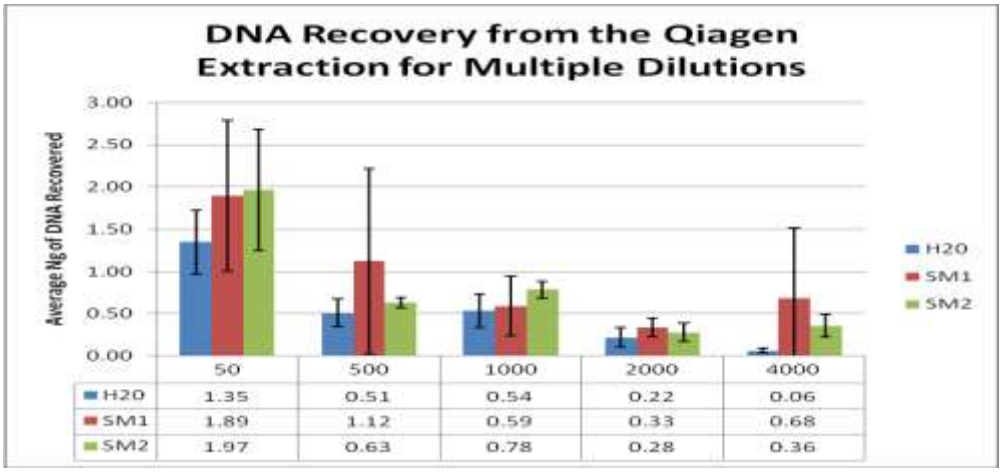


Fig. 52: DNA Recovery for Qiagen Extraction Method for Each Dilution

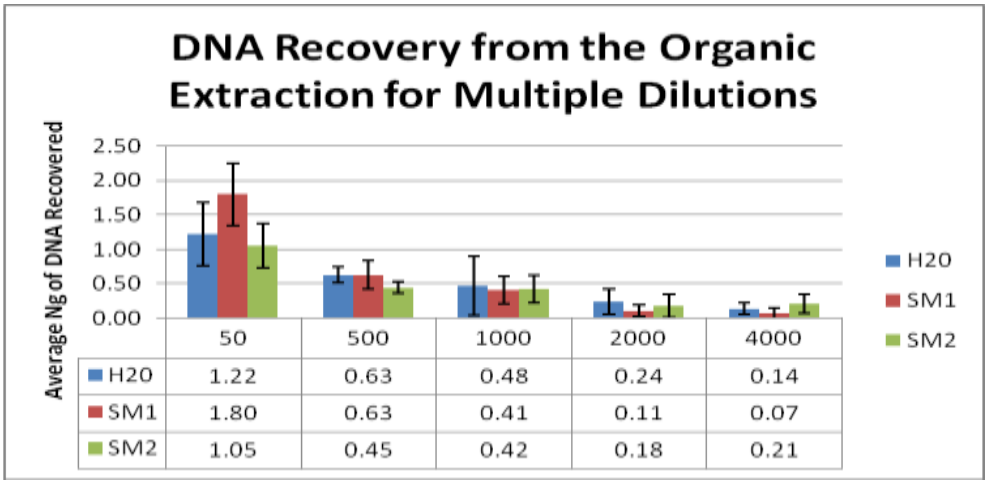


Fig. 53: DNA Recovery for Organic Extraction for Each Dilution

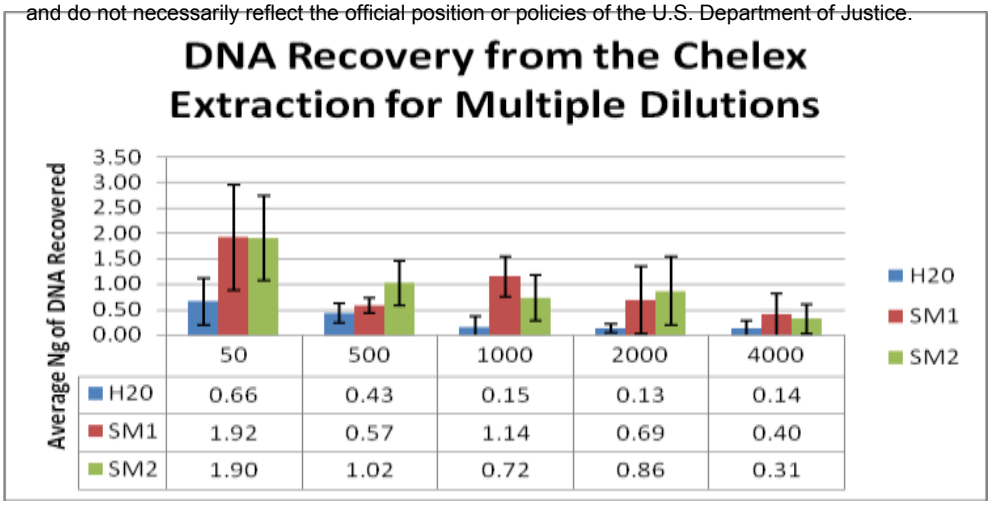


Fig. 54: DNA Recovery from the Chelex Extraction at Multiple Dilutions

For the organic extraction, SampleMatrix™ resulted in higher or equivalent yields as compared to water for the 1:500 dilutions (Fig. 53). SM1 had a greater recovery than water for the 1:50 (48% more DNA recovery) and an equivalent recovery for the 1:500 dilutions. At higher dilutions, water either provides comparable recovery or results in higher yields than SM1 and SM2. Overall, SM1 and SM2 recovered greater amounts of DNA at all dilutions relative to water when employing a Chelex extraction (Fig. 54). The statistical analyses support that there was no significant difference when comparing the mean recovery with the three wetting agents.

Comparison of the SampleMatrix™ Formulations

When comparing SM1 and SM2 in terms of total DNA recovered by extraction method, SM2 resulted in higher yields compared with SM1 for the Qiagen extraction (Fig. 55) whereas the results for the Chelex extraction were comparable for the two formulations. The Qiagen and Chelex extractions gave comparable yields. The organic extraction recovered the least amount of DNA (Fig. 55). The statistical analyses support that there was no significant difference when comparing the mean recovery with the two different SampleMatrix™ formulations.

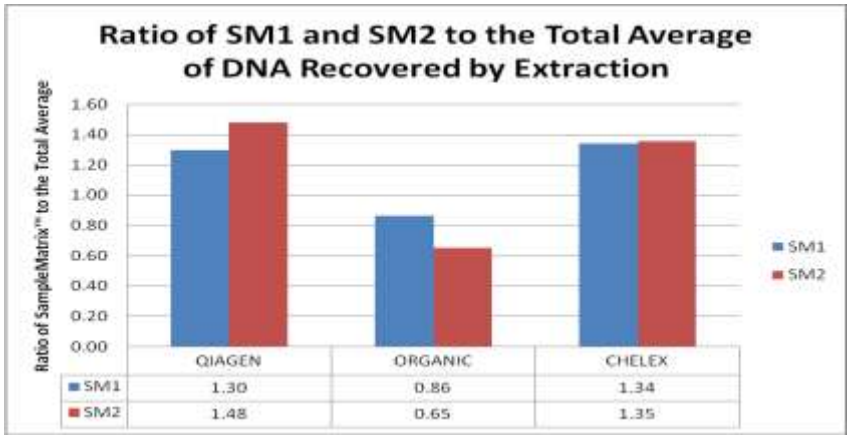


Fig. 55: Ratio of SM1 and SM2 to the Total Mean DNA Recovered for Extraction

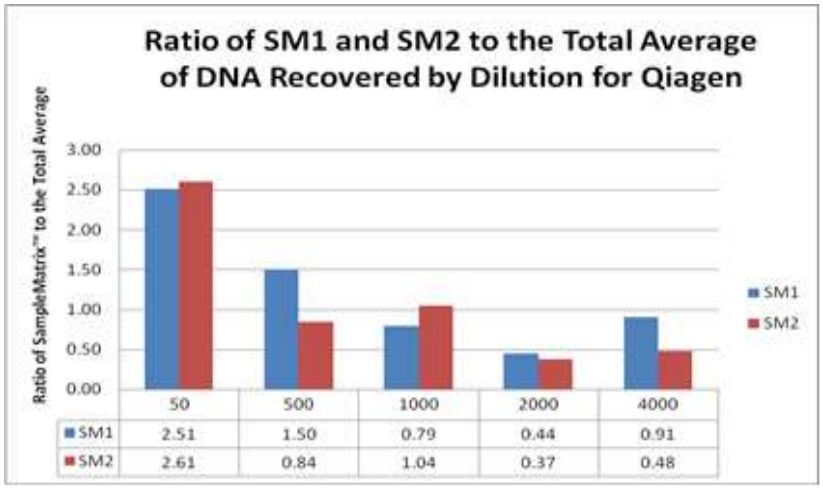


Fig. 56: Ratio of SM1 and SM2 to the Total Mean DNA Recovered *v.* Dilution of Semen

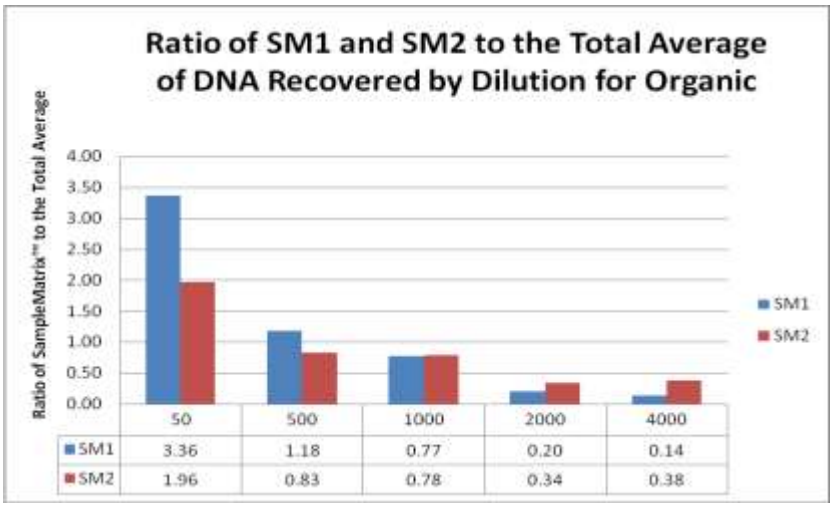


Fig. 56: Ratio of SM1 and SM2 to the Total Mean DNA Recovered *v.* Dilution of Semen

The ratio of the total DNA recovered of each SampleMatrix™ formulation to the total average of DNA recovered was calculated for the each of the extraction methods as a function of dilution factor (Fig 56-57). The statistical analyses support that there is no significant difference in the mean recovery when comparing the SM1 and SM2 formulations

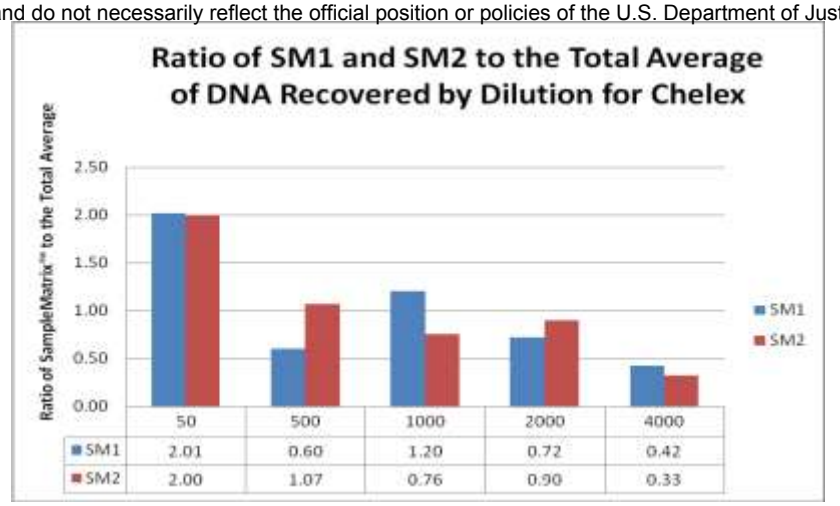


Fig. 57: Ratio of SM1 and SM2 to Total Mean DNA *v.* Dilution of Semen

Given the overall comparable recovery rates obtained with the Chelex extraction method, the variables of dilution and substrate were examined in detail to determine whether dilution or substrate played a role in the consistency of recovery. The standard deviation values were evident and variable throughout each substrate. The least variation and therefore most precise recovery values were obtained from the glass substrate, as evidenced by the smallest standard deviations overall [Figure 59]. Cement, cotton, and carpet substrates exhibited low recovery and high standard deviations regardless of the wetting agent used.

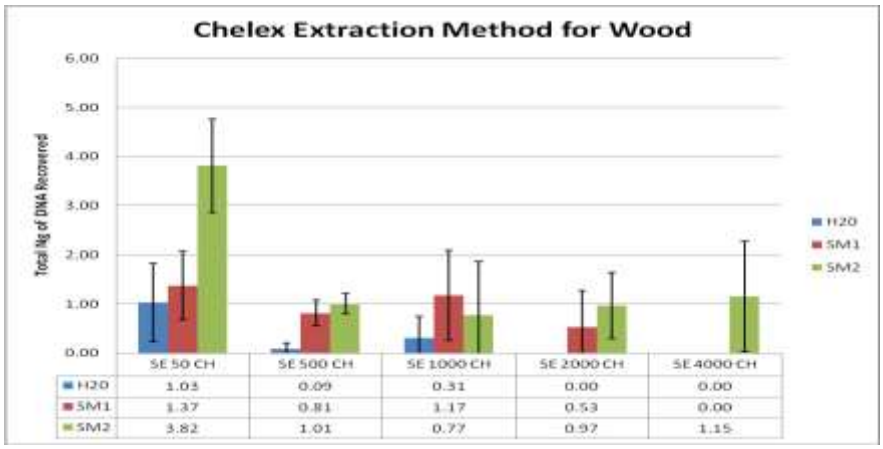


Fig. 58: Total DNA Recovered for Each Wetting Agent *v.* Dilution Factor

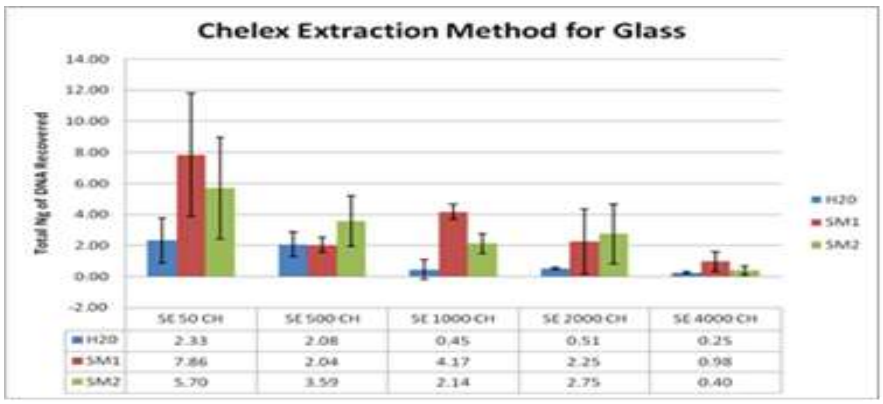


Fig. 59: Total DNA Recovered for Each Wetting Agent *v.* Dilution Factor

When examining the Chelex extraction as a function of dilution, the standard deviations for the highest dilution (1:4000) as well as for the lowest (1:50 dilution) showed the greatest variation. This trend was similar for each substrate [Figures 60-64].

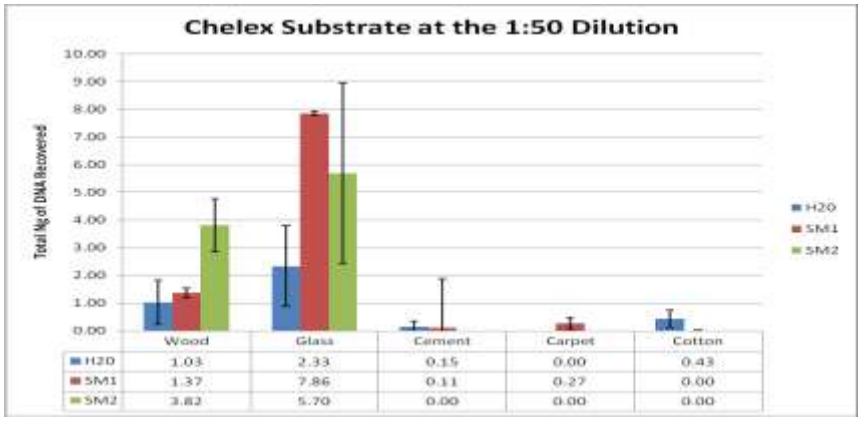


Fig. 60: Total DNA Recovered for Each Wetting Agent *v.* Substrate for 1:50 Dilution

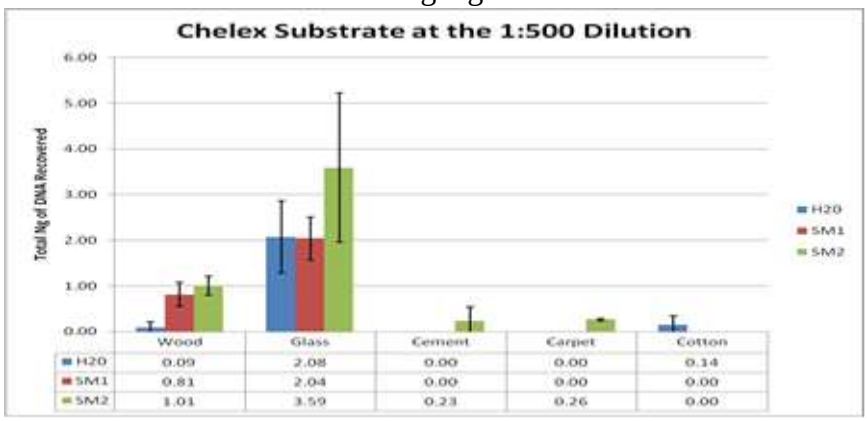


Fig. 61: Total DNA Recovered for Each Wetting Agent *v.* Substrate for 1:500 Dilution

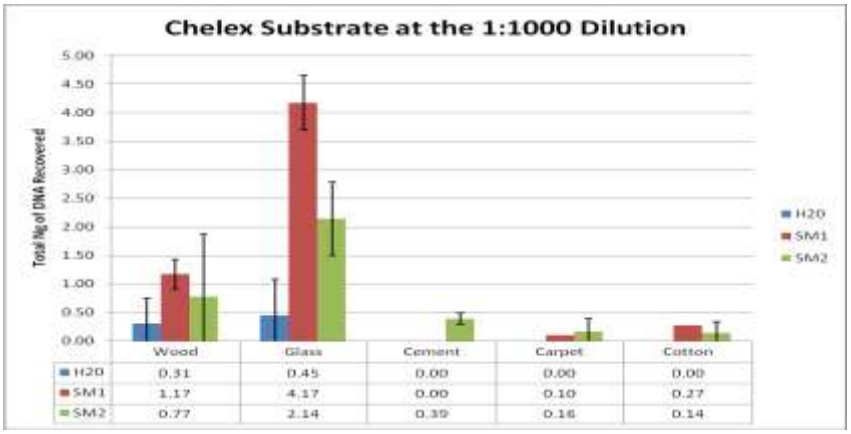


Fig. 62: Total DNA Recovered for Each Wetting Agent *v.* Substrate for 1:1,000 Dilution

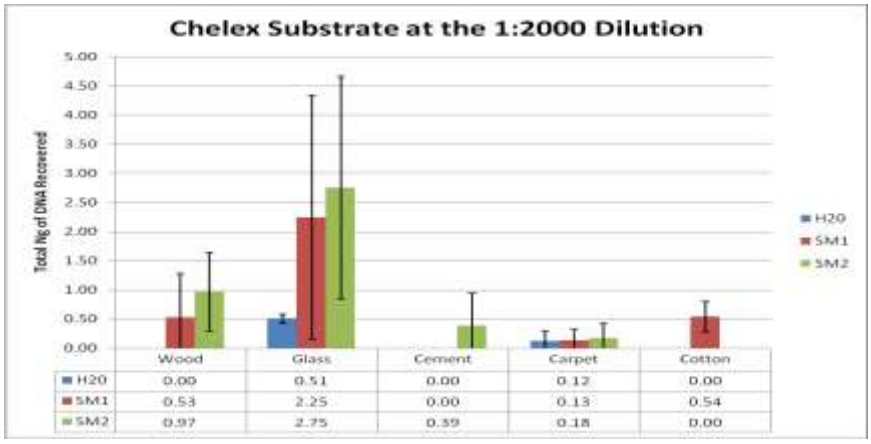


Fig. 63: Total DNA Recovered for Each Wetting Agent *v.* Substrate for 1:2,000 Dilution

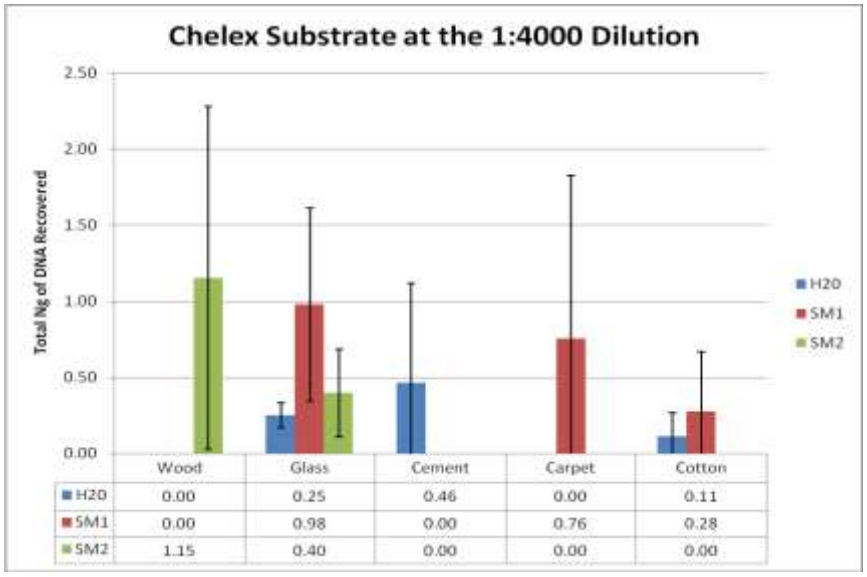


Fig. 64: Total DNA Recovered for Each Wetting Agent *v.* Substrate for 1:4000 Dilution

T-Test Statistical Analysis (Independent Samples) - SampleMatrix™ as Wetting Agent for Semen (6M)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations as compared to the unprotected control for each substrate and the specific extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison.

SEMEN COTTON QIAGEN 1:50 Dilution Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	.5150	.55000	.27500
	NO SM	2	.2800	.14142	.1,0000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	8.465	.044	.564	4	.603	.23500	.41702	.92284	1.39284
	Equal variances not assumed			.803	3.654	.471	.23500	.29262	.60868	1.07868

SEMEN GLASS QIAGEN 1:50 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	10.1475	3.26593	1.63296
	NO SM	2	5.0050	1.97283	1.39500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.184	.690	1.982	4	.118	5.14250	2.59414	2.05998	12.34498
	Equal variances not assumed			2.394	3.455	.085	5.14250	2.14770	1.20998	11.49498

SEMEN GLASS CHELEX 1:50 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	6.7750	3.21018	1.60509
	NO SM	2	2.3300	1.45664	1.03000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.512	.188	1.786	4	.149	4.44500	2.48888	2.46524	11.35524
	Equal variances not assumed			2.331	3.963	.081	4.44500	1.90715	.86949	9.75949

SEMEN WOOD QIAGEN 1:50 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.2550	.96738	.48369
	NO SM	2	.9200	1.30108	.92000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.370	.576	.365	4	.734	.33500	.91859	2.21541	2.88541
	Equal variances not assumed			.322	1.589	.785	.33500	1.03940	5.45206	6.12206

C. SALIVA (N = 450)

Extraction Chemistry

One observation that is apparent in recovering saliva samples from various substrates is that the organic and Qiagen extraction methods are both far superior to the Chelex extraction. Poor recovery was noted with Chelex, regardless of the substrate or wetting agent applied. SM2 gave the lowest mean yield; however, there was no clear difference between SM1 and H_2O. There is considerable variation as to which of these two wetting agents recovers the highest mean yield, depending on the dilution factor and substrate. The comparison between SM1 and H_2O is compounded by the lower recovery of DNA from saliva as compared to blood and semen. Statistical analyses (ANOVA and t test) support that there is no statistical difference in the mean recovery of DNA from each of the three wetting agents when collecting saliva from various substrates for the vast majority of samples. Two exceptions were identified: 1) neat saliva recovered from carpet and extracted with Qiagen chemistry indicated a significant difference at the 95% confidence level in favor of the unprotected samples 2) a 1:100 saliva dilution recovered from glass and extracted with PCI chemistry indicated a significant difference at the 95% confidence level in favor of the SampleMatrix™ protected samples.

ANOVA Statistical Analysis - SampleMatrix™ as Wetting Agent for Saliva (6M)

The ANOVA analysis was performed on data that combined all of the saliva dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

GLASS QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	21.6860	36.29654	11.47797	-4.2790	47.6510	.42	104.42
SM2	10	36.7670	78.68997	24.88395	-19.5244	93.0584	.23	253.44
H_2O	10	60.7470	89.51096	28.30585	-3.2853	124.7793	.59	219.37
Total	30	39.7333	71.30717	13.01885	13.1068	66.3599	.23	253.44

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	7760.796	2	3880.398	.750	.482
Within Groups	139695.851	27	5173.920		
Total	147456.646	29			

GLASS PCI COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	21.6860	36.29654	11.47797	-4.2790	47.6510	.42	104.42
SM2	10	36.7670	78.68997	24.88395	-19.5244	93.0584	.23	253.44
H2O	10	32.7157	69.91860	22.11020	-17.3011	82.7324	.59	219.37
Total	30	30.3896	62.36759	11.38671	7.1011	53.6780	.23	253.44

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1218.345	2	609.173	.147	.864
Within Groups	111583.440	27	4132.720		
Total	112801.785	29			

WOOD CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SM1	10	.7320	1.18447	.37456	-.1153	1.5793	.00	3.94
SM2	10	.5400	.82180	.25988	-.0479	1.1279	.00	2.36
H2O	10	.5560	.69439	.21959	.0593	1.0527	.00	1.76
Total	30	.6093	.89581	.16355	.2748	.9438	.00	3.94

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.227	2	.113	.133	.876
Within Groups	23.045	27	.854		
Total	23.272	29			

T Test Statistical Analysis (Independent Samples) - SampleMatrix™ as Wetting Agent for Saliva (6M Storage)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations as compared to the unprotected control for each substrate and the specific extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison.

COTTON CHELEX NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.9925	1.74261	.87131
	NO SM	2	3.5650	1.71827	1.21500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.137	.730	-1.046	4	.355	-1.57250	1.50390	-5.74800	2.60300
	Equal variances not assumed			-1.052	2.107	.398	-1.57250	1.49512	-7.70175	4.55675

COTTON PCI NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	6.2725	7.43968	3.71984
	NO SM	2	7.2100	2.13546	1.51,000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.562	.280	-.166	4	.876	-.93750	5.65586	-16.64069	14.76569
	Equal variances not assumed			-.234	3.764	.828	-.93750	4.01464	-12.36559	10.49059

WOOD CHELEX NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	2.1900	1.28691	.64345
	NO SM	2	1.3850	.51619	.36500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.473	.292	.813	4	.462	.80500	.99072	-1.94569	3.55569
	Equal variances not assumed			1.088	3.999	.338	.80500	.73977	-1.24911	2.85911

WOOD PCI NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	20.8400	24.02948	12.01474
	NO SM	2	19.4550	5.23966	3.70500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.492	.190	.076	4	.943	1.38500	18.16436	-49.04735	51.81735
	Equal variances not assumed			.110	3.503	.918	1.38500	12.57303	-35.56772	38.33772

WOOD PCI 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	22.1350	32.62412	16.31206
	NO SM	2	71.2500	68.03781	48.11,000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.602	.131	-1.282	4	.269	-49.11500	38.29689	-155.44421	57.21421
	Equal variances not assumed			-.967	1.24	.485	-49.11500	50.80015	-464.22310	365.9931 0

WOOD QIAGEN NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	17.4425	12.51482	6.25741
	NO SM	2	13.7650	2.94864	2.08500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.905	.240	.388	4	.718	3.67750	9.47256	-22.62255	29.97755
	Equal variances not assumed			.558	3.571	.610	3.67750	6.59564	-15.53586	22.89086

WOOD QIAGEN 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	4.9400	4.06887	2.03443
	NO SM	2	3.6550	2.02940	1.43500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.970	.380	.405	4	.706	1.28500	3.17565	-7.53203	10.10203
	Equal variances not assumed			.516	3.861	.634	1.28500	2.48961	-5.72669	8.29669

WOOD QIAGEN 1:50

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.1225	.76360	.38180
	NO SM	2	2.8000	1.40007	.99000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	13.135	.022	-2.011	4	.115	-1.67750	.83398	-3.99301	.63801
	Equal variances not assumed			-1.581	1.310	.314	-1.67750	1.06107	-9.53499	6.17999

CARPET PCI NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	37.6875	39.14540	19.57270
	NO SM	2	13.9800	3.64867	2.58000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.218	.211	.806	4	.465	23.70750	29.40153	-57.92423	105.33923
	Equal variances not assumed			1.201	3.102	.313	23.70750	19.74201	-37.96391	85.37891

CARPET PCI 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	8.7850	14.07161	7.03581
	NO SM	2	1.6200	1.99404	1.41,000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.976	.160	.677	4	.536	7.16500	10.58897	-22.23469	36.56469
	Equal variances not assumed			.999	3.230	.387	7.16500	7.17570	-14.77773	29.10773

CARPET QIAGEN NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	12.2500	1.82680	.91340
	NO SM	2	19.3600	3.18198	2.25000

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.439	.297	-3.659	4	.022	-7.11,000	1.94309	-12.50489	-1.71511
	Equal variances not assumed			-2.928	1.345	.155	-7.11,000	2.42833	-24.33665	10.11665

CARPET QIAGEM 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	2.3425	2.13244	1.06622
	NO SM	2	10.7250	13.35725	9.44500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	69.143	.001	-1.397	4	.235	-8.38250	6.00091	-25.04368	8.27868
	Equal variances not assumed			-.882	1.026	.537	-8.38250	9.50499	-122.26042	105.49542

CARPET CHELEX 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.8575	1.81055	.90528
	NO SM	2	3.7850	.68589	.48500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.195	.336	-1.387	4	.238	-1.92750	1.39001	-5.78679	1.93179
	Equal variances not assumed			-1.877	3.985	.134	-1.92750	1.02701	-4.78331	.92831

GLASS PCI NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	123.6525	88.22094	44.11047
	NO SM	2	149.5950	98.67675	69.77500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.020	.896	-.329	4	.758	-25.94250	78.76298	-244.62360	192.73860
	Equal variances not assumed			-.314	1.860	.785	-25.94250	82.54868	-408.01047	356.12547

GLASS PCI 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	16.4750	6.62669	3.31334
	NO SM	2	9.4350	2.24153	1.58500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.663	.267	1.390	4	.237	7.04000	5.06390	-7.01965	21.09965
	Equal variances not assumed			1.917	3.915	.129	7.04000	3.67294	-3.24555	17.32555

GLASS PCI 1:100 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.5750	.30447	.15223
	NO SM	2	.7800	.26870	.19000

SIG DIFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.057	.823	3.102	4	.036	.79500	.25628	.08344	1.50656
	Equal variances not assumed			3.265	2.370	.065	.79500	.24346	-.11030	1.70030

GLASS QIAGEN NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	143.8350	50.36139	25.18070
	NO SM	2	167.8850	75.62507	53.47500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.702	.449	-.481	4	.656	-24.05000	49.98993	-162.84428	114.74428
	Equal variances not assumed			-.407	1.469	.735	-24.05000	59.10705	-390.53536	342.43536

GLASS QIAGEN 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	29.6050	3.93014	1.96507
	NO SM	2	33.8150	.86974	.61500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	176.146	.000	-1.417	4	.230	-4.21,000	2.97157	-12.46040	4.04040
	Equal variances not assumed			-2.045	3.515	.120	-4.21,000	2.05906	-10.25158	1.83158

GLASS QIAGEN 1:50 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	6.6025	2.50070	1.25035
	NO SM	2	7.5650	2.22739	1.57500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	5.577	.078	-.456	4	.672	-.96250	2.10899	-6.81798	4.89298
	Equal variances not assumed			-.479	2.347	.673	-.96250	2.01097	-8.49781	6.57281

GLASS QIAGEN 1:100 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	3.5675	1.11924	.55962
	NO SM	2	2.6350	.07778	.05500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.078	.223	1.110	4	.329	.93250	.84010	-1.40000	3.26500
	Equal variances not assumed			1.658	3.057	.194	.93250	.56231	-.83819	2.70319

GLASS QIAGEN 1:200 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.0700	.84376	.42188
	NO SM	2	2.4550	.86974	.61500

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.130	.737	-1.881	4	.133	-1.38500	.73641	-3.42960	.65960
	Equal variances not assumed			-1.857	2.014	.204	-1.38500	.74579	-4.57271	1.80271

GLASS CHELEX NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	6.0100	1.61446	.80723
	NO SM	2	4.1500	3.11127	2.20000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.135	.151	1.027	4	.363	1.86000	1.81139	-3.16923	6.88923
	Equal variances not assumed			.794	1.280	.548	1.86000	2.34342	-16.19634	19.91634

GLASS CHELEX 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	4	1.3000	1.03405	.51703
	NO SM	2	2.2600	1.48492	1.05000

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.504	.517	-.953	4	.395	-.96000	1.00742	-3.75705	1.83705
	Equal variances not assumed			-.820	1.514	.521	-.96000	1.17039	-7.91108	5.99108

II. 6 - 24 MONTH STUDIES

A. BLOOD (N = 180)

The objective of these studies was to evaluate the total mean recovery of DNA from swabs that were used to collected blood from different substrates and then stored for a period of 6 – 24 months. The independent variables include stain dilution factor, wetting agent (SampleMatrix™ as compared with the conventional approach of using water). The Qiagen extraction chemistry was selected for this study and the effectiveness was evaluated by comparing the total mean DNA (ng) recovered for three different serial dilutions (neat, 1:100, 1:800). A total of four substrates were compared: wood, glass, cotton, and carpet. Although the data reflect that the mean recovery of DNA from SampleMatrix™ protected samples exceeds that of the unprotected controls for neat blood, the difference is not statistically significant (t test analysis). The reported results were based on five replicate sampling for each storage condition and are representative of the mean obtained. Separate negative controls were analyzed tested simultaneously for each dilution and storage period. For the purpose of performing the t test analysis, the two SampleMatrix™ formulations were collapsed into a single category in order to compare the mean recovery for treated v untreated samples.

T-Test Statistical Analysis (Independent Samples) - SampleMatrix™ as Wetting Agent for Blood (2+yrs)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations as compared to the unprotected control for each substrate and the specific extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison.

WOOD NEAT QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	106.8581	50.55089	15.98559
	NO SM	5	54.0910	50.89368	22.76035

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.053	.821	1.902	13	.080	52.76711	27.74577	7.17398	112.70820
	Equal variances not assumed			1.897	8.049	.094	52.76711	27.81317	11.30210	116.83632

WOOD 1:100 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	1.5603	1.88503	.59610
	NO SM	5	1.0960	.18474	.08262

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	5.530	.035	.539	13	.599	.46434	.86090	1.39552	2.32420
	Equal variances not assumed			.772	9.341	.459	.46434	.60180	-.88947	1.81815

WOOD 1:800 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	8	.5481	.26955	.09530
	NO SM	5	.7765	.55071	.24629

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.686	.221	1.013	11	.333	-.22841	.22554	.72483	.26800
	Equal variances not assumed			-.865	5.221	.425	-.22841	.26408	.89872	.44189

COTTON NEAT QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	28.1953	17.89145	5.65777
	NO SM	5	23.6046	5.89855	2.63791

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.976	.108	.550	13	.592	4.59072	8.34834	13.44477	22.62621
	Equal variances not assumed			.735	12.056	.476	4.59072	6.24251	-9.00350	18.18494

COTTON 1:100 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	1.3490	.92156	.29142
	NO SM	5	4.3300	3.71045	1.65936

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	19.837	.001	2.478	13	.028	-2.98100	1.20301	5.57994	-.38206
	Equal variances not assumed			1.769	4.249	.147	-2.98100	1.68476	7.55259	1.59059

GLASS NEAT QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	353.6166	148.43473	46.93918
	NO SM	5	203.5436	139.10136	62.20802

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.031	.862	1.881	13	.082	150.07298	79.76300	22.24451	322.39047
	Equal variances not assumed			1.926	8.611	.088	150.07298	77.93025	27.43946	327.58542

GLASS 1:100 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	15.8853	10.42820	3.29769
	NO SM	5	17.3879	6.36014	2.84434

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.398	.146	-.293	13	.774	-1.50265	5.13030	12.58599	9.58069
	Equal variances not assumed			-.345	12.191	.736	-1.50265	4.35488	10.97467	7.96937

GLASS 1:800 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	3.3614	2.73093	.86360
	NO SM	5	6.0763	4.13277	1.84823

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.268	.094	-1.536	13	.149	-2.71487	1.76793	6.53424	1.10450
	Equal variances not assumed			-1.331	5.814	.233	-2.71487	2.04004	7.74560	2.31586

CARPET NEAT QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	161.6666	94.22849	29.79766
	NO SM	5	80.9894	56.35785	25.20399

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.859	.071	1.745	13	.105	80.67723	46.23083	19.19841	180.553
	Equal variances not assumed			2.067	12.309	.060	80.67723	39.02745	4.12026	165.475

CARPET 1:100 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	1.7376	1.26250	.39924
	NO SM	5	2.5145	1.29574	.57947

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.002	.969	1.114	13	.285	-.77692	.69715	2.28303	.72919
	Equal variances not assumed			1.104	7.907	.302	-.77692	.70369	2.40297	.84913

CARPET 1:800 DILUTION QIAGEN Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	10	.6887	.51188	.16187
	NO SM	5	.4033	.45156	.20194

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.248	.627	1.054	13	.311	.28537	.27063	.29929	.87003
	Equal variances not assumed			1.103	9.118	.298	.28537	.25881	.29894	.86968

CHAPTER 5: RESULTS OF COATING AGENT STUDIES

I. SIX MONTH STUDIES

The objective of these studies was to evaluate the total amount of DNA recovered from a blood, semen and saliva samples deposited on a cotton swab that were subjected to a six month storage period. The reported results for each biological fluid is based on duplicate sampling and are representative of the mean obtained across both trials. Separate negative controls were tested simultaneously for each dilution and storage period.

A. BLOOD STUDIES (N = 180)

Extraction Chemistry Compatibility

A mean was obtained of the total DNA recovered over six storage mediums: SM1 Dry, SM1 Wet, SM2 Dry, SM2 Wet, Frozen, and Room Temperature. In addition, a ratio was determined of the total DNA recovered from samples stored with SampleMatrix™ to the mean across all storage conditions. Approximately 40 % of samples stored with SampleMatrix™ resulted in a higher DNA recovery than the total mean.

Table 25: Mean DNA Recovered and Average Standard Deviation for Three Extraction Methods: Qiagen, Chelex, and Organic.

STORAGE MEDIUM	*MEAN DNA (NG)*			*STD DEV (NG)*		
	CHELEX	*ORGANIC*	*QIAGEN*	*CHELEX*	*ORGANIC*	*QIAGEN*
SM1 DRY	37.87	77.81	58.77	14.91	62.81	28.06
SM1 WET	47.77	31.08	66.60	26.38	27.66	30.48
SM2 DRY	33.66	43.26	71.68	18.10	40.32	17.95
SM2 WET	37.33	66.37	40.05	2.29	79.11	15.19
FROZEN	35.38	38.22	48.00	24.05	42.16	29.95
ROOM TEMP	102.47	79.71	56.39	7.69	45.25	24.96
Mean	49.08	56.08	56.92	15.57	49.55	24.43

The following graphs (Figures 65-67) demonstrate the DNA recovered based on extraction method for the 1:100 dilution samples. The data is not consistent within each storage condition (as evident from the standard deviation values) and the total DNA recovered does not appear to be directly related to the extraction method used. These preliminary findings are consistent across the dilution series although neat samples appear to have the least variability.

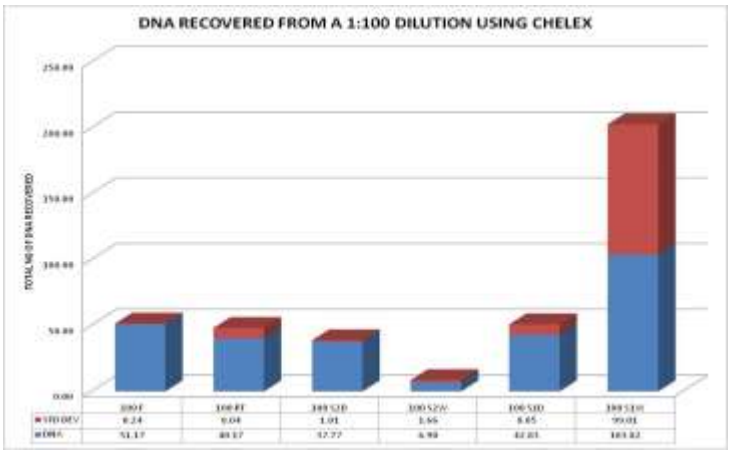


Fig. 65: DNA Recovered from 1:100 Dilutions by Chelex Extraction with Storage Condition

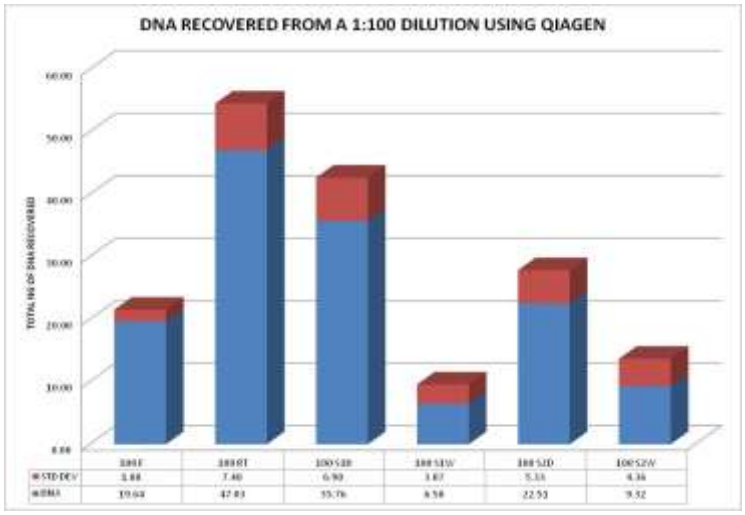


Fig. 66: DNA Recovered from 1:100 Dilutions by Qiagen Extraction with Storage Condition

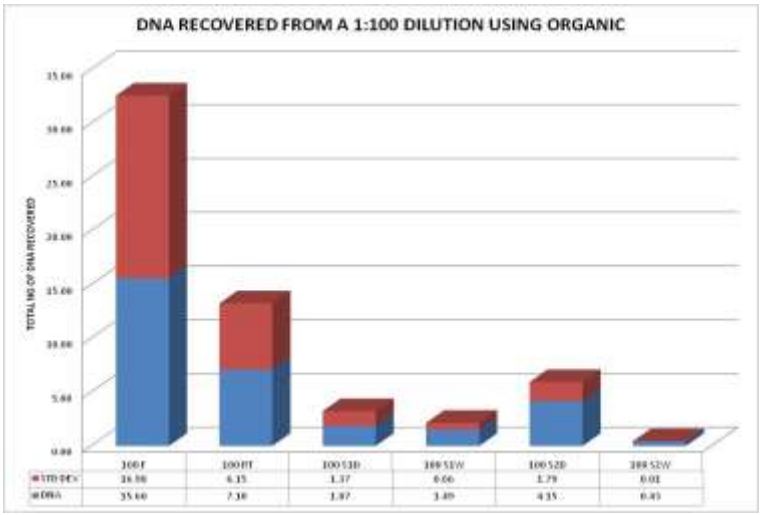


Fig. 67: DNA Recovered from 1:100 Dilutions by Organic Extraction with Storage Conditions

Storage Conditions

The overall recovery was observed by calculating the mean for each extraction method and the following figures demonstrate the results (Table 26 and Figure 68-69). Figure 69 compares the DNA recovery of samples stored at room temperature to samples stored at -20°C. An interesting observation is that the room temperature samples produced a higher mean recovery than comparable samples stored at -20°C across each extraction method. The recovery of DNA from samples stored with SampleMatrix™ was not as consistent as those samples stored without SampleMatrix™. Samples with SampleMatrix™ formulation SM1 (applied dry) have a higher mean recovery relative to samples with SampleMatrix™ SM2 (wet or dry). Room temperature samples have a higher mean recovery than samples stored with SampleMatrix™ for the Chelex and Organic extraction methods. However, SampleMatrix™ protected samples result in higher mean yields for the Qiagen extraction method. A comparison was made by calculating the ratio of DNA recovery from samples stored with SampleMatrix™ to samples without SampleMatrix™ stored at either room temperature or -20°C. Figure 70 shows that approximately 75% of the samples on average resulted in a greater DNA yield with SampleMatrix™ as compared with the control samples stored at -20°C. Specifically for Qiagen, higher recovery is observed regardless of the formulation, method of application or extraction method. In contrast, the data in Figure 71 shows that in comparison to samples stored at room temperature, only approximately 25% of the samples with SampleMatrix™ showed greater DNA recovery. Given the large standard deviations manifested across all storage conditions, any differences in the mean recovery are not statistically significant.

Table 26: Mean DNA Recovery and Std. Dev. for Each Extraction Method/Storage Medium

	STORAGE MEDIUM	CHELEX	ORGANIC	QIAGEN	MEAN
AVERAGE DNA (NG)	SM1 DRY	37.87	77.81	58.77	58.15
	SM1 WET	47.77	31.08	66.60	48.48
	SM2 DRY	33.66	43.26	71.68	49.53
	SM2 WET	37.33	66.37	40.05	47.92
	FROZEN	35.38	38.22	48.00	40.53
	ROOM TEMP	102.47	79.71	56.39	79.52
MEAN STD DEV (NG)	SM1 DRY	14.91	62.81	28.06	35.26
	SM1 WET	26.38	27.66	30.48	28.17
	SM2 DRY	18.10	40.32	17.95	25.46
	SM2 WET	2.29	79.11	15.19	32.20
	FROZEN	24.05	42.16	29.95	32.05
	ROOM TEMP	7.69	45.25	24.96	25.97

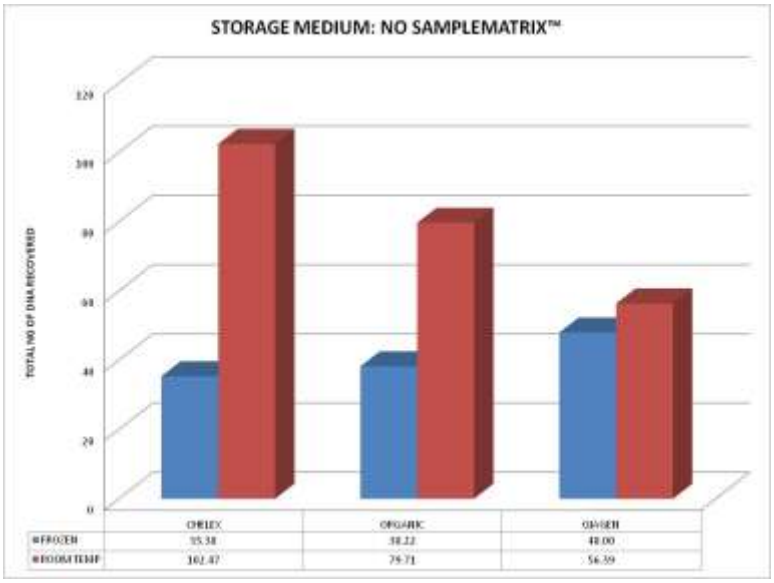


Fig. 68: Comparison of DNA Recovery Between Samples Stored at Room Temp and -20°C

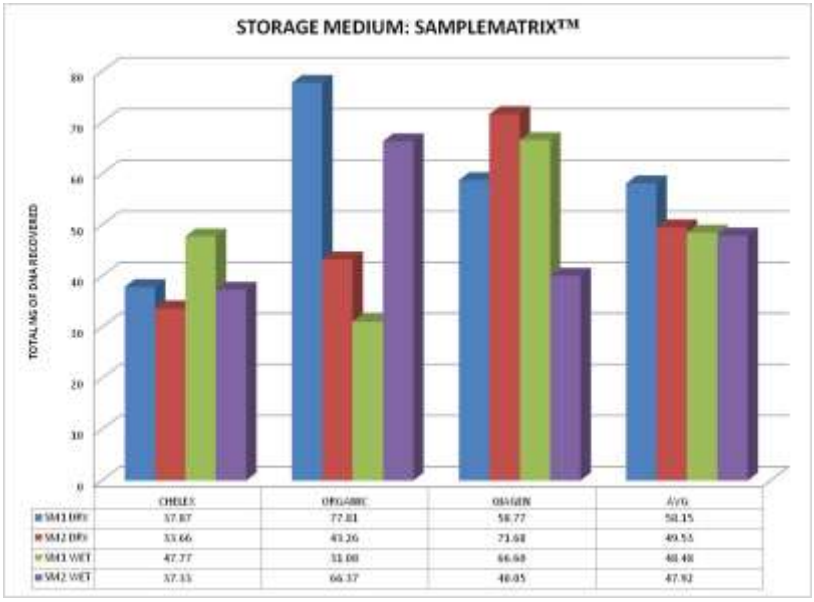


Fig. 69: Comparison of DNA Recovery Between Samples Stored at Room Temp and -20°C.

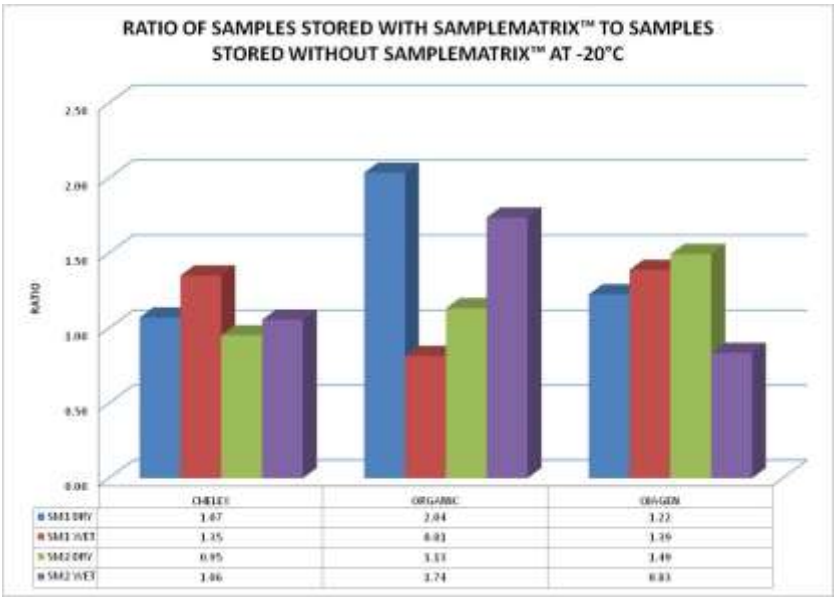


Fig. 70: Ratio of Samples Stored with SampleMatrix™ to Samples Stored without SampleMatrix™ at -20°C.

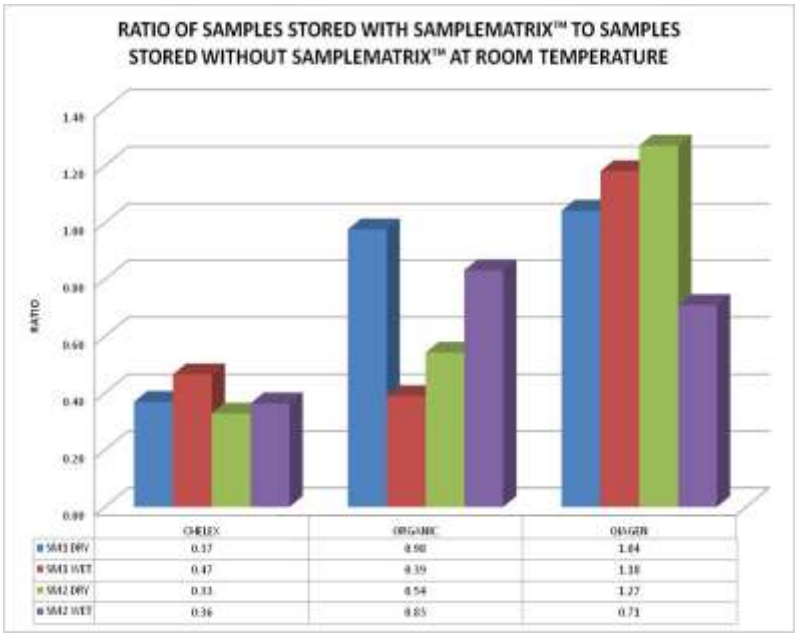


Fig. 71: Ratio of Samples Stored with SampleMatrix™ to Samples Stored without SampleMatrix™ at Room Temperature.

Formulations of SampleMatrix™

An average was calculated for both application methods (dry vs. wet) for each formulation and was compared as a function of each extraction method. Although both formulations are comparable in terms of total DNA recovered, it appears that SampleMatrix™

SM1 is slightly more effective. The Organic extraction method resulted in a marginally higher recovery (0.4ng) using SampleMatrix™ SM2. The data is also presented in an expanded form by considering both the extraction method and SampleMatrix™ application method in Figures 72-76.

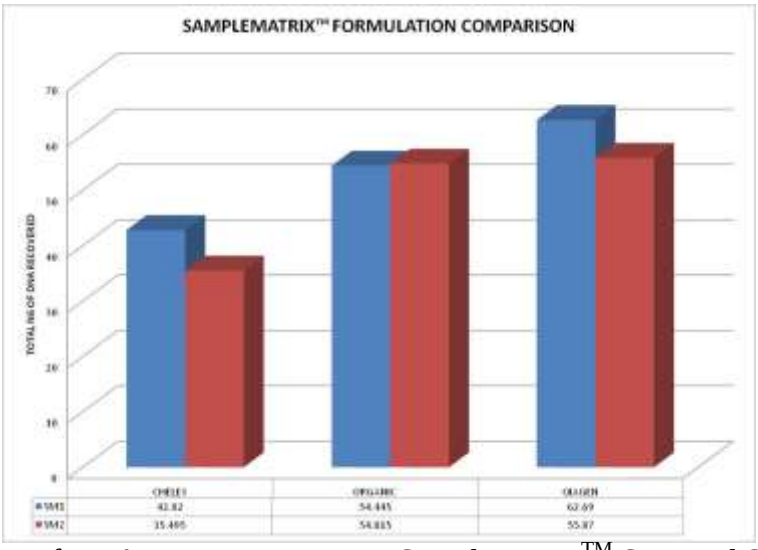


Fig. 72: Comparison of DNA Recovery Between SampleMatrix™ SM1 and SM2.

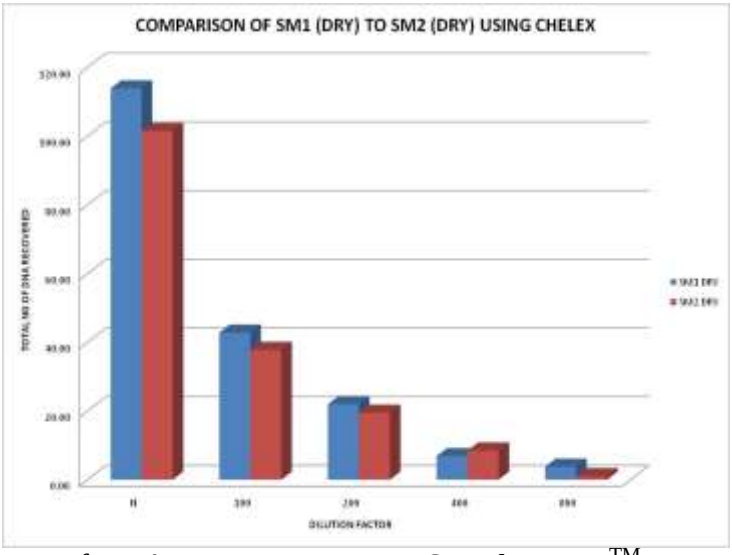


Fig. 73: Comparison of DNA Recovery Between SampleMatrix™ Formulation Using Dry Application Method for Chelex Extraction

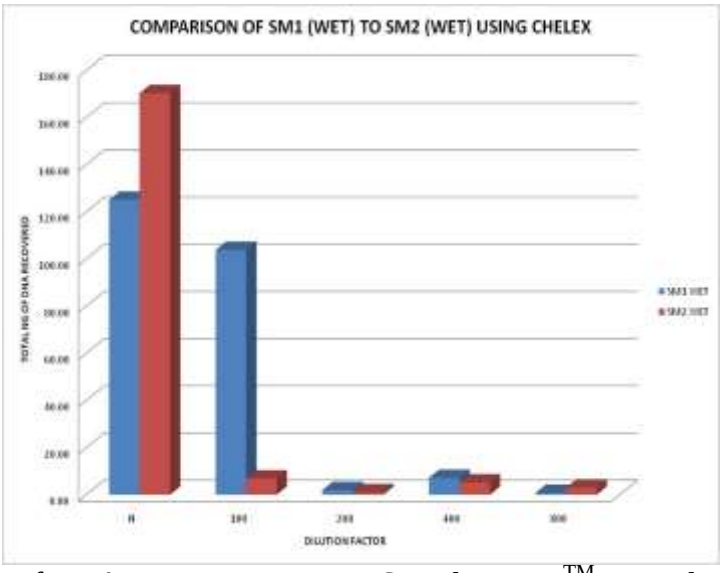


Fig. 74: Comparison of DNA Recovery Between SampleMatrix™ Formulations Using Wet Application Method and Chelex Extraction.

SampleMatrix™ Application Method

Figure 75 shows that the wet method results in a higher mean DNA recovery for the Chelex extraction method. However, the dry method produces a more predictable correlation for both formulations whereby the higher dilutions result in the highest recovery and decrease in descending order with increasing dilution (Figure 76). The Qiagen and Organic methods produce less consistent results as evident in the standard deviation values; however, SampleMatrix™ (mean of both formulations) applied using the dry method results in a higher DNA recovery relative to comparable samples prepared using the wet application method (Figures 77-79). The difference in the mean recovery is not statistically significant (ANOVA).

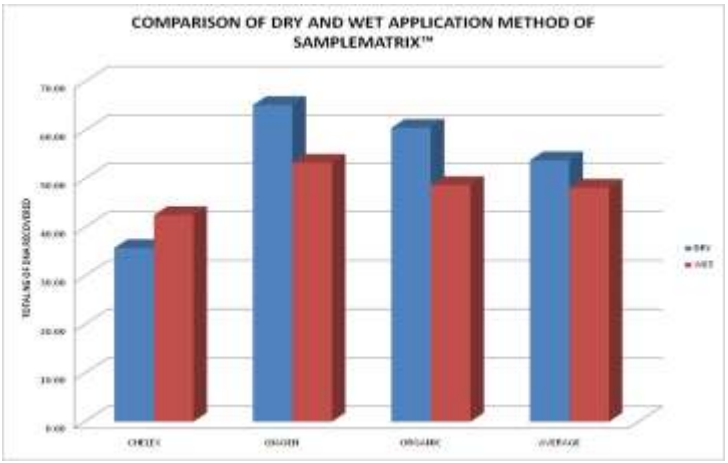


Fig. 75: DNA Recovery Comparison of Wet and Dry Application Methods.

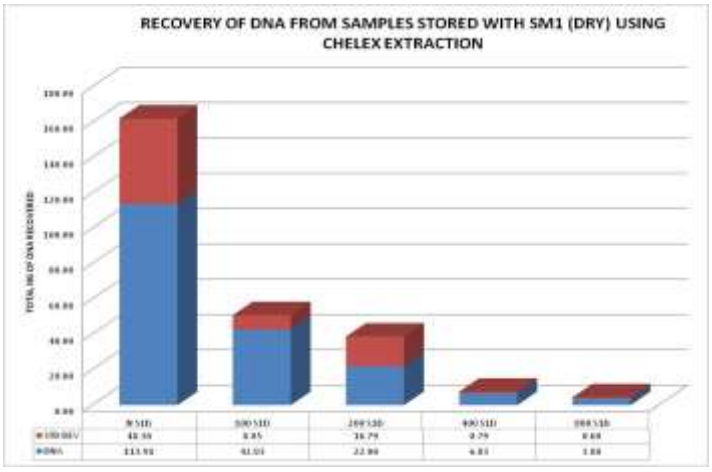


Fig.76: Recovery of DNA from Samples Stored with SM1 (Dry) Using Chelex Extraction.

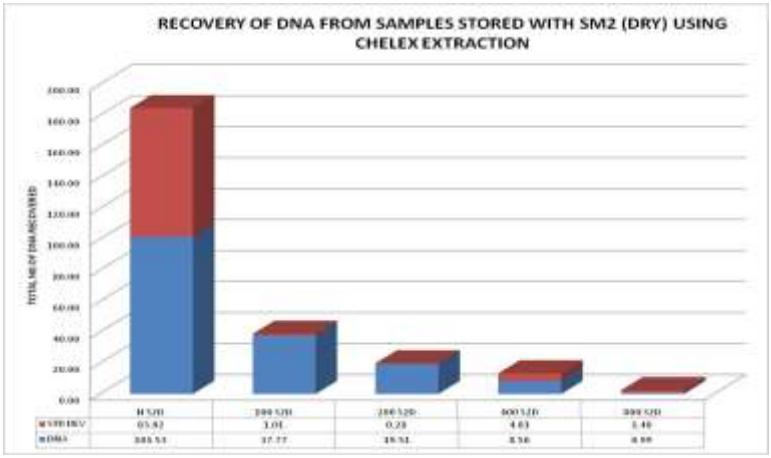


Fig. 77: Recovery of DNA from Samples Stored with SM2 (Dry) Using Chelex Extraction.

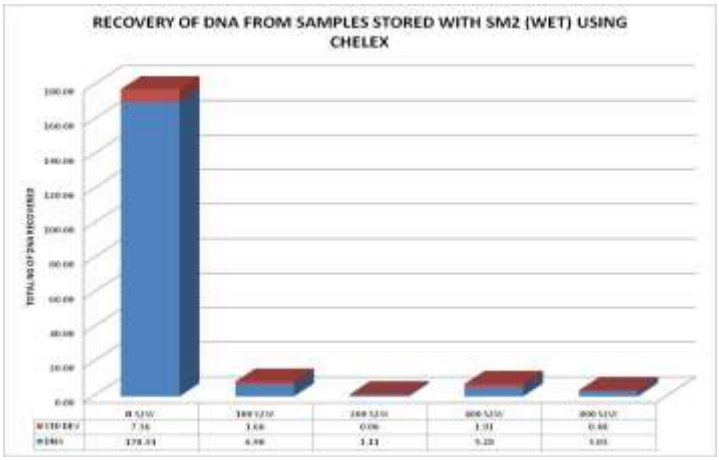


Fig. 78: Recovery of DNA from Samples Stored with SM2 (Wet) Using Chelex Extraction

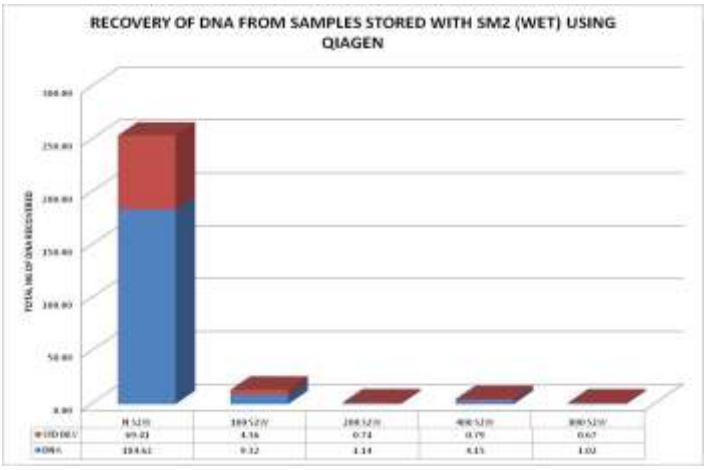


Fig. 79: Recovery of DNA from Samples Stored with SM2 (Wet) Using Qiagen Extraction

ANOVA Statistical Analysis - SampleMatrix™ as Coating Agent for Blood (6M Storage)

The ANOVA analysis was performed on data that combined all of the blood dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	10	35.3840	54.10513	17.10954	-3.3205	74.0885	1.62	180.02
RT	10	1.0247E2	183.33263	57.97487	-28.6773	233.6193	2.45	466.62
SM1D	10	37.8710	46.00339	14.54755	4.9622	70.7798	3.37	148.06
SM1W	10	47.7740	67.26258	21.27029	-.3427	95.8907	.53	173.83
SM2D	10	33.6630	47.10039	14.89445	-.0306	67.3566	.00	160.24
SM2W	10	37.3330	70.18234	22.19361	-12.8724	87.5384	1.06	175.55
Total	60	49.0827	90.97561	11.74490	25.5812	72.5842	.00	466.62

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35412.025	5	7082.405	.844	.524
Within Groups	452905.139	54	8387.132		
Total	488317.163	59			

B. SEMEN STUDIES (N = 180)

Storage Condition

The overall recovery was calculated by taking an average of all dilutions for a certain storage condition for each extraction method. These values are displayed in Table 27. When extracted via the Chelex or Organic methods, a higher recovery of DNA was seen when the samples were stored frozen (-20 °C). However, samples extracted using the Qiagen method showed a higher recovery of DNA when samples were stored at room temperature rather than -20 °C. Considering only the Qiagen extraction method, SampleMatrix™ protected semen samples gave a higher DNA recovery relative to the other storage conditions when considering the optimal coating approach of applying to a dried semen swab. SampleMatrix™ formulation SM2 applied dry resulted in the highest mean DNA recovery, followed by SM1 applied dry, SM2 applied wet and finally SM1 applied wet. These results were not consistent with samples extracted using the Chelex method. For Chelex extractions, recovery of DNA was highest when stored using SM1 applied wet, followed by SM1 applied dry, then SM2 applied wet and finally SM2 applied dry. Figure 80 presents a comparison of samples treated with SampleMatrix™ to samples that were untreated and stored at -20 °C. Figure 81 presents a similar comparison of the ratios of DNA recovery of each treated samples to those stored at room temperature. ANOVA statistical analysis was performed by combining the dilution series and comparing the mean DNA recovery across for each storage condition and each extraction chemistry. The results support that there is no significant difference in the mean yield, regardless of storage condition or extraction chemistry

Table 27: Mean DNA Recovered and Mean Standard Deviation of Samples for Each Storage Condition (Including All Extraction Methods)

Mean DNA Recovered (ng)				
Storage Condition	**Chelex**	**Qiagen**	**Organic**	**MEAN (ALL EXTRACTIONS)**
SM1 DRY	5.64	92.99	27.04	41.89
SM1 WET	16.16	23.04	7.05	15.41
SM2 DRY	2.71	103.52	69.67	58.64
SM2 WET	4.80	41.97	10.06	18.94
ROOM TEMP	4.63	110.04	77.49	64.06
FROZEN	10.41	89.63	113.36	71.13
Mean Standard Deviation (ng)				
Storage Condition	**Chelex**	**Qiagen**	**Organic**	**MEAN (ALL EXTRACTIONS)**
SM1 DRY	1.02	38.28	18.57	19.29
SM1 WET	21.01	20.93	7.68	16.54
SM2 DRY	2.47	5.88	11.87	6.74
SM2 WET	1.60	16.22	10.53	9.45
ROOM TEMP	3.81	9.63	42.03	18.49
FROZEN	9.90	18.92	146.64	58.49

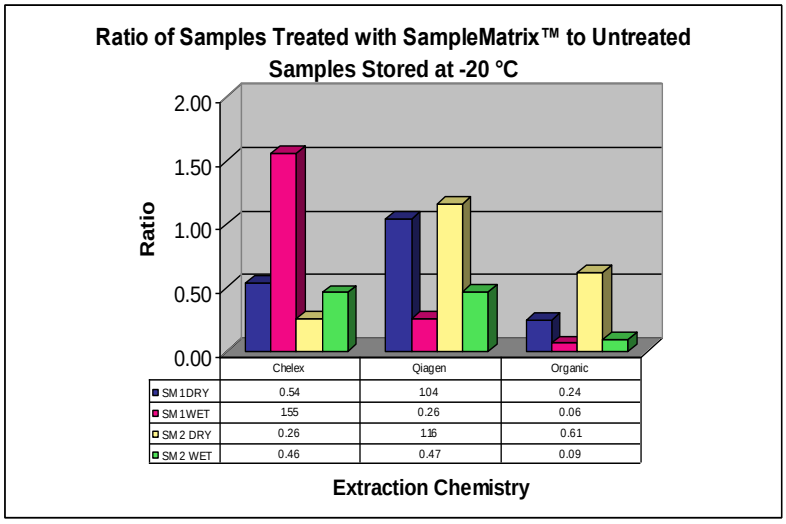


Fig. 80: Comparison of Ratios of Samples Treated with SampleMatrix™ to Untreated Samples Stored at -20 °C.

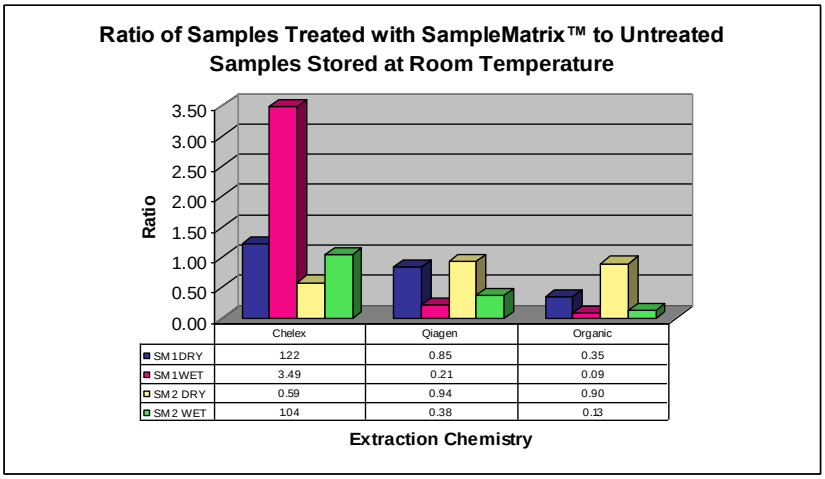


Fig. 81: Comparison of Ratios of Samples Treated with SampleMatrix™ to Untreated Samples Stored at Room Temperature.

Extraction Chemistry

The mean DNA recovery was calculated for all dilutions using each extraction method and at each storage condition. These values are shown in Table 28. DNA was recovered in all instances. However, the mean standard deviation was greater than the mean DNA recovered for the following four samples: SM1 applied wet (Chelex), SM1 applied wet (Organic), SM2 applied wet (Organic) and frozen (-20 °C/Organic). Ratios were also calculated to compare the samples treated with SampleMatrix™ with respect to an average of all of the samples (Figure 82).

Table 28: Average DNA Recovered and Average Standard Deviation of Samples for Each Extraction Method (Including All Storage Conditions).

	Mean DNA Recovered (ng)			Mean Standard Deviation (ng)		
Storage Condition	**Chelex**	**Qiagen**	**Organic**	**Chelex**	**Qiagen**	**Organic**
SM1 DRY	5.64	92.99	27.04	1.02	38.28	18.57
SM1 WET	16.16	23.04	7.05	21.01	20.93	7.68
SM2 DRY	2.71	103.52	69.67	2.47	5.88	11.87
SM2 WET	4.80	41.97	10.06	1.60	16.22	10.53
ROOM TEMP	4.63	110.04	77.49	3.81	9.63	42.03
FROZEN	10.41	89.63	113.36	9.90	18.92	146.64
MEAN	7.39	76.87	50.78	6.64	18.31	39.55

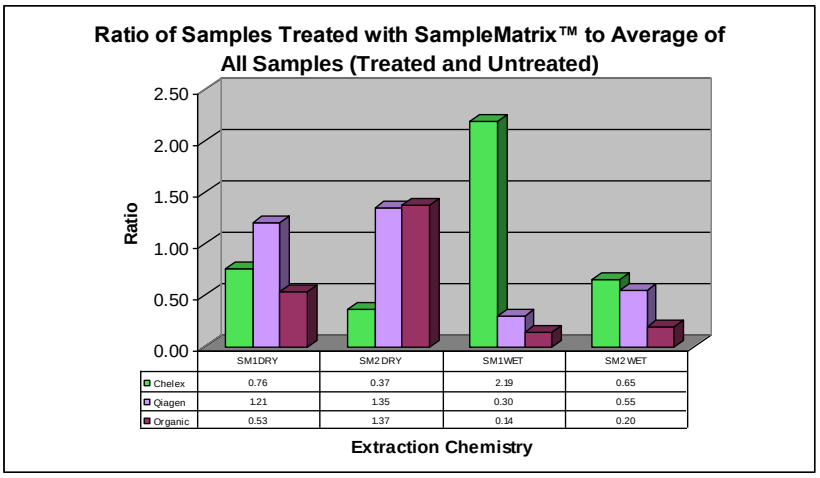


Fig. 82: Comparison of Ratios of Samples Treated with SampleMatrix™ to the Mean of All Samples (Treated and Untreated).

Figures 83-85 show the DNA recovery of the 1:50 dilution samples using each extraction method at the different storage conditions. Each of the extraction methods appears to recover DNA when the sample is either treated with SampleMatrix™ or left untreated. There does not appear to be a trend in DNA recovery values between the storage conditions and extraction method. However, it is notable that the DNA recovery values are the highest for Qiagen, slightly lower for Organic and the lowest for the samples extracted with Chelex. Figures 142-144 demonstrate the DNA recovery for the remaining dilutions using each extraction method with the different storage conditions. More visible trends based on storage condition can be seen within extraction methods between dilutions; samples treated with SampleMatrix™ showed lower DNA recovery when SampleMatrix™ was applied wet opposed to dry but the difference in the mean recovery was not statistically significant.

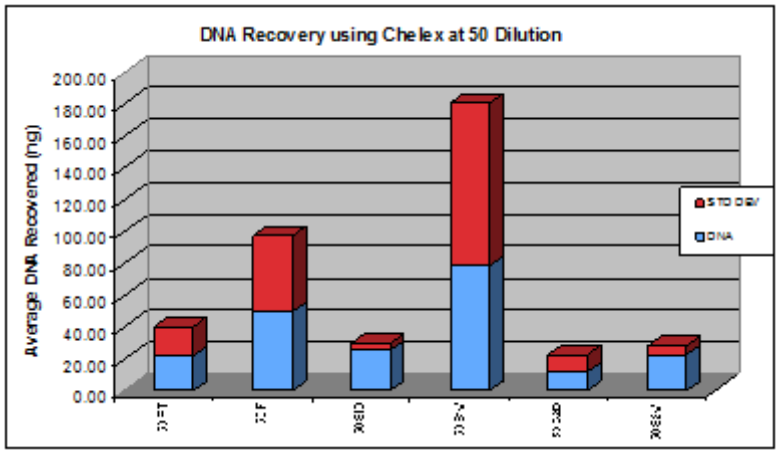


Fig. 83: DNA Recovery Using Chelex Extraction at 1:50 Dilution.

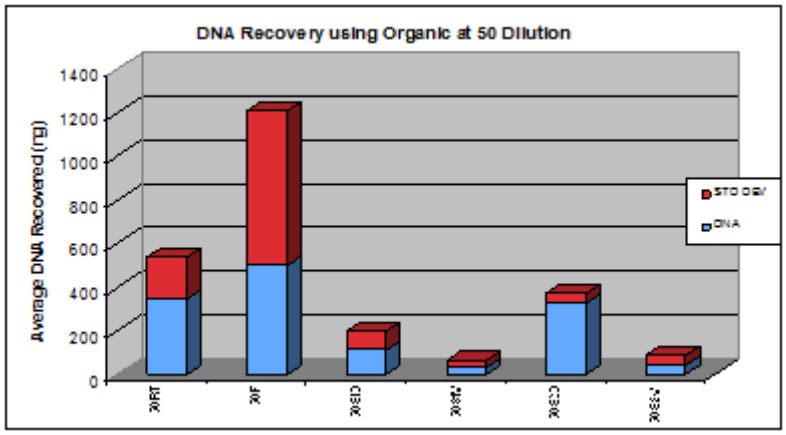


Fig. 84: DNA Recovery Using Organic Extraction at 1:50 Dilution.

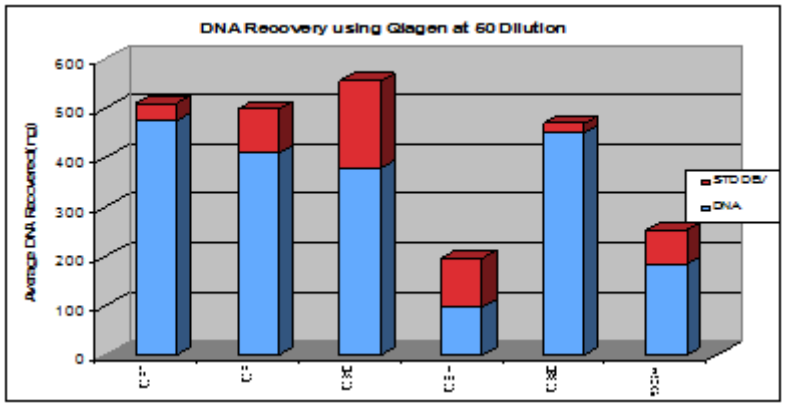


Fig. 85: DNA Recovery Using Qiagen Extraction at 1:50 Dilution.

SampleMatrix™ Application Time-Delay

To examine the possible effect of a time delay in the application of SampleMatrix™ on DNA recovery, the mean DNA yield of samples subjected to the dry and wet application methods were compared for each extraction method (Fig. 86). The Qiagen and Organic methods show a trend with the dry application of SampleMatrix™ in producing a higher mean recovery of DNA whereas the Chelex extracted samples, while exhibiting very low recovery, still show a slightly higher recovery with the wet application. A representative sample of the results is illustrated in Figures 87-90.

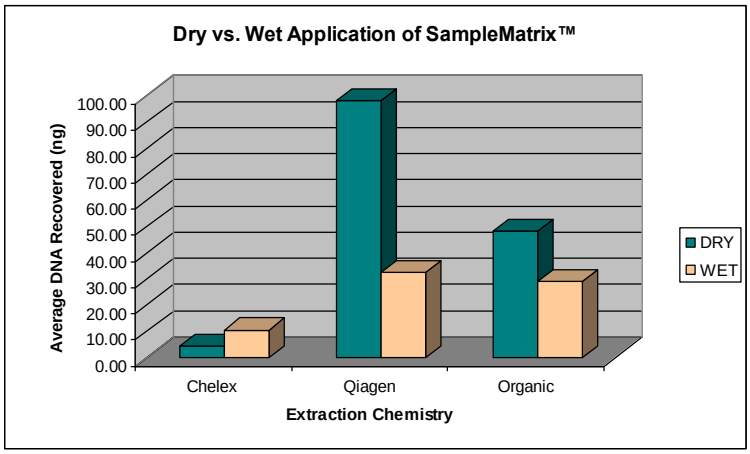


Fig. 86: Comparison of Average DNA Recoveries of SampleMatrix™ Applied Wet *v.* Dry

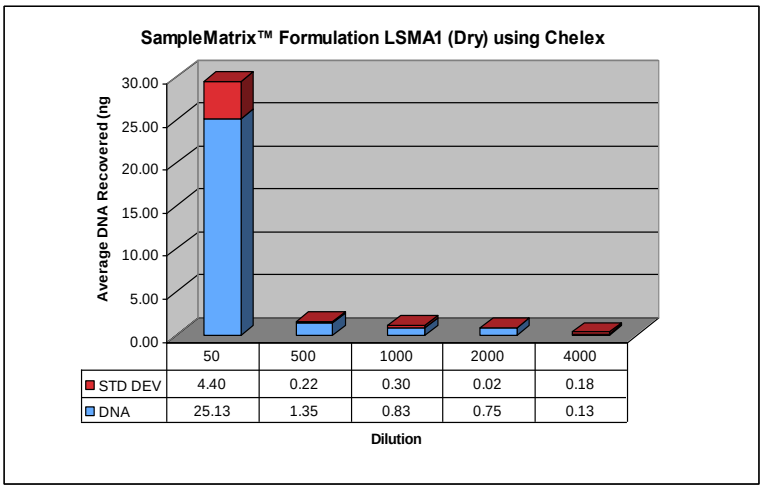


Fig. 87: Recovery of DNA for SM1 Applied Dry Using the Chelex Extraction Method.

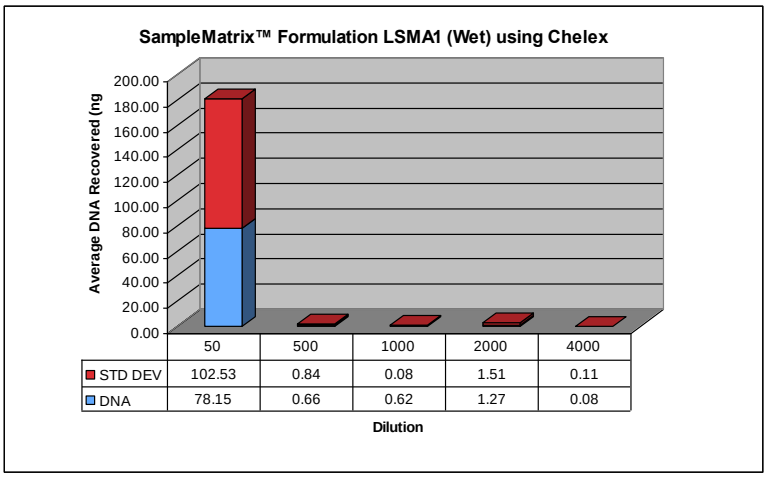


Fig. 88: Recovery of DNA for SM1 Applied Wet Using the Chelex Extraction Method.

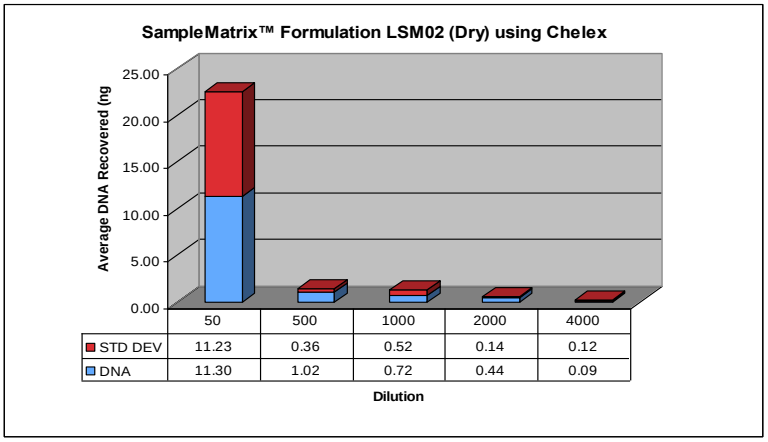


Fig. 89: Recovery of DNA for SM2 Applied Dry Using the Chelex Extraction Method.

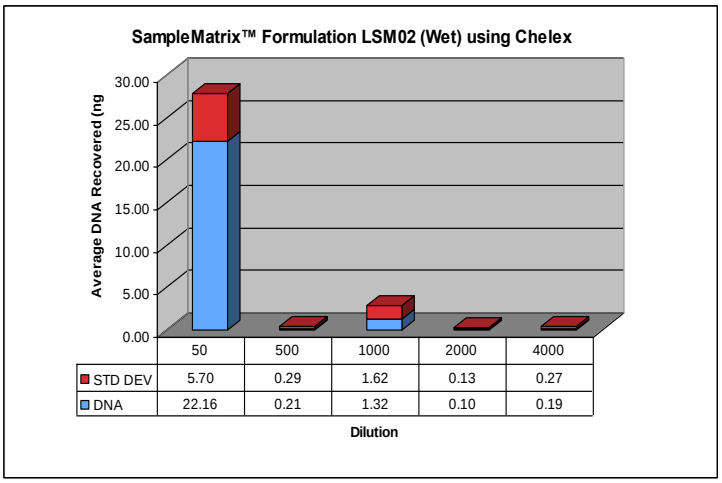


Fig. 90: Recovery of DNA for SM2 Applied Wet Using the Chelex Extraction Method.

ANOVA Statistical Analysis - SampleMatrix™ as Coating Agent for Semen (6M Storage)

The ANOVA analysis was performed on data that combined all of the semen dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	10	10.4080	25.98452	8.21703	-8.1802	28.9962	.00	83.20
RT	10	4.6330	10.89221	3.44442	-3.1588	12.4248	.00	34.77
SM1D	10	5.6370	10.38670	3.28456	-1.7932	13.0672	.00	28.24
SM1W	10	16.1550	47.28799	14.95378	-17.6728	49.9828	.00	150.65
SM2D	10	2.7140	5.88626	1.86140	-1.4968	6.9248	.00	19.24
SM2W	10	4.7940	9.37625	2.96503	-1.9134	11.5014	.00	26.19
Total	60	7.3902	22.77228	2.93989	1.5075	13.2729	.00	150.65

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1252.118	5	250.424	.461	.804
Within Groups	29343.919	54	543.406		
Total	30596.037	59			

QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	10	89.6280	170.62087	53.95506	-32.4268	211.6828	1.34	471.96
RT	10	110.0370	193.03976	61.04453	-28.0553	248.1293	4.90	499.23
SM1D	10	92.9910	162.29049	51.32076	-23.1046	209.0866	6.27	503.87
SM1W	10	23.0434	51.37709	16.24686	-13.7096	59.7964	.27	167.48
SM2D	10	103.5220	183.45590	58.01385	-27.7144	234.7584	3.50	463.84
SM2W	10	41.9720	78.47922	24.81731	-14.1686	98.1126	.00	232.76
Total	60	76.8656	147.29196	19.01531	38.8160	114.9151	.00	503.87

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	63482.054	5	12696.411	.564	.727
Within Groups	1216518.244	54	22528.116		
Total	1280000.298	59			

PCI COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	10	113.3640	312.00498	98.66464	-109.8309	336.5589	.00	1,000.73
RT	10	77.4950	155.45785	49.16009	-33.7128	188.7028	.00	481.38
SM1D	10	27.0420	53.14874	16.80711	-10.9783	65.0623	1.84	172.02
SM1W	10	7.0450	16.70279	5.28188	-4.9035	18.9935	.00	54.27
SM2D	10	69.6710	135.30799	42.78814	-27.1225	166.4645	.80	357.48
SM2W	10	10.0610	22.36734	7.07318	-5.9396	26.0616	.05	72.77
Total	60	50.7797	153.05118	19.75882	11.2424	90.3170	.00	1,000.73

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	91215.980	5	18243.196	.763	.580
Within Groups	1290839.146	54	23904.429		
Total	1382055.126	59			

C. SALIVA STUDIES (N = 180)

The results indicate that samples protected with SM1 or SM2 and stored at room temperature exceed the recovery obtained from the unprotected controls and tend to outperform frozen samples, except for the more concentrated dilutions extracted with the Qiagen method. However, the difference in the mean recovery of DNA is not statistically significant (ANOVA).

ANOVA Statistical Analysis - SampleMatrix™ as Coating Agent for Saliva (6M Storage)

The ANOVA analysis was performed on data that combined all of the saliva dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	10	2.2230	4.04988	1.28068	-.6741	5.1201	.00	12.18
RT	10	2.0350	3.59877	1.13803	-.5394	4.6094	.00	9.56
SM1D	10	2.5900	4.83076	1.52762	-.8657	6.0457	.00	13.41
SM1W	10	1.8430	2.40735	.76127	.1209	3.5651	.00	5.95
SM2D	10	.7440	1.17708	.37223	-.0980	1.5860	.00	3.92
SM2W	10	.5940	.76516	.24197	.0466	1.1414	.00	1.97
Total	60	1.6715	3.12834	.40387	.8634	2.4796	.00	13.41

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.306	5	6.661	.661	.654
Within Groups	544.097	54	10.076		
Total	577.403	59			

PCI COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	10	16.6370	28.08189	8.88027	-3.4516	36.7256	.06	89.05
RT	10	14.8040	28.13528	8.89716	-5.3228	34.9308	.05	80.78
SM1D	10	74.0150	202.13764	63.92153	-70.5856	218.6156	.02	646.46
SM1W	10	7.8890	11.72300	3.70714	-.4971	16.2751	.00	34.31
SM2D	10	12.8030	19.56342	6.18650	-1.1918	26.7978	.00	47.12
SM2W	10	12.0150	17.23567	5.45040	-.3147	24.3447	.02	44.86
Total	60	23.0272	84.46693	10.90463	1.2070	44.8473	.00	646.46

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	31631.793	5	6326.359	.878	.502
Within Groups	389313.328	54	7209.506		
Total	420945.121	59			

II. 17 - 24 MONTH STUDIES

The objective of these studies was to quantitate the total amount of DNA recovered from a blood, semen and saliva samples that was deposited on a cotton swab and stored for a 17-24 month period in order to assess if the storage condition and extraction method affected the results. The reported results for each biological fluid are based on quadruple (untreated) or duplicate (treated) sampling and are representative of the mean obtained across all trials. Separate negative controls were analyzed tested simultaneously for each dilution and storage period.

A. BLOOD (N = 258)

Extraction Chemistry Compatibility

The averages and standard deviations were calculated of the total DNA recovered from six diverse storage conditions: SM1 Dry, SM1 Wet, SM2 Dry, SM2 Wet, Frozen, and Room Temperature (Table 29).

Table 29: Average and Std Dev. of Recovered DNA for Chelex, Qiagen, and Organic Extraction

STORAGE MEDIUM	AVG DNA (ng) CHELEX	QIAGEN	ORGANIC	STD DEV (ng) CHELEX	QIAGEN	ORGANIC
SM1 DRY	177.84	153.00	788.01	240.52	307.90	1722.68
SM1 WET	131.47	80.78	769.66	201.09	166.10	1702.88
SM2 DRY	124.13	84.45	455.80	182.58	166.03	999.51
SM2 WET	209.95	65.31	179.36	277.51	138.49	384.38
FROZEN	63.33	65.08	815.08	60.72	129.45	1766.71
ROOM TEMP	332.58	114.43	926.50	564.35	235.39	2045.30
AVERAGE	173.22	93.84	655.74	254.46	190.56	1436.91

From the calculated data, it appears that the standard deviation values for all six storage conditions exceed their respective averages. This indicates that there is considerable variance in the data. Thus, although a ratio was determined of the total DNA recovered from samples stored with SampleMatrix™ to the average of all storage mediums with SampleMatrix™, the large standard deviation values should be considered (Figure 91). Differences in the mean recovery are not statistically significant.

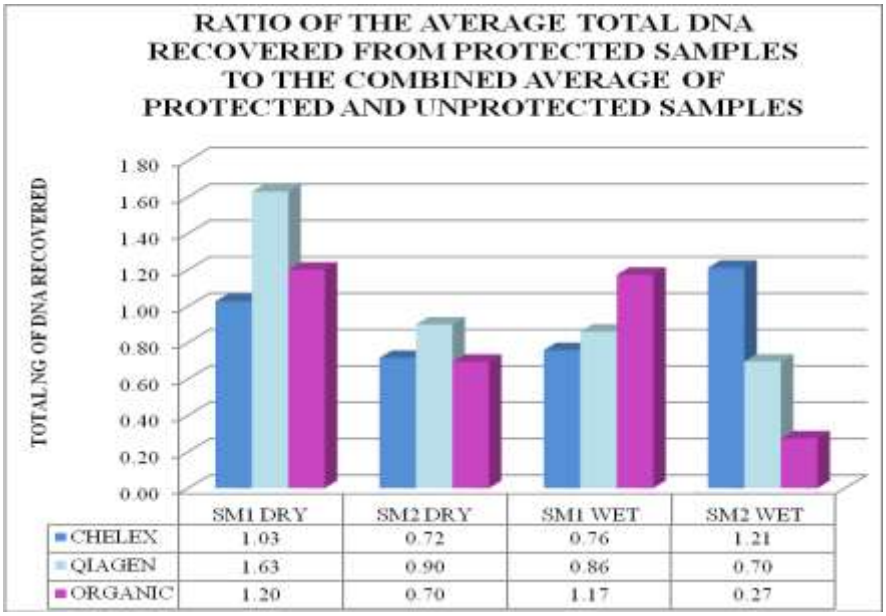


Fig. 91: Ratio of Mean Total DNA Recovered from Protected Samples to the Combined Average of Protected and Unprotected Samples.

Table 30: Ratio Comparisons of Protected Samples to Combined Mean of Protected and Unprotected Samples

	SM1 DRY	SM2 DRY	SM1 WET	SM2 WET	NSM RT	NSM F
CHELEX	1.03	0.72	0.76	1.21	1.92	0.37
QIAGEN	1.63	0.90	0.86	0.70	1.22	0.69
ORGANIC	1.20	0.70	1.17	0.27	1.41	1.24

Although unprotected (NSM) samples stored in frozen conditions indicate, for the most part, result in lower amounts of DNA recovery than both SampleMatrix™ formulations, DNA recovery from the room temperature control samples appears to be higher than either formulation. However, it is possible to conclude that the extraction methods are compatible with the SampleMatrix™ formulations. Overall, SampleMatrix™ formulations exceed or result in comparable DNA recovery when combining the total number of samples per extraction method (Figure 92).

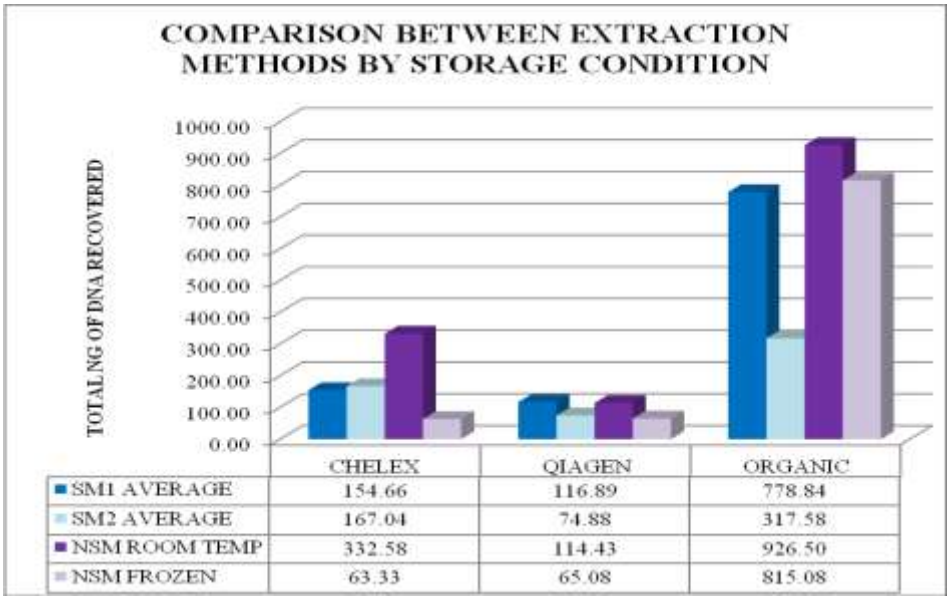


Fig. 92: Extraction Method Comparison and Total Recovered DNA.

The results indicate that the average DNA recovery from the Organic extraction is higher than either the Chelex or the Qiagen extraction (Figures 93-95). Furthermore, Figures 96-98 exhibit the total amount of DNA that is recovered as a function of dilution factor and storage condition for the three extraction techniques.

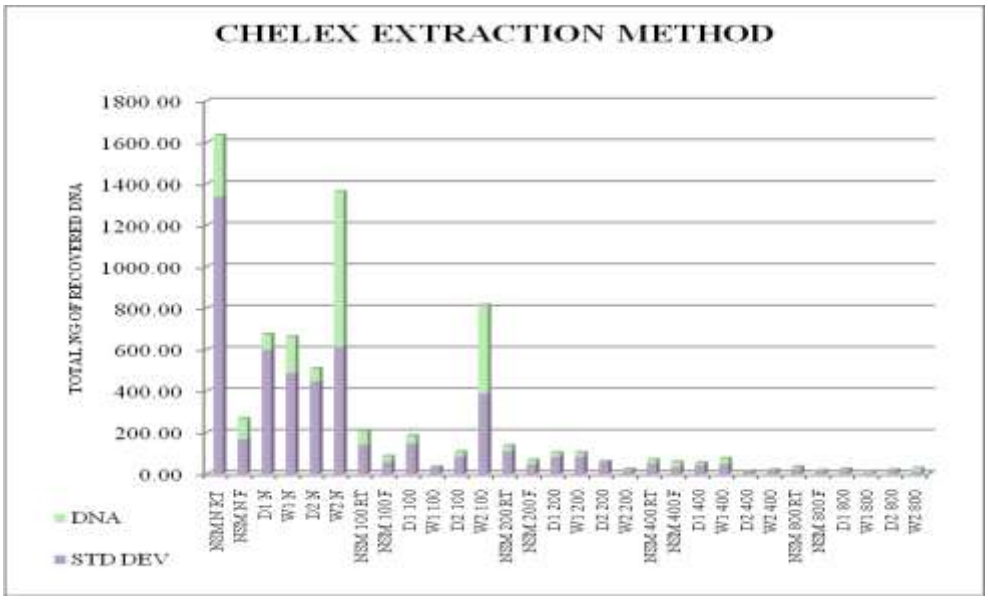


Fig. 93: Average Recovered DNA from Chelex Extraction Method for Multiple Dilutions.

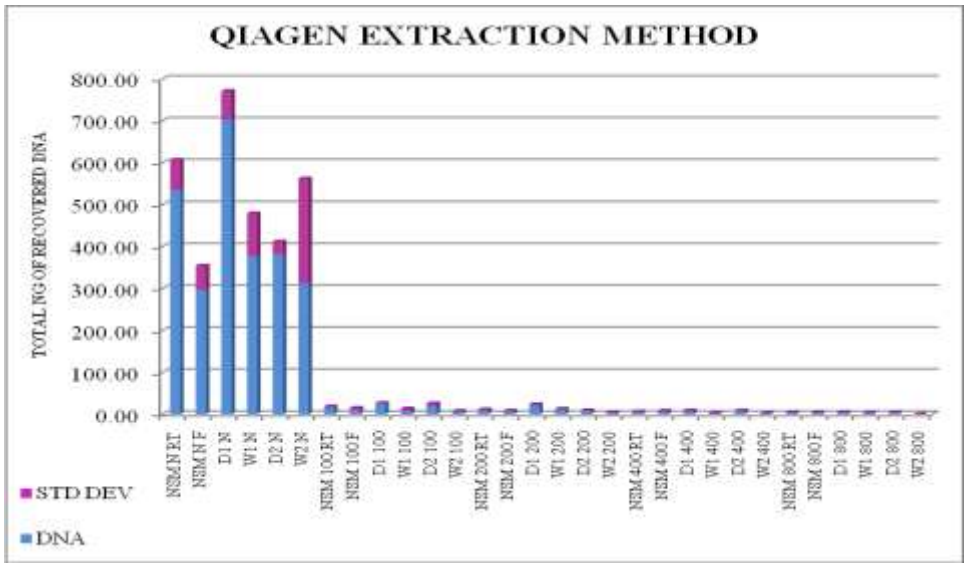


Fig. 94: Average Recovered DNA from Qiagen Extraction Method for Multiple Dilutions.

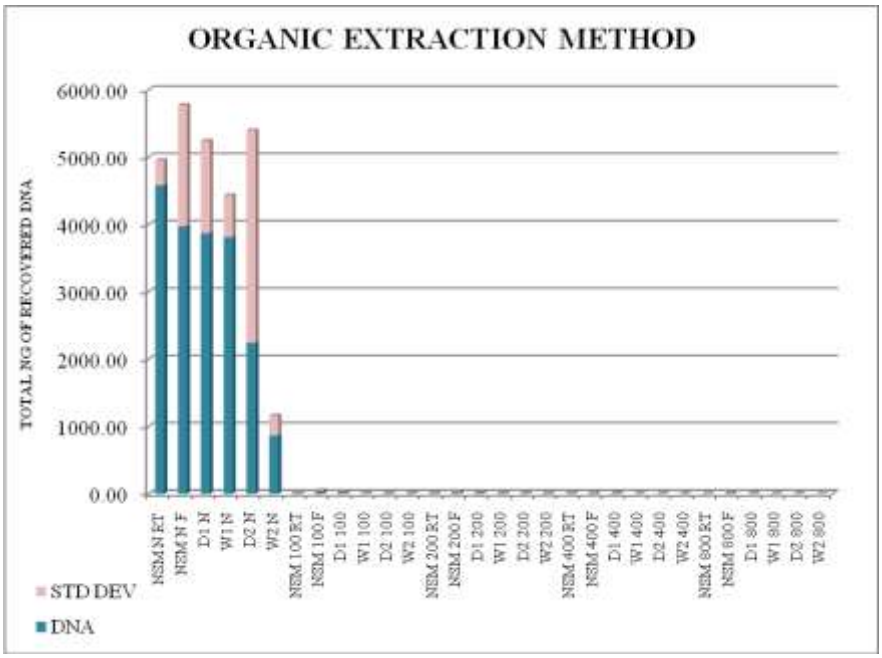


Fig. 95: Average Recovered DNA from Organic Extraction Method for Multiple Dilutions.

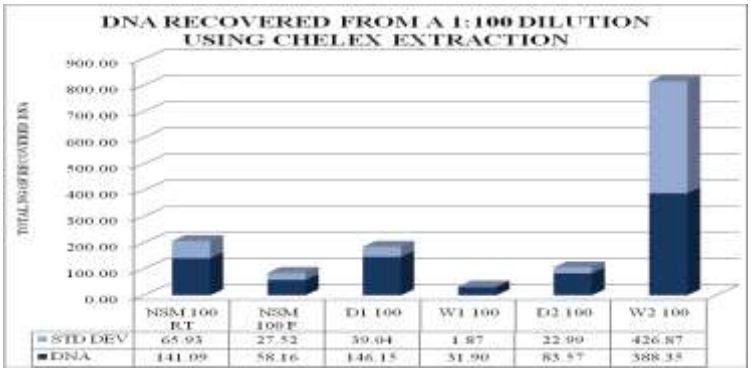


Fig. 96: DNA Recovery from a 1:100 Dilution Using the Chelex Extraction in Varying Storage Conditions.

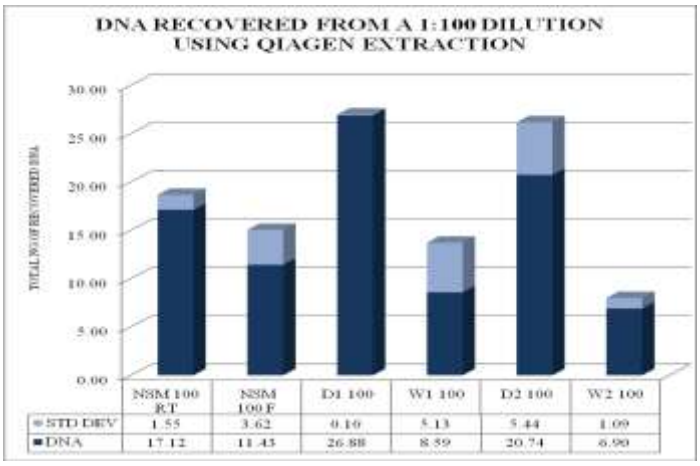


Fig. 97: DNA Recovery from 1:100 Dilutions Using Qiagen Extraction *v.* Storage Condition

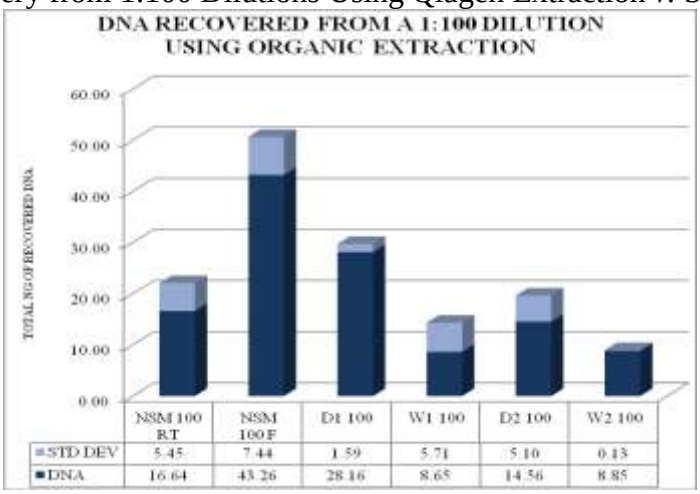


Fig. 98: DNA Recovery from 1:100 Dilutions Using Organic Extraction *v.* Storage Conditions

Generally, there does not seem to be a correlation between the amount of recovered DNA and a particular extraction method.

Storage Conditions

Averages were calculated for each extraction method in order to observe the overall recovery of DNA. Two different comparisons are made across the three diverse extraction techniques. The first comparison involves the examination of average recovered DNA quantities without protection from any of the SampleMatrix™ formulations. The second includes a comparison of average recovered DNA quantities with the protection of SM1 and SM2 formulations. These results are depicted in Table 31. It is evident that the standard deviations for each of the storage conditions are larger than their respective averages of recovered DNA. As a result, it was found that the difference in the mean recovery is not statistically significant. Only two exceptions were noted. The difference in the mean DNA recovery for 1) neat blood extracted with Chelex chemistry was statistically significant in favor of the unprotected samples stored at room temperature and 2) 1:100 dilution blood extracted with PCI chemistry was statistically significant in favor of the unprotected samples stored frozen.

Table 31: Mean Recovered DNA and Standard Deviation Amounts for Each Extraction Method and Storage Medium.

	STORAGE MEDIUM	CHELEX	QIAGEN	ORGANIC	AVERAGE
MEAN DNA (NG)	SM1 DRY	177.84	153.00	788.01	372.95
	SM1 WET	131.47	80.78	769.66	327.30
	SM2 DRY	124.13	84.45	455.80	221.46
	SM2 WET	209.95	65.31	179.36	151.54
	FROZEN	63.33	65.08	815.08	314.50
	ROOM TEMP	332.58	114.43	926.50	457.84
MEAN STD DEV (NG)	SM1 DRY	240.52	307.90	1722.68	757.03
	SM1 WET	201.09	166.10	1702.88	690.02
	SM2 DRY	182.58	166.03	999.51	449.37
	SM2 WET	277.51	138.49	384.38	266.79
	FROZEN	60.72	129.45	1766.71	652.29
	ROOM TEMP	564.35	235.39	2045.30	948.35

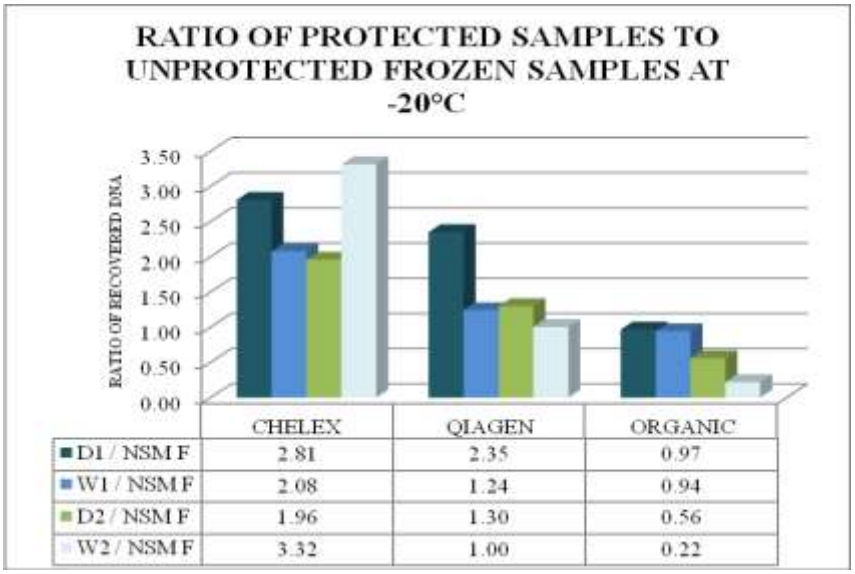


Fig. 99: Comparison of Ratios of Protected Samples at Room Temperature *v.* Unprotected Samples Stored at -20°C for Each Extraction.

Figure 99 reveals that DNA recovery is greater at room temperature with both SampleMatrix™ formulations than the unprotected samples stored at -20°C. While the Organic extraction displayed the least amount of DNA recovery when comparing the ratio of SampleMatrix™ samples to unprotected frozen samples, the Chelex extraction had the highest ratio. It was also observed that unprotected samples stored at room temperature exhibit higher DNA yields as compared with samples stored frozen; this holds true across each extraction chemistry.

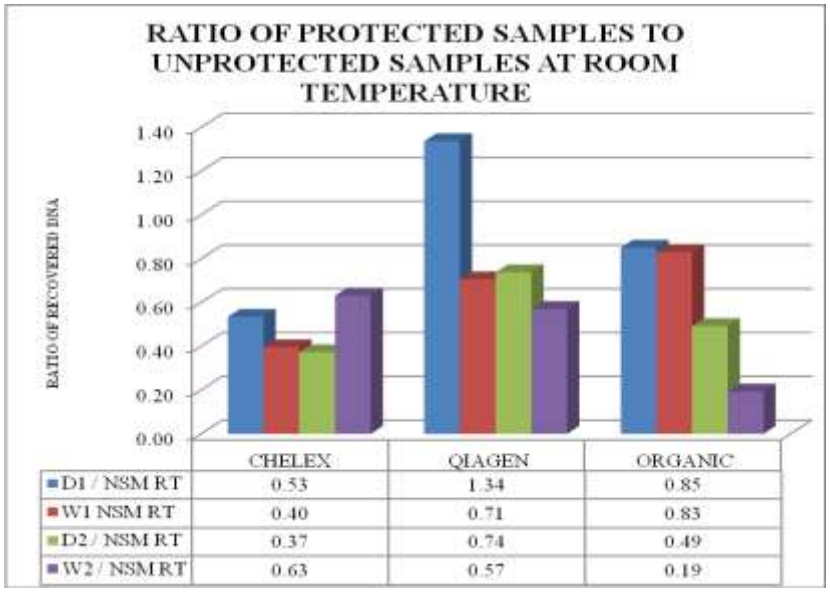


Fig. 100: Comparison of Ratios of Protected Samples at Room Temperature to Unprotected Samples Stored at Room Temperature for Each Extraction

It is also apparent that the unprotected samples for a given extraction method displayed a greater consistency than protected samples stored with either of the two SampleMatrix™ formulations. Unprotected samples stored at room temperature also resulted in a higher average DNA recovery than protected samples stored with either SampleMatrix™ formulation. In addition, comparisons were made of each extraction by calculating the ratios of recovered DNA from protected samples to unprotected samples both at room temperature and at -20°C. As indicated earlier, the Chelex extraction exhibited the greatest DNA recovery of protected sample to unprotected sample at -20°C, regardless of the method of application and the type of formulation.

Formulations of SampleMatrix™

For each formulation, a comparison was made of each of the three extraction techniques for both wet and dry application methods. For the dry application method of the Chelex and Organic extractions, the SM1 formulation resulted in an overall greater DNA recovery than the SM 2 formulation. With the exception of the 1:400 and 1: 800 dilution series, the dry application method with Qiagen extraction also exhibited a similar pattern whereby the SM1 formulation resulted in a higher DNA yield as compared with the SM2 formulation. For the wet application method, the recovery for samples protected with SM1 and SM2 formulations varied based on extraction technique and no clear trend was evident.

SampleMatrix™ Application Method

The objective of this part of the study was to determine whether the time delay in the application of the SampleMatrix™ formulation affects DNA recovery.

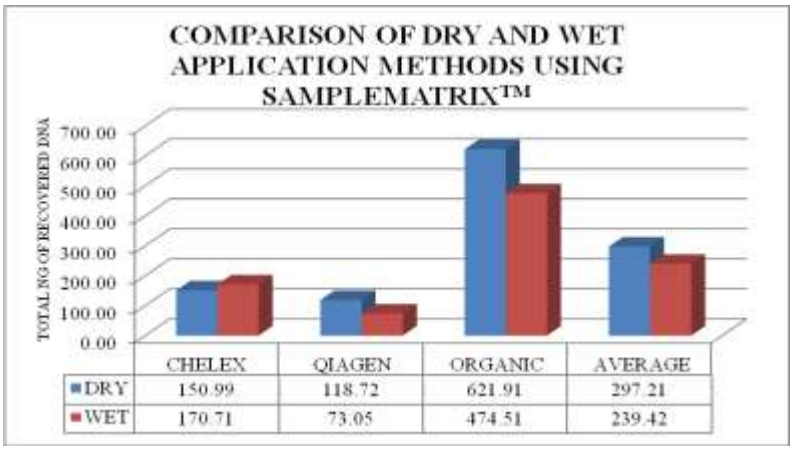


Fig. 101: Average DNA Recovery Comparison for All Dilutions of the Dry and Wet Application Methods.

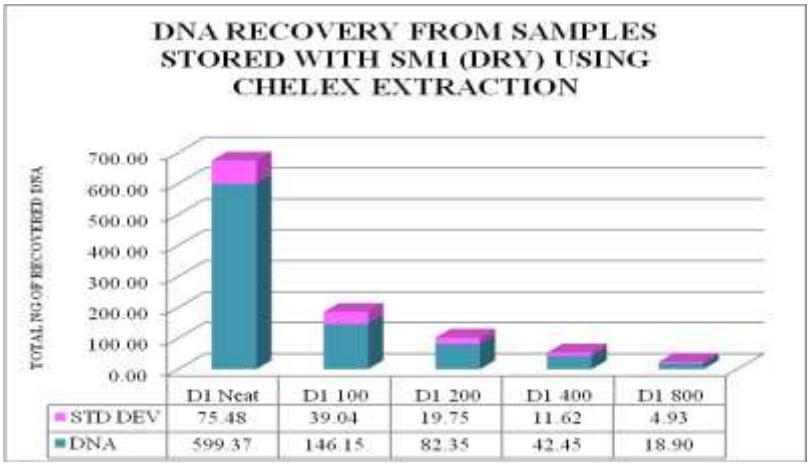


Fig. 102: DNA Recovery From Samples Stored with SM1 (Dry) Using Chelex Extraction.

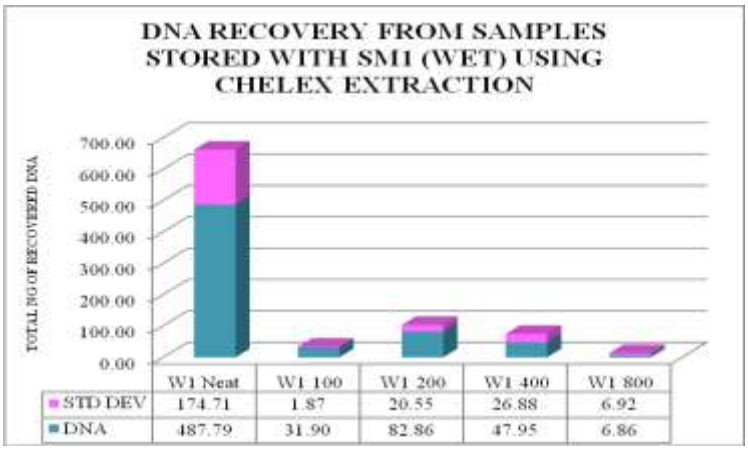


Fig. 103: DNA Recovery from Samples Stored with SM1 (Wet) Using Chelex Extraction.

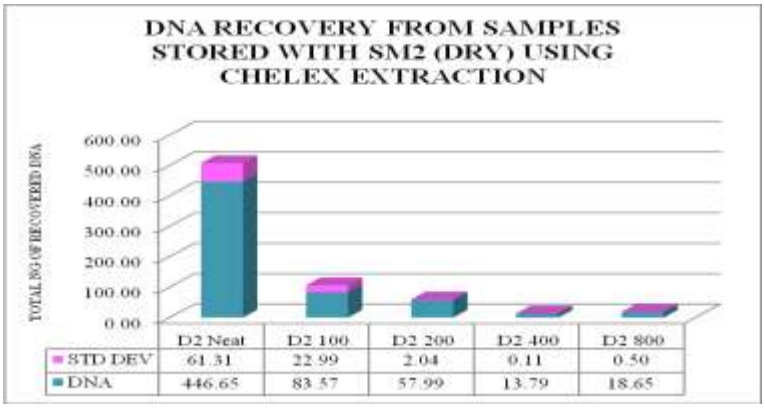


Fig. 104: DNA Recovery from Samples Stored with SM2 (Dry) Using Chelex Extraction.

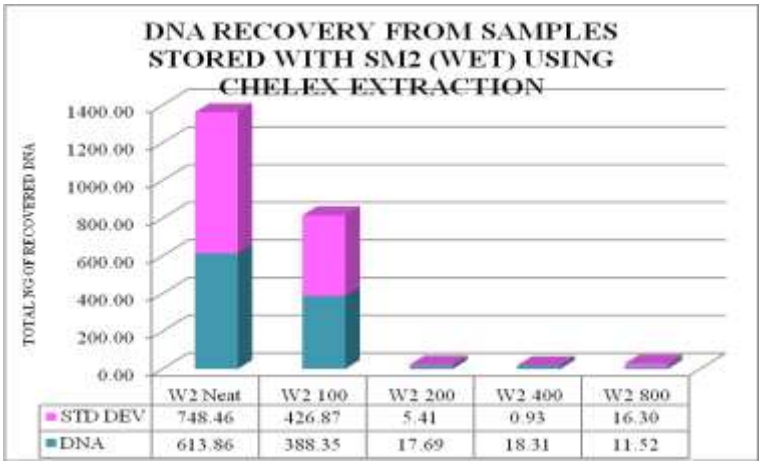


Fig. 105: DNA Recovery from Samples Stored with SM2 (Wet) Using Chelex Extraction.

From Figure 101, it appears that the Organic extraction method resulted in the greatest DNA recovery for both dry and wet application methods. Using the dry application method and the SM1 formulation, a consistent pattern was exhibited of decreasing recovered DNA displayed with increasing dilution concentrations (Figures 102-105). These resultant DNA yields associated with the dry application method for each extraction also had low standard deviation values, with the exception of the neat sample from the Organic SM2 extraction. In addition, despite the exception for the SM2 formulation extracted with Chelex, the dry technique resulted in an overall higher recovery than the wet application method for the remaining extractions and SM formulations (Figures 102-105). Again, the large standard deviation values meant that any differences observed in the mean recovery are not statistically significant.

ANOVA Statistical Analysis - SampleMatrix™ as Coating Agent for Blood (17-24M)

The ANOVA analysis was performed on data that combined all of the blood dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	20	63.3311	70.44463	15.75190	30.3620	96.3002	7.78	272.98
RT	20	332.5757	531.99527	118.95776	83.5943	581.5572	13.83	1697.96
SM1D	10	177.8447	228.66111	72.30899	14.2704	341.4190	15.41	652.74
SM1W	10	131.4700	198.66536	62.82350	-10.6466	273.5866	1.97	611.33
SM2D	10	124.1301	173.51649	54.87073	.0039	248.2563	13.71	490.00
SM2W	10	209.9475	388.55523	122.87195	-68.0082	487.9032	.00	1143.10
Total	80	179.4007	332.40115	37.16358	105.4285	253.3730	.00	1697.96

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	801572.034	5	160314.407	1.497	.201
Within Groups	7927179.511	74	107124.047		
Total	8728751.545	79			

QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	20	65.0761	121.02656	27.06236	8.4340	121.7183	2.80	352.13
RT	20	114.4321	217.86346	48.71575	12.4689	216.3953	3.18	620.49
SM1D	10	152.9959	291.17260	92.07686	-55.2964	361.2882	4.59	751.43
SM1W	10	80.7806	160.23756	50.67157	-33.8464	195.4077	1.15	449.74
SM2D	10	84.4479	156.88438	49.61120	-27.7804	196.6763	3.57	403.13
SM2W	10	65.3145	154.80103	48.95239	-45.4235	176.0524	.37	489.41
Total	80	92.8194	184.25672	20.60053	51.8151	133.8238	.37	751.43

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	70663.415	5	14132.683	.400	.847
Within Groups	2611429.036	74	35289.582		
Total	2682092.452	79			

PCI COMBINATION ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	20	815.0837	1771.69808	396.16373	-14.0965	1644.2639	8.64	6576.20
RT	18	1028.1576	1962.55895	462.57958	52.2000	2004.1152	2.38	5068.80
SM1D	10	788.0141	1687.25242	533.55606	-418.9736	1995.0018	1.92	4839.20
SM1W	10	769.6601	1618.54323	511.82831	-388.1760	1927.4962	3.18	4251.00
SM2D	10	455.8007	1411.71835	446.42454	-554.0818	1465.6831	2.55	4473.60
SM2W	10	179.3565	375.39960	118.71178	-89.1882	447.9012	1.02	1074.64
Total	78	727.3952	1608.00891	182.07116	364.8452	1089.9452	1.02	6576.20

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5577739.328	5	1115547.866	.415	.837
Within Groups	1.935E8	72	2687786.053		
Total	1.991E8	77			

T Test (Independent Samples) Statistical Analysis - SampleMatrix™ as Coating Agent for Blood (Stored 6-24M)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations (including wet v dry application) as compared to the unprotected control with each extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison. The results are based on samples that were stored for a period of 6-24 months. Representative results are presented below:

CHELEX 1:100 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	105.1616	164.46690	41.11672
	NO SM Frozen	6	55.8330	21.61839	8.82567

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.935	.179	.721	20	.479	49.32858	68.38044	93.31052	191.96768
	Equal variances not assumed			1.173	16.310	.258	49.32858	42.05327	39.68277	138.33993

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	105.1616	164.46690	41.11672
	NO SM RT	6	107.4478	73.05708	29.82543

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.328	.574	-.032	20	.974	-2.28627	70.39101	149.11934	144.54680
	Equal variances not assumed			-.045	19.086	.965	-2.28627	50.79509	108.56929	103.99675

CHELEX 1:200 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	37.3460	33.63207	8.40802
	NO SM F	6	37.0749	21.45888	8.76055

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.950	.178	.018	20	.986	.27108	14.85909	30.72443	31.26660
	Equal variances not assumed			.022	14.385	.982	.27108	12.14257	25.70686	26.24902

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	35.6579	34.31458	8.57864
	NO SM RT	6	77.2185	54.06323	22.07122

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.754	.067	-2.161	20	.043	-41.56063	19.23110	81.67599	-1.44526
	Equal variances not assumed			-1.755	6.575	.125	-41.56063	23.67978	98.29688	15.17563

CHELEX NEAT Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	332.3146	297.34154	74.33539
	NO SM F	6	144.4556	99.64413	40.67955

SIG DIFF AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	4.794	.041	1.496	20	.150	187.85906	125.55739	-74.04906	449.76718
	Equal variances not assumed			2.217	19.959	.038	187.85906	84.73827	11.07503	364.64308

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	332.3146	297.34154	74.33539
	NO SM RT	6	1042.0733	514.06338	209.86549

SIG DIFF AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.718	.115	-4.075	20	.001	-709.7587	174.17168	-1073.0745	-346.443
	Equal variances not assumed			-3.188	6.300	.018	-709.7587	222.64158	-1248.3109	-171.2065

CHELEX 1:800 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	18.7897	18.08849	4.52212
	NO SM F	6	23.2540	24.45825	9.98504

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.844	.190	-.469	20	.644	-4.46430	9.51360	24.30932	15.38072
	Equal variances not assumed			-.407	7.161	.696	-4.46430	10.96132	30.26603	21.33743

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	18.7897	18.08849	4.52212
	NO SM RT	6	35.5838	26.01522	10.62067

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.238	.279	-1.723	20	.100	-16.79407	9.74735	37.12669	3.53856
	Equal variances not assumed			-1.455	6.902	.190	-16.79407	11.54332	44.16873	10.58060

CHELEX 1:400 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.0690	8.63806	2.15952
	NO SM F	6	9.4896	6.61776	2.70169

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.058	.167	-.363	20	.721	-1.42051	3.91583	9.58879	6.74777
	Equal variances not assumed			-.411	11.822	.689	-1.42051	3.45870	-8.96901	6.12799

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.0690	8.63806	2.15952
	NO SM RT	6	17.0140	13.67287	5.58192

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.875	.186	-1.844	20	.080	-8.94496	4.85131	19.06461	1.17469
	Equal variances not assumed			-1.495	6.560	.182	-8.94496	5.98510	23.29230	5.40238

PCI NEAT Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	1483.2275	1801.52186	450.38047
	NO SM F	6	3111.9633	2301.70457	939.66696

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.099	.307	-1.755	20	.095	1628.73583	928.08370	3564.68451	307.21285
	Equal variances not assumed			-1.563	7.430	.160	1628.73583	1042.02522	4064.09251	806.62084

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	1483.2275	1801.52186	450.38047
	NO SM RT	6	2778.6400	2320.85132	947.48358

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.022	.885	-1.392	20	.179	1295.41250	930.81151	3237.05129	646.22629
	Equal variances not assumed			-1.235	7.389	.255	1295.41250	1049.07946	3749.85808	1159.03308

PCI 1:100 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.5198	9.19269	2.29817
	NO SM F	6	34.0380	17.17279	7.01076

SIG DIFF AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.319	.143	-4.552	20	.000	25.51823	5.60535	-37.21080	-13.82567
	Equal variances not assumed			-3.459	6.109	.013	-25.51823	7.37783	-43.49349	-7.54298

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.5198	9.19269	2.29817
	NO SM RT	6	13.4585	7.04638	2.87667

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.592	.451	-1.185	20	.250	4.93873	4.16761	13.63223	3.75476
	Equal variances not assumed			-1.341	11.815	.205	4.93873	3.68196	12.97500	3.09754

PCI 1:200 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.9301	8.53784	2.13446
	NO SM F	6	20.4883	12.33440	5.03550

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.098	.758	-2.508	20	.021	-11.55822	4.60923	-21.17290	-1.94354
	Equal variances not assumed			-2.113	6.884	.073	-11.55822	5.46920	-24.53509	1.41865

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.9301	8.53784	2.13446
	NO SM RT	6	11.5803	6.85676	2.79926

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.127	.301	-.679	20	.505	-2.65024	3.90158	-10.78879	5.48832
	Equal variances not assumed			-.753	11.238	.467	-2.65024	3.52019	-10.37815	5.07768

PCI 1:400 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	4.2163	4.24963	1.06241
	NO SM F	6	11.1261	8.89971	3.63329

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	10.778	.004	-2.500	20	.021	-6.90971	2.76437	-12.67608	-1.14334
	Equal variances not assumed			-1.825	5.877	.119	-6.90971	3.78543	-16.21942	2.40001

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	4.2163	4.24963	1.06241
	NO SM RT	4	6.1031	7.04418	3.52209

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.243	.152	-.699	18	.494	-1.88681	2.69951	-7.55827	3.78466
	Equal variances not assumed			-.513	3.565	.638	-1.88681	3.67883	-12.61189	8.83827

QIAGEN NEAT Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	349.7221	175.45051	43.86263
	NO SM F	6	264.1840	92.92876	37.93801

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.577	.224	1.125	20	.274	85.53813	76.06290	-73.12630	244.20255
	Equal variances not assumed			1.475	17.110	.158	85.53813	57.99330	-36.75698	207.83323

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	349.7221	175.45051	43.86263
	NO SM RT	6	428.9453	181.03780	73.90837

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.063	.804	-.936	20	.361	-79.22321	84.66714	-255.83576	97.38934
	Equal variances not assumed			-.922	8.779	.381	-79.22321	85.94404	-274.38960	115.94318

QIAGEN 1:100 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	17.1493	11.05334	2.76333
	NO SM F	6	14.1663	5.15125	2.10299

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	4.778	.041	.629	20	.537	2.98294	4.74545	-6.91590	12.88177
	Equal variances not assumed			.859	18.644	.401	2.98294	3.47255	-4.29458	10.26045

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	17.1493	11.05334	2.76333
	NO SM RT	6	27.0885	15.85020	6.47082

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.156	.158	-1.671	20	.110	-9.93920	5.94915	-22.34891	2.47051
	Equal variances not assumed			-1.413	6.913	.201	-9.93920	7.03616	-26.61944	6.74105

QIAGEN 1:200 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	16	13.8961	17.06077	4.26519
	NO SM F	6	9.3452	3.62681	1.48064

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.683	.209	.639	20	.530	4.55087	7.12610	-10.31391	19.41564
	Equal variances not assumed			1.008	18.047	.327	4.55087	4.51488	-4.93278	14.03452

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	13.8961	17.06077	4.26519
	NO SM RT	6	10.5826	1.48508	.60628

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.611	.122	.468	20	.645	3.31353	7.08195	-11.45915	18.08622
	Equal variances not assumed			.769	15.593	.453	3.31353	4.30807	-5.83857	12.46563

QIAGEN 1:400 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	5.0586	2.73086	.68271
	NO SM F	6	5.9895	2.41665	.98659

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.444	.513	-.732	20	.473	-.93087	1.27136	-3.58289	1.72115
	Equal variances not assumed			-.776	10.159	.456	-.93087	1.19978	-3.59849	1.73676

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	4.8718	2.62518	.65629
	NO SM RT	6	5.0028	1.25951	.51419

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.074	.095	-.116	20	.909	-.13108	1.12932	-2.48681	2.22465
	Equal variances not assumed			-.157	18.338	.877	-.13108	.83374	-1.88038	1.61822

QIAGEN 1:800 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM	15	2.3068	1.67578	.43268
	NO SM F	6	3.2321	1.19058	.48605

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.704	.412	-1.226	19	.235	-.92535	.75489	-2.50535	.65465
	Equal variances not assumed			-1.422	13.122	.178	-.92535	.65074	-2.32986	.47916

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	15	2.3068	1.67578	.43268
	NO SM RT	6	3.8045	1.42033	.57985

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.587	.453	-1.923	19	.070	-1.49775	.77890	-3.12801	.13251
	Equal variances not assumed			-2.070	10.910	.063	-1.49775	.72349	-3.09174	.09623

B. SEMEN (N = 258)

Extraction Compatibility and Protection

An average was taken of the total DNA recovered over six storage conditions (Table 41). To determine the extraction compatibility, a ratio was determined of the SampleMatrix™ protected samples to the average of all storage conditions (Figure 106). Figure 185 shows that approximately 30% of samples stored with SampleMatrix™ resulted in a slightly higher DNA recovery than the total average of all storage conditions. Also illustrated is that approximately another 15% of SampleMatrix™ protected samples gave an equivalent recovery when compared to the total average of all storage conditions (Figure 106). These preliminary findings suggest that the three extraction methods are compatible with both SampleMatrix™ formulations.

Figures 107-109 depicts the total DNA recovered at various dilutions for each extraction method. It appears that the Organic extraction method recovered a higher amount of DNA when compared to either the Qiagen or the Chelex method; however, the Organic method also exhibits the highest standard deviation of the three extraction chemistries (Table 32). In addition, Figure 110 shows that unprotected control samples extracted with the Organic method across all dilutions also recovered the highest amount of DNA. As a general trend, it appears that the Qiagen extraction method recovered the lowest average amount of DNA when compared to either the Organic or Chelex method; however, the Qiagen method did exhibit the lowest standard deviation of the three extraction chemistries. It was observed that the Chelex method recovered the lowest amount DNA (an average of 7ng) from the unprotected sample stored at room temperature across all dilutions (Table 32).

Table 32: Average ng of DNA across the different extraction methods.

Storage Medium	Average (ng)			Std Dev (ng)		
	Organic	Qiagen	Chelex	Organic	Qiagen	Chelex
Room Temperature	54.36	35.07	7.04	15.39	10.51	2.49
Frozen	74.13	20.74	50.32	21.30	6.26	17.77
SM1 Dry	58.30	20.69	31.76	18.55	5.56	10.76
SM1 Wet	54.98	11.51	30.29	17.29	2.32	10.04
SM2 Dry	50.66	11.94	32.15	15.52	3.41	11.61
SM2 Wet	58.57	15.21	18.49	18.75	4.59	7.18
Average (ng)	58.50	19.19	28.34	17.80	5.44	9.98

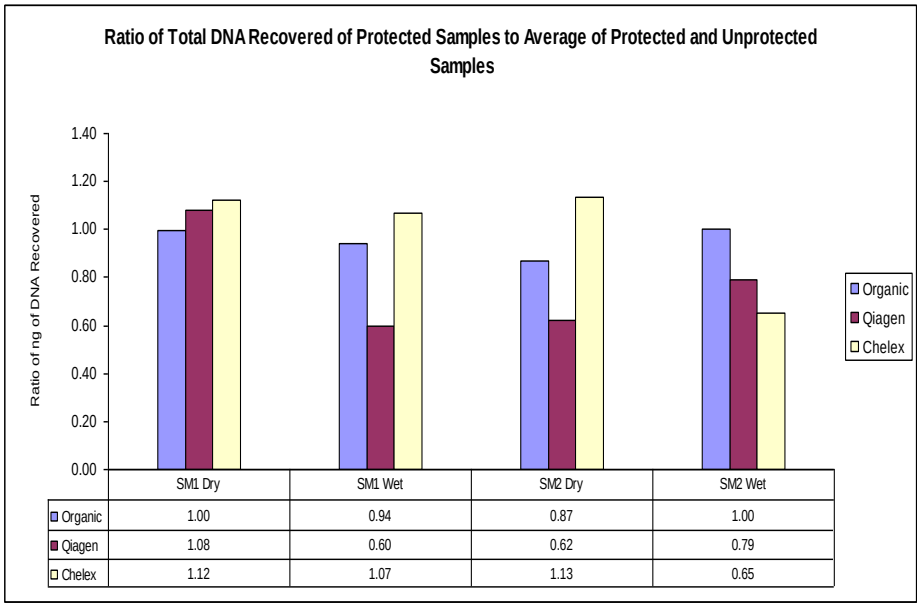


Fig. 106: Ratio of Total DNA Recovered of Protected Samples to Average of Protected and Unprotected Samples.

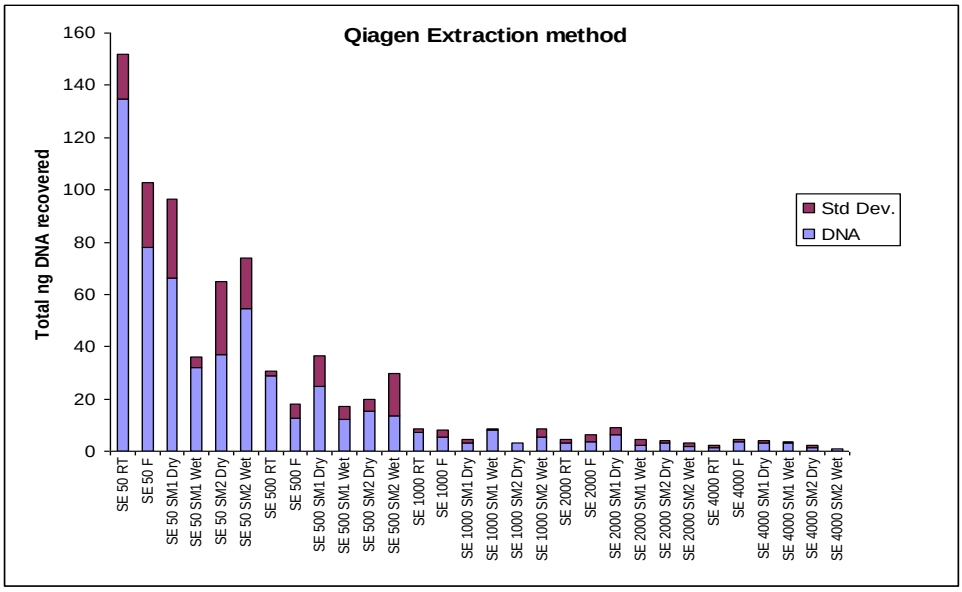


Fig. 107: DNA recovery (ng) with Qiagen extraction at multiple dilution and storage condition

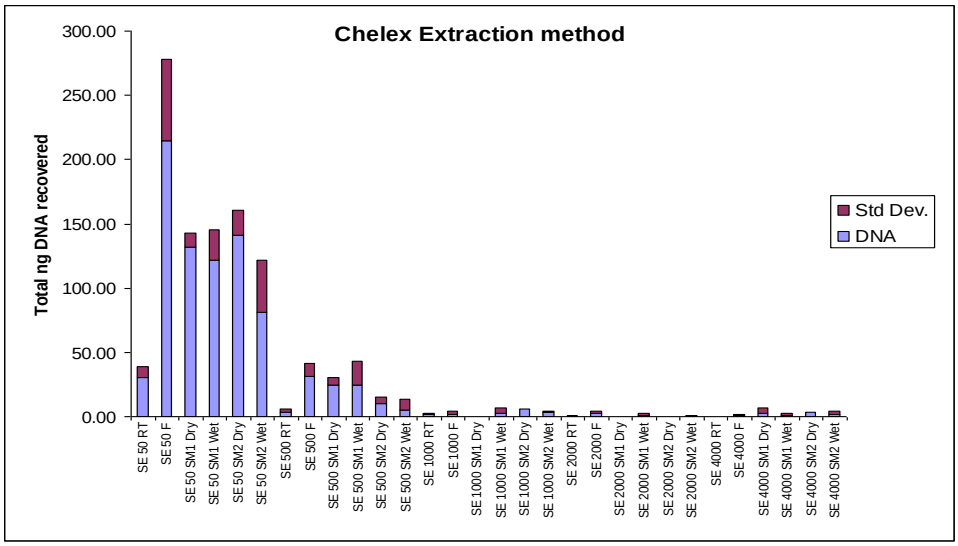


Fig. 108: DNA recovery (ng) with chelex extraction at multiple dilution and storage condition

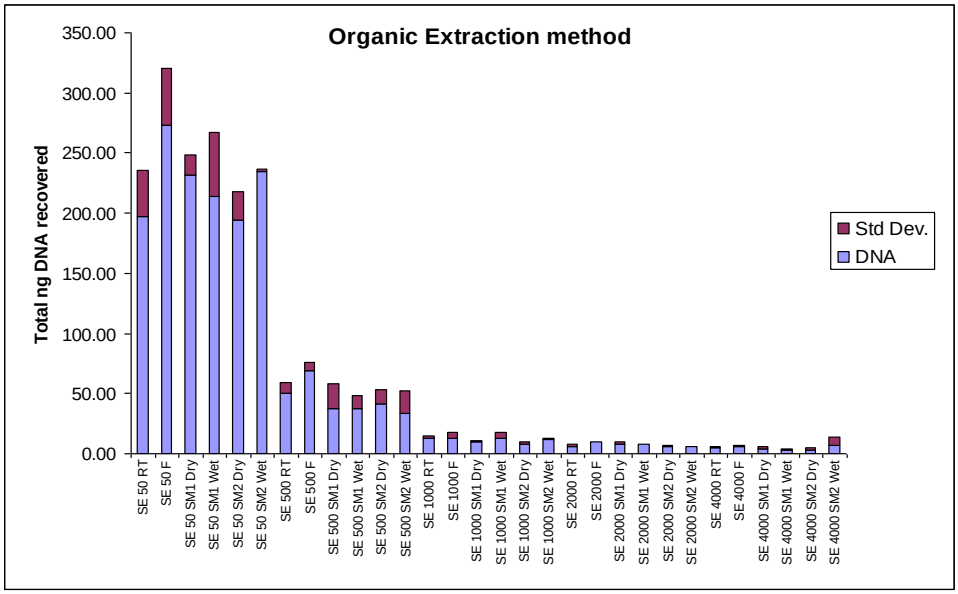
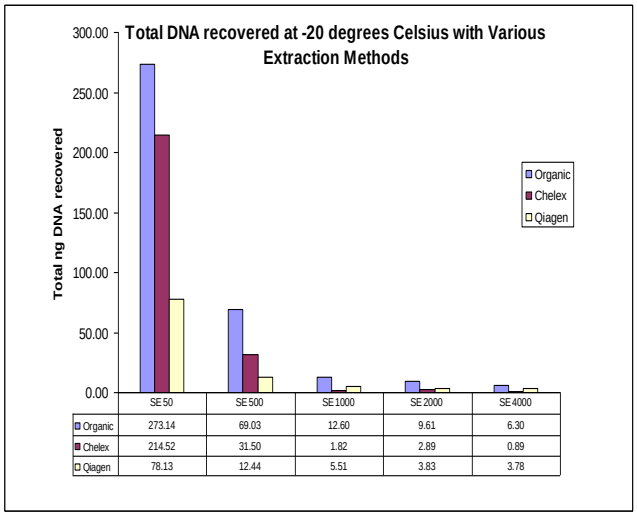


Fig. 109: DNA recovery (ng) with Organic extraction at multiple dilution and storage condition



	SE 50	SE 500	SE 1000	SE 2000	SE 4000
Organic	273.14	69.03	12.60	9.61	6.30
Chelex	214.52	31.50	1.82	2.89	0.89
Qiagen	78.13	12.44	5.51	3.83	3.78

Fig. 110: Comparison of DNA recovery at -20 degrees Celsius with various extraction method and multiple dilutions.

The ratio of SampleMatrix™ protected samples to unprotected samples at -20°C and room temperature was measured. Samples protected by SampleMatrix™ did not appear to show a higher mean DNA recovery when compared to either unprotected samples stored at -20°C or room temperature. There was some inconsistent results; SampleMatrix™ protected samples extracted with the Chelex method appear to show a higher average DNA recovery when compared to the unprotected control samples stored at room temperature.

Storage Conditions

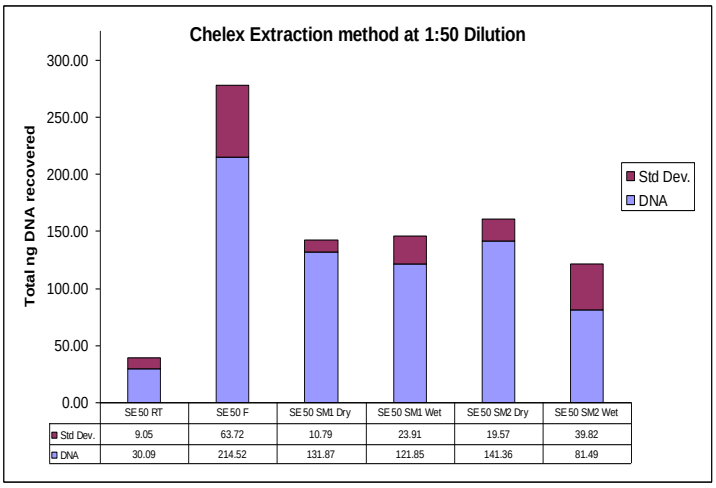
Regardless of the extraction method utilized, Table 42 shows that the average ng of DNA recovered for room temperature, frozen and SampleMatrix™ room temperature storage are comparable. An unexpected result was the observation that unprotected semen samples stored at frozen temperatures demonstrated a ~20-30% higher average DNA recovery as compared to the SampleMatrix™ protected samples (Table 33). In addition, unprotected semen samples stored at frozen temperature and extracted with the Organic and Chelex extraction methods at dilutions of 1:50 and 1:500 showed the highest amount of DNA recovery (Figures 207-208 and 200-201).

The following graphs demonstrate that the DNA recovery was not consistent within each storage conditions and extraction method. Unprotected samples stored at room temperature and extracted with the Chelex method at dilutions of 1:50 and 1:500 had the lowest amount of DNA recovery (Figures 111-112). It was also observed that the Chelex extraction method at the following dilutions (1:1,000, 1:2,000, 1:4000) exhibited the largest variability. (Figure 113). Conversely, unprotected samples stored at room temperature and extracted with the Qiagen method at dilutions of 1:50 and 1:500 showed the highest amount of DNA (Figure 114-115) when compared to other storage conditions. Generally, the statistical analyses indicated that there is no significant difference in the mean DNA yield across the various storage conditions. However, there were some exceptions. For example, the 1:500 and 1:1,000 diluted semen samples extracted with PCI chemistry showed a significant difference in favor of unprotected samples stored frozen. The 1:50 diluted semen samples extracted with chelex chemistry showed a significant difference in favor of unprotected samples stored frozen. Further, the 1:500 diluted

semen samples extracted with Qiagen chemistry showed a significant difference in favor of unprotected samples stored at room temperature.

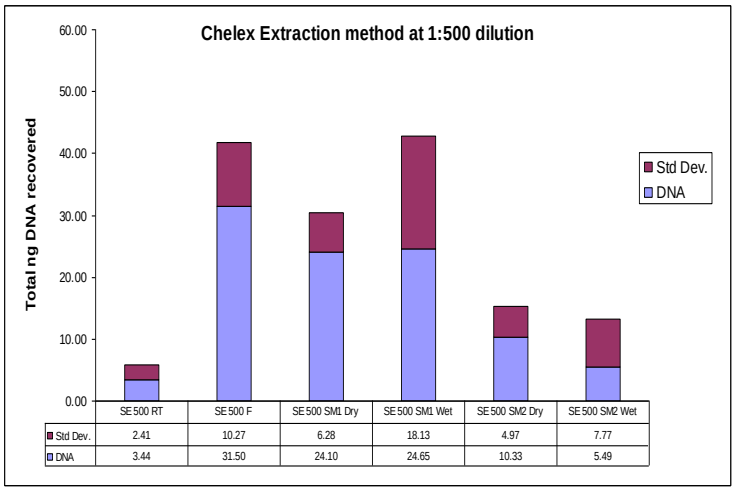
Table 33: Average ng of DNA across each storage condition

Storage Medium	**Average (ng)**			
	Organic	Qiagen	Chelex	Average
Room Temperature	54.36	35.07	7.04	32.16
Frozen	74.13	20.74	50.32	48.40
SM1 Dry	58.30	20.69	31.76	36.92
SM1 Wet	54.98	11.51	30.29	32.26
SM2 Dry	50.66	11.94	32.15	31.58
SM2 Wet	58.57	15.21	18.49	30.76
Storage Medium Room Temperature	**Std Dev (ng)**			
	Organic	Qiagen	Chelex	Average
Frozen	15.39	10.51	2.49	9.46
SM1 Dry	21.30	6.26	17.77	15.11
SM1 Wet	18.55	5.56	10.76	11.62
SM2 Dry	17.29	2.32	10.04	9.88
SM2 Wet	15.52	3.41	11.61	10.18
	18.75	4.59	7.18	10.17



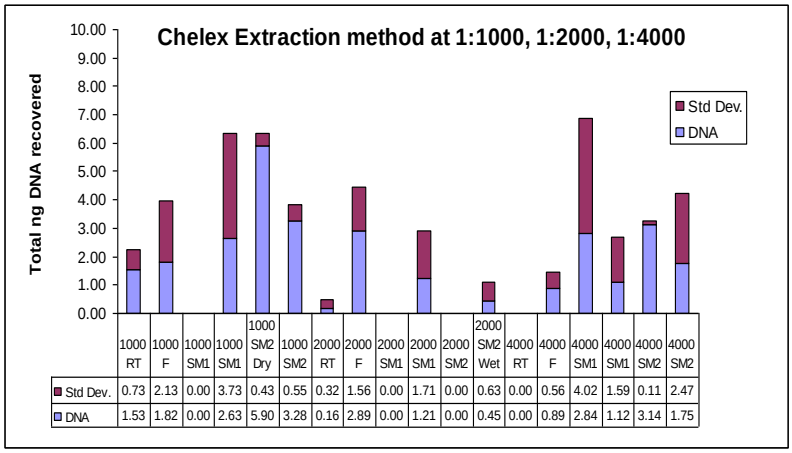
	SE 50 RT	SE 50 F	SE 50 SM1 Dry	SE 50 SM1 Wet	SE 50 SM2 Dry	SE 50 SM2 Wet
Std Dev.	9.05	63.72	10.79	23.91	19.57	39.82
DNA	30.09	214.52	131.87	121.85	141.36	81.49

Fig. 111: DNA recovered by Chelex Extraction at 1:50 Dilution with Various Storage Conditions



	SE 500 RT	SE 500 F	SE 500 SM1 Dry	SE 500 SM1 Wet	SE 500 SM2 Dry	SE 500 SM2 Wet
Std Dev.	2.41	10.27	6.28	18.13	4.97	7.77
DNA	3.44	31.50	24.10	24.65	10.33	5.49

Fig. 112: DNA recovered by Chelex Extraction at 1:500 dilution with Various Storage Conditions.



	1000 RT	1000 F	1000 SM1	1000 SM1	1000 SM2 Dry	1000 SM2	2000 RT	2000 F	2000 SM1	2000 SM1	2000 SM2	2000 SM2 Wet	4000 RT	4000 F	4000 SM1	4000 SM1	4000 SM2	4000 SM2
Std Dev.	0.73	2.13	0.00	3.73	0.43	0.55	0.32	1.56	0.00	1.71	0.00	0.63	0.00	0.56	4.02	1.59	0.11	2.47
DNA	1.53	1.82	0.00	2.63	5.90	3.28	0.16	2.89	0.00	1.21	0.00	0.45	0.00	0.89	2.84	1.12	3.14	1.75

Fig. 113: DNA recovered by Chelex Extraction at 1:1,000, 1:2,000, 1:4000 dilutions with Various Storage Conditions.

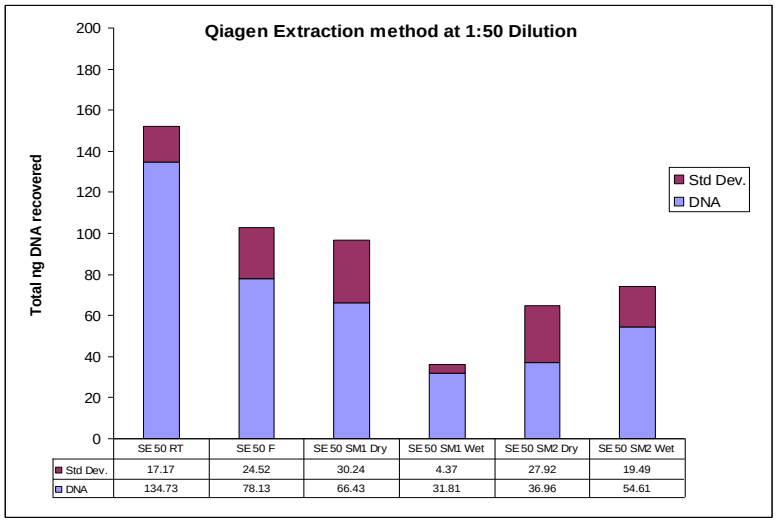


Fig. 114: DNA recovered by Qiagen Extraction at 1:50 dilutions with Various Storage Conditions.

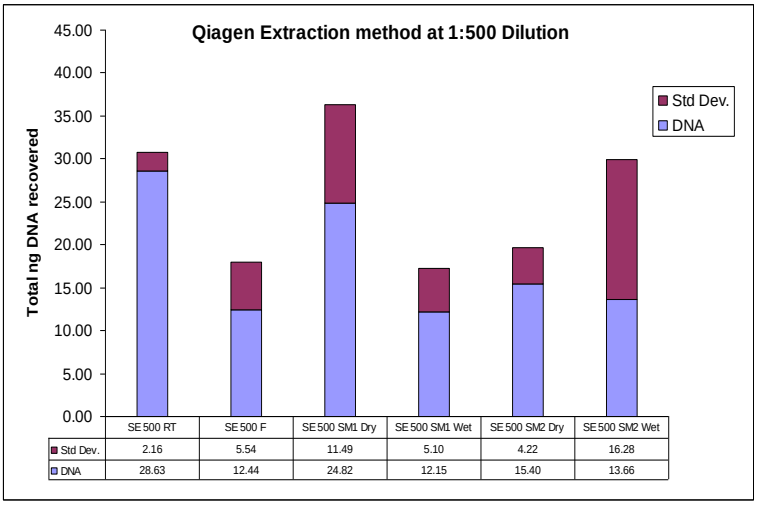


Fig. 115: DNA recovered by Qiagen Extraction at 1:500 dilution with Various Storage Conditions.

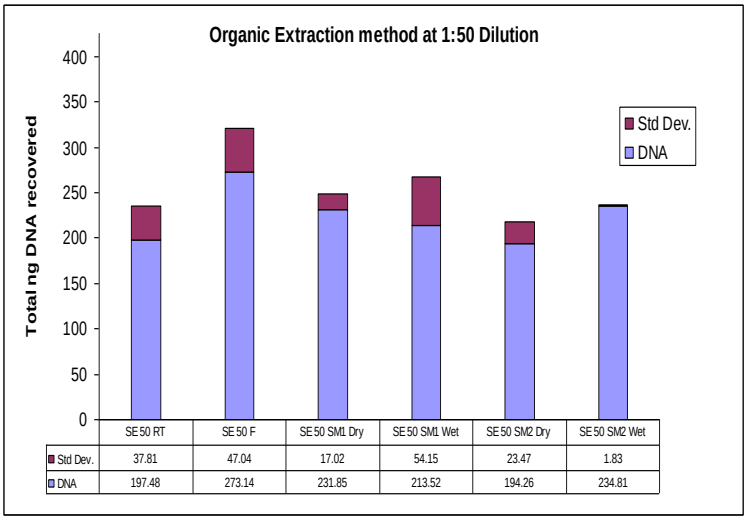


Fig. 116: DNA recovered by Organic Extraction at 1:50 dilutions with Various Storage Conditions.

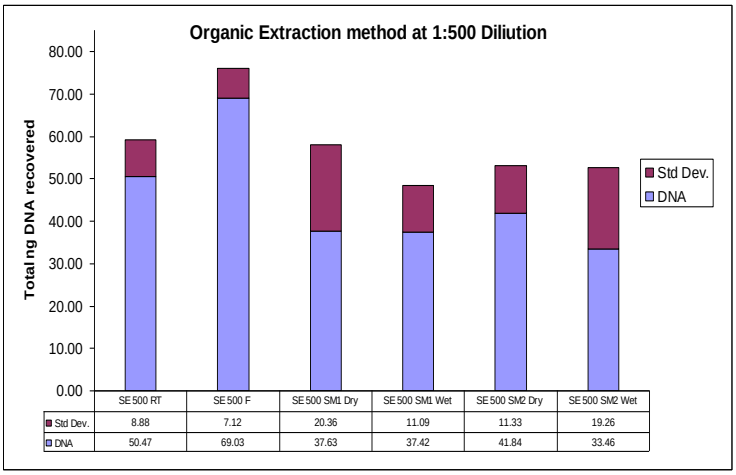


Fig. 117: DNA recovered by Organic Extraction at 1:500 dilutions with Various Storage Conditions.

Comparison of SampleMatrix™ Formulations

According to Figure 202, where the average amount of DNA recovered between the two different formulations is compared, it appears that SM1 is slightly more effective than SM2 in recovering DNA regardless of the extraction method. Analysis of the individual extraction methods comparing SM1 effectiveness against SM2 shows a few inconsistent results.

ANOVA Statistical Analysis - SampleMatrix™ as a Coating Agent for Semen (17-24M)

The ANOVA analysis was performed on data that combined all of the semen dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

CHELEX COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	20	50.2805	88.86668	19.87119	8.6896	91.8714	.00	277.93
RT	20	7.0425	12.46191	2.78657	1.2101	12.8749	.00	41.76
SMID	10	31.7620	53.79035	17.01,000	-6.7173	70.2413	.00	139.50
SM1W	10	30.2930	50.18848	15.87099	-5.6097	66.1957	.00	138.75
SM2D	10	32.1460	58.06343	18.36127	-9.3901	73.6821	.00	155.20
SM2W	10	18.4900	35.90846	11.35525	-7.1974	44.1774	.00	109.65
Total	80	28.4171	57.76710	6.45856	15.5617	41.2726	.00	277.93

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	19969.229	5	3993.846	1.213	.312
Within Groups	243656.724	74	3292.658		
Total	263625.954	79			

PCI COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	20	74.1355	106.52056	23.81872	24.2823	123.9887	5.19	338.40
RT	20	54.3605	76.96298	17.20945	18.3407	90.3803	4.09	231.84
SMID	10	58.2960	92.74195	29.32758	-8.0476	124.6396	3.47	243.88
SM1W	10	54.9800	86.47230	27.34494	-6.8786	116.8386	3.13	251.81
SM2D	10	50.6620	77.61331	24.54348	-4.8592	106.1832	2.49	210.86
SM2W	10	58.5710	93.73540	29.64174	-8.4833	125.6253	2.25	236.11
Total	80	59.9376	88.01124	9.83996	40.3517	79.5236	2.25	338.40

ANOVA ANALYSIS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5805.458	5	1161.092	.142	.982
Within Groups	606126.765	74	8190.902		
Total	611932.223	79			

T Test (Independent Samples) Statistical Analysis - SampleMatrix™ as a Coating Agent for Semen (6-24M Storage)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations (including wet v dry application) as compared to the unprotected control with each extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison. The results are based on samples that were stored for a period of 6-24 months. Representative results are presented below:

PCI 1:50 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	172.9131	104.40311	26.10078
	NO SM FROZEN	6	348.8800	339.47374	138.58957

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	4.525	.046	-1.911	20	.070	175.96687	92.06448	368.01002	16.07627
	Equal variances not assumed			-1.248	5.359	.264	175.96687	141.02596	531.30652	179.37277

GROUP STATISTICS

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	172.9131	104.40311	26.10078
	NO SM RT	6	246.7183	118.72127	48.46776

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.030	.865	-1.425	20	.169	-73.80521	51.77789	-181.812	34.20158
	Equal variances not assumed			-1.341	8.094	.216	-73.80521	55.04883	-200.493	52.88246

PCI 1:500 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	21.7575	18.74588	4.68647
	NO SM FROZEN	6	53.5517	24.61963	10.05092

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.126	.301	-3.260	20	.004	-31.79417	9.75317	-52.13892	-11.44942
	Equal variances not assumed			-2.867	7.295	.023	-31.79417	11.08982	-57.80363	-5.78470

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	21.7575	18.74588	4.68647
	NO SM RT	6	44.0417	12.75781	5.20835

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.086	.164	-2.669	20	.015	-22.28417	8.35004	-39.70204	-4.86629
	Equal variances not assumed			-3.181	13.438	.007	-22.28417	7.00642	-37.37065	-7.19768

PCI 1:1,000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	7.2231	4.61316	1.15329
	NO SM FROZEN	6	14.9350	5.36629	2.19078

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.024	.878	-3.347	20	.003	-7.71188	2.30381	-12.51754	-2.90621
	Equal variances not assumed			-3.115	7.952	.014	-7.71188	2.47580	-13.42712	-1.99663

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	7.2231	4.61316	1.15329
	NO SM RT	6	10.4267	4.70655	1.92144

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.088	.770	-1.443	20	.164	-3.20354	2.21964	-7.83364	1.42655
	Equal variances not assumed			-1.430	8.868	.187	-3.20354	2.24099	-8.28453	1.87745

PCI 1:2,000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	5.3394	3.23121	.80780
	NO SM FROZEN	6	13.8300	10.86295	4.43478

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	6.291	.021	-2.903	20	.009	-8.49063	2.92491	-14.59189	-2.38936
	Equal variances not assumed			-1.884	5.335	.115	-8.49063	4.50775	-19.86250	2.88125

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	5.3394	3.23121	.80780
	NO SM RT	6	4.0767	2.18548	.89222

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.957	.101	.878	20	.390	1.26271	1.43810	-1.73713	4.26254
	Equal variances not assumed			1.049	13.527	.313	1.26271	1.20358	-1.32720	3.85261

PCI 1:4000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	2.9719	2.80777	.70194
	NO SM FROZEN	6	4.8617	2.47573	1.01071

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.037	.849	-1.447	20	.163	-1.88979	1.30619	-4.61446	.83488
	Equal variances not assumed			-1.536	10.196	.155	-1.88979	1.23055	-4.62452	.84493

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	2.9719	2.80777	.70194
	NO SM RT	6	4.5533	1.19510	.48790

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.850	.367	-1.319	20	.202	-1.58146	1.19867	-4.08184	.91893
	Equal variances not assumed			-1.850	19.406	.080	-1.58146	.85485	-3.36815	.20523

CHELEX 1:50 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	76.6644	58.73302	14.68325
	NO SM FROZEN	6	159.4183	100.91981	41.20034

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.731	.114	-2.413	20	.026	-82.75396	34.29867	-154.29974	-11.20818
	Equal variances not assumed			-1.892	6.317	.105	-82.75396	43.73861	-188.49073	22.98281

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	76.6644	58.73302	14.68325
	NO SM RT	6	27.2600	11.73213	4.78962

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	43.479	.000	2.016	20	.057	49.40438	24.51082	-1.72429	100.53304
	Equal variances not assumed			3.199	17.759	.005	49.40438	15.44469	16.92466	81.88409

CHELEX 1:500 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.4744	11.43587	2.85897
	NO SM FROZEN	6	21.4033	17.55002	7.16477

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.574	.224	-2.041	20	.055	-12.92896	6.33432	-26.14213	.28421
	Equal variances not assumed			-1.676	6.663	.140	-12.92896	7.71411	-31.35881	5.50089

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	8.4744	11.43587	2.85897
	NO SM RT	6	2.4783	2.38771	.97478

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	6.423	.020	1.256	20	.224	5.99604	4.77538	-3.96523	15.95731
	Equal variances not assumed			1.985	17.962	.063	5.99604	3.02058	-.35092	12.34300

CHELEX 1:1,000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	1.6831	2.16368	.54092
	NO SM FROZEN	6	1.4650	1.75465	.71633

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.329	.573	.220	20	.828	.21813	.99046	-1.84795	2.28420
	Equal variances not assumed			.243	11.122	.812	.21813	.89762	-1.75488	2.19113

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	1.6831	2.16368	.54092
	NO SM RT	6	1.3017	.66352	.27088

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	6.188	.022	.419	20	.680	.38146	.91096	-1.51878	2.28169
	Equal variances not assumed			.631	19.742	.536	.38146	.60495	-.88151	1.64443

CHELEX 1:2,000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	.5269	.78907	.19727
	NO SM FROZEN	6	2.1650	1.65337	.67499

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	5.565	.029	-3.190	20	.005	-1.63813	.51345	-2.70916	-.56709
	Equal variances not assumed			-2.329	5.876	.060	-1.63813	.70322	-3.36766	.09141

QIAGEN 1:50 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	162.6194	168.25327	42.06332
	NO SM FROZEN	6	188.0933	176.11536	71.89880

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.070	.795	-.313	20	.758	-25.47396	81.50234	-195.48485	144.53694
	Equal variances not assumed			-.306	8.670	.767	-25.47396	83.29922	-215.00942	164.06150

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	162.6194	168.25327	42.06332
	NO SM RT	6	247.9967	176.66370	72.12265

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.141	.711	-1.047	20	.308	-85.37729	81.57030	-255.52995	84.77537
	Equal variances not assumed			-1.023	8.646	.334	-85.37729	83.49251	-275.43421	104.67963

QIAGEN 1:500 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	21.5650	13.40712	3.35178
	NO SM FROZEN	6	15.1533	6.01532	2.45574

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	6.078	.023	1.117	20	.277	6.41167	5.74175	-5.56542	18.38875
	Equal variances not assumed			1.543	19.001	.139	6.41167	4.15513	-2.28510	15.10843

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	21.5650	13.40712	3.35178
	NO SM RT	6	34.2500	8.92148	3.64218

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	2.143	.159	-2.130	20	.046	-12.68500	5.95438	-25.10563	-.26437
	Equal variances not assumed			-2.563	13.764	.023	-12.68500	4.94974	-23.31821	-2.05179

QIAGEN 1:1,000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	9.8944	8.20974	2.05244
	NO SM FROZEN	6	7.6083	3.85097	1.57215

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.576	.224	.648	20	.524	2.28604	3.52619	-5.06945	9.64154
	Equal variances not assumed			.884	18.578	.388	2.28604	2.58537	-3.13353	7.70561

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	9.8944	8.20974	2.05244
	NO SM RT	6	9.3583	5.27168	2.15216

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	1.215	.283	.148	20	.884	.53604	3.62995	-7.03590	8.10798
	Equal variances not assumed			.180	14.290	.859	.53604	2.97393	-5.83027	6.90235

QIAGEN 1:2,000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	4.9456	4.18160	1.04540
	NO SM FROZEN	6	4.2717	2.06081	.84132

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	3.040	.097	.374	20	.712	.67396	1.80241	-3.08581	4.43372
	Equal variances not assumed			.502	18.031	.622	.67396	1.34190	-2.14491	3.49283

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	4.9456	4.18160	1.04540
	NO SM RT	6	5.7983	3.86947	1.57970

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.010	.920	-.434	20	.669	-.85271	1.96550	-4.95267	3.24725
	Equal variances not assumed			-.450	9.717	.662	-.85271	1.89429	-5.09016	3.38474

QIAGEN 1:4000 DILUTION Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	2.7813	2.28676	.57169
	NO SM FROZEN	6	3.3767	1.10654	.45174

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	4.796	.041	-.605	20	.552	-.59542	.98434	-2.64872	1.45789
	Equal variances not assumed			-.817	18.243	.424	-.59542	.72863	-2.12475	.93392

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAMT	SM RT	16	2.7813	2.28676	.57169
	NO SM RT	6	2.8900	2.38906	.97533

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAMT	Equal variances assumed	.006	.941	-.098	20	.923	-.10875	1.10715	-2.41823	2.20073
	Equal variances not assumed			-.096	8.684	.926	-.10875	1.13053	-2.68043	2.46293

SALIVA (N = 258)

Extraction Chemistry Compatibility

A ratio was determined of the total DNA recovered from protected samples to the average across all storage conditions. Approximately 18% of the samples stored with SampleMatrix™ produced a higher DNA recovery than the total average. It appears from Figure 118 that the Qiagen extraction method produced the lowest mean recovery of DNA across all storage conditions. The largest deviation between a protected sample and the combined average appears to be the sample using Qiagen extraction method stored with SM2 (applied dry). Figure 119 shows samples protected with SampleMatrix™ recovered DNA either higher or comparable to unprotected samples stored at room temperature or frozen. These preliminary findings suggest that the three extraction methods are all compatible with SampleMatrix™.

Figures 118-124 show the total DNA recovered for each extraction method as a function of storage condition and dilution factor. The samples for Chelex were stored for ~17 months, while the samples for Organic method were stored for ~24 months, and samples for Qiagen fell within a 17-24 month timeframe. The Chelex and Organic methods are comparable in that there is no apparent difference between protected and unprotected samples in terms of DNA recovery. However, the Qiagen method appears to have a lower DNA recovery when compared to both the Chelex and Organic methods.

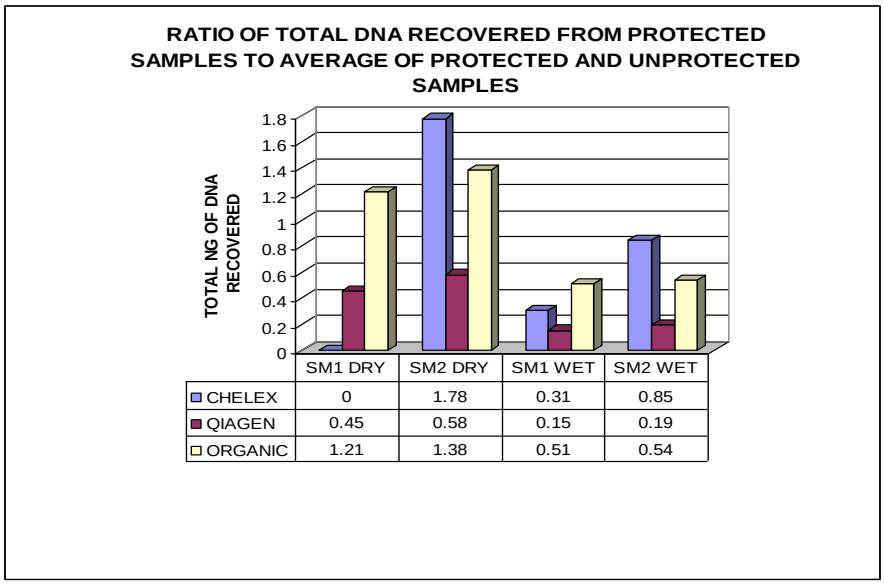


Fig. 118: Ratio of Total DNA Recovered from Protected Samples to Combined Average of Protected and Unprotected Samples (Chelex SM1 DRY not analyzed).

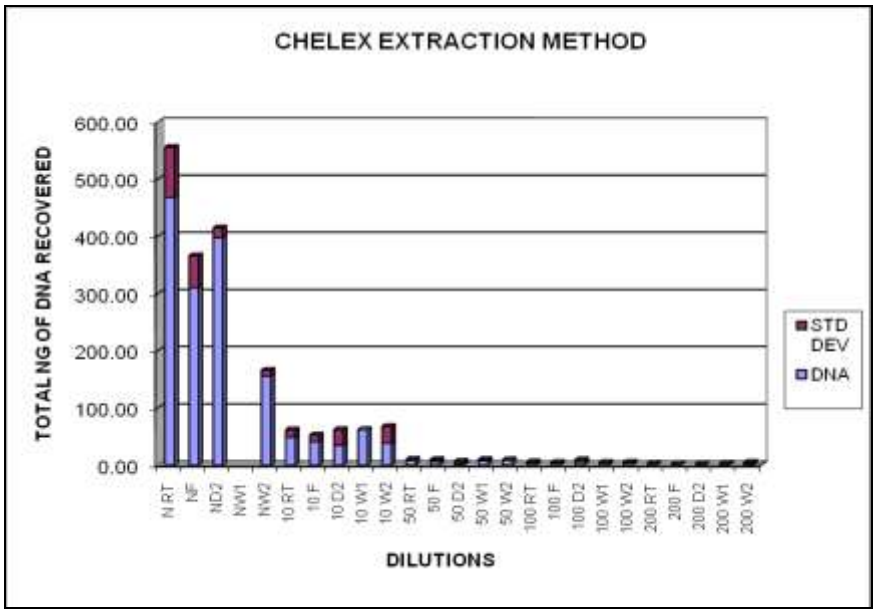


Fig. 119: DNA Recovery from Chelex Extraction Method for Multiple Dilutions.

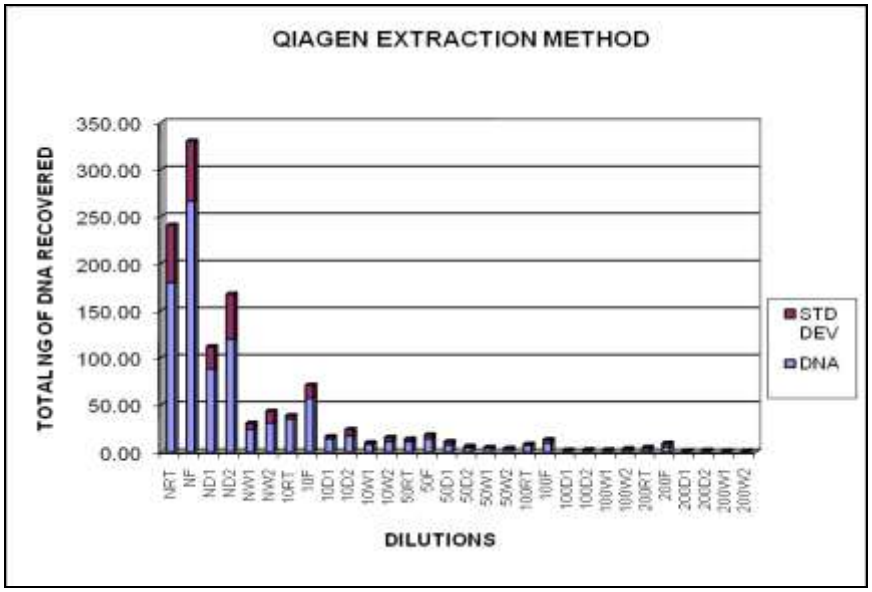


Fig. 120: DNA Recovery from Qiagen Extraction Method for Multiple Dilutions.

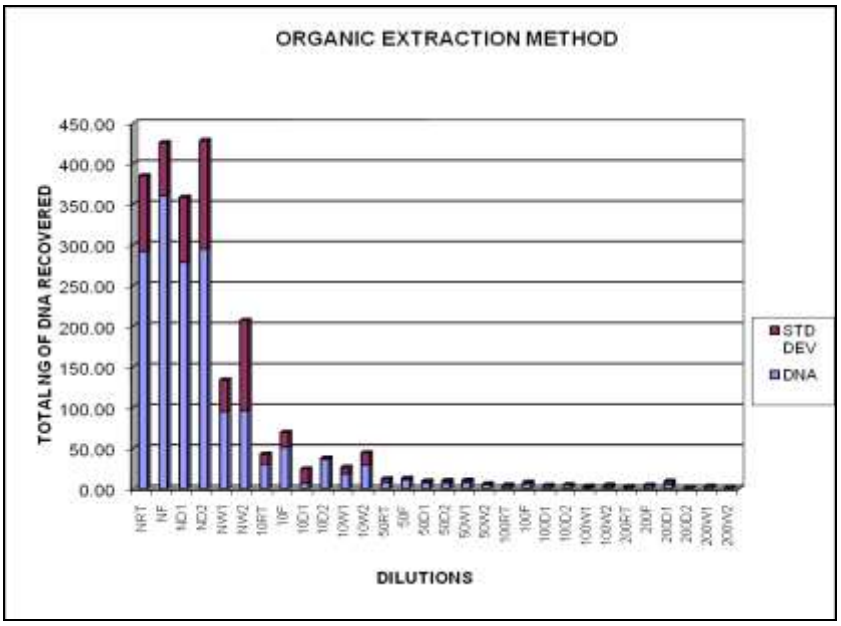


Fig. 121: DNA Recovery from Organic Extraction Method for Multiple Dilutions.

In the next series of graphs, DNA recovery is based on extraction method at a 1:100 dilution. As can be seen from the standard deviation values, the data is not consistent within each storage condition. The DNA recovered varies among extraction methods and storage conditions; recovery does not appear to be directly related to the extraction method used.

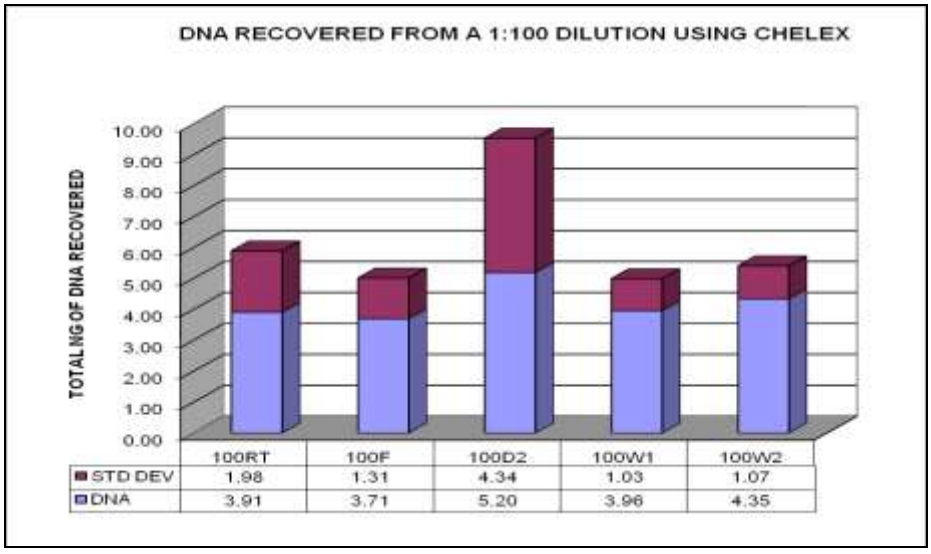


Fig. 122: Average DNA Recovered from 1:100 Dilutions by Chelex Extraction with Varying Storage Conditions.

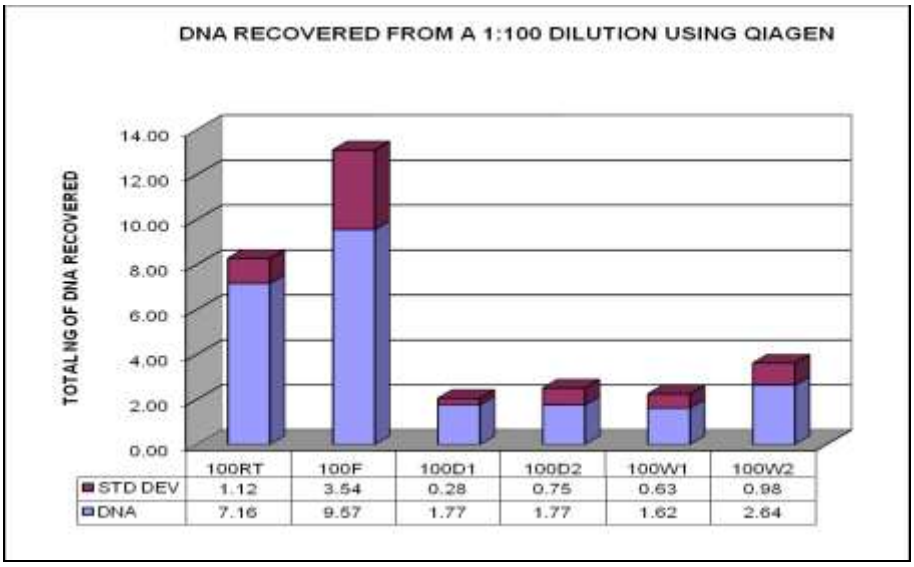


Fig. 123: Average DNA Recovered from 1:100 Dilutions by Qiagen Extraction with Varying Storage Conditions.

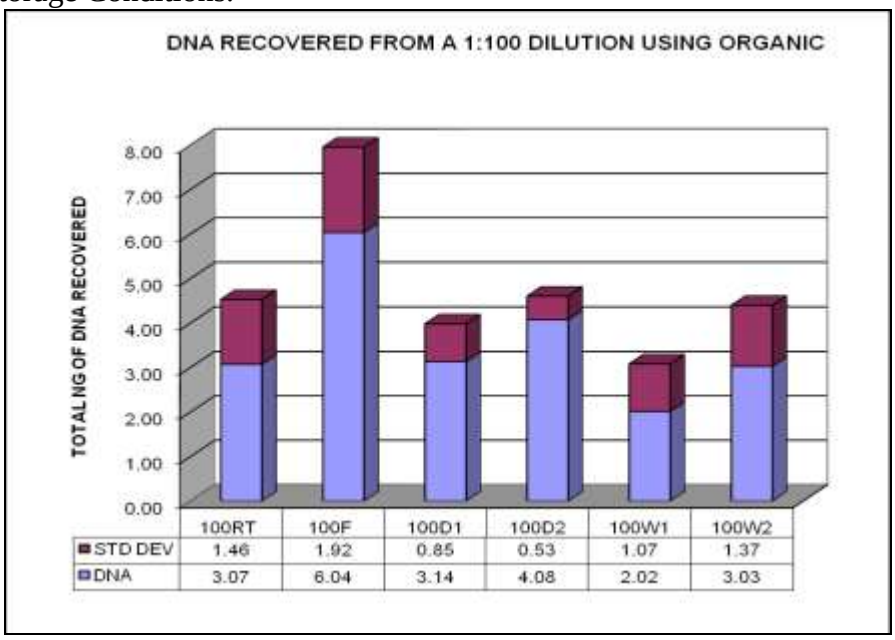


Fig. 124: Average DNA Recovered from 1:100 Dilutions by Organic Extraction with Varying Storage Conditions.

Storage Conditions

One of the objectives of this study was to determine if the recovery of DNA varies as a function of storage condition. An average of the overall recovery was taken of each extraction method (Table 35).

Table 35: Average DNA Recovery and Standard Deviation of Samples for Each Extraction Method and Storage Medium.

	Storage Medium	CHELEX	QIAGEN	ORGANIC	AVERAGE
Average DNA (NG)	SM1 DRY	N/A	22.48	60.22	41.35
	SM1 WET	19.09	7.69	25.22	17.33
	SM2 DRY	88.25	28.76	68.56	61.86
	SM2 WET	42.03	9.64	26.76	26.14
	FROZEN	72.55	70.41	86.40	76.46
	ROOM TEMP	106.11	47.19	66.90	73.40
Average STD DEV (NG)	SM1 DRY	N/A	37.09	122.22	79.65
	SM1 WET	28.80	9.46	39.58	25.95
	SM2 DRY	173.51	51.24	16.39	80.38
	SM2 WET	65.18	12.49	40.30	39.32
	FROZEN	133.74	111.29	154.49	133.17
	ROOM TEMP	203.36	75.18	126.24	134.93

The next graph shows a comparison of the ratio of DNA recovered from samples stored with SampleMatrix™ to samples without SampleMatrix™ stored frozen. Figure 125 shows that 91% of the samples on average resulted in lower DNA recovery with SampleMatrix™ than those stored at -20°C without SampleMatrix™. The data in Figure 126 is consistent with figure 125, also showing that 91% of the samples resulted in lower DNA recovery in comparison to samples stored at room temperature without SampleMatrix.™ The majority of the statistical analyses indicate that there is no significant difference in the mean DNA yield across storage conditions. However, some exceptions were observed. For example, unprotected saliva samples stored frozen resulted in higher yields that were statistically significant for the following samples: Qiagen neat, 1:10, 1:100 dilution series and PCI 1:10, 1:50 dilution series. In addition, unprotected saliva samples stored at room temperature resulted in higher yields that were statistically significant for the neat samples extracted with Chelex chemistry.

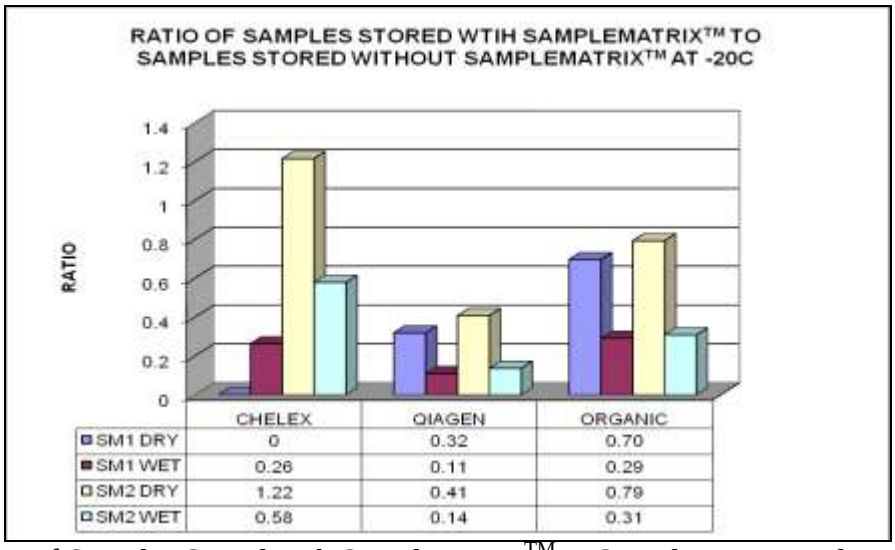


Fig. 125: Ratio of Samples Stored with SampleMatrix™ v. Stored Unprotected at -20°C.

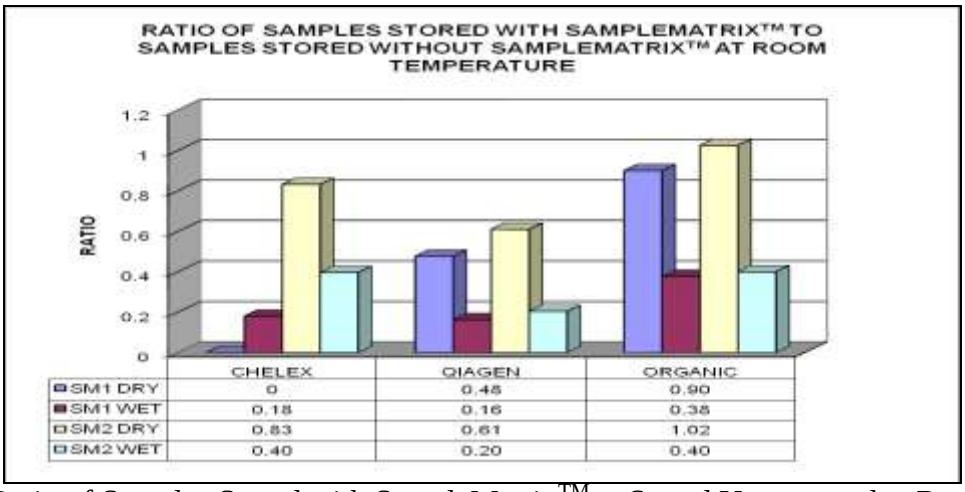


Fig. 126: Ratio of Samples Stored with SampleMatrix™ *v.* Stored Unprotected at Room Temp.

Formulations of SampleMatrix™

An average was calculated for both application methods (dry vs wet) for each formulation and was compared as a function of each extraction method. SampleMatrix™ SM2 has a greater recovery; however, the value of Chelex for SM1 is a biased on the low side due to missing data as this value does not include data for the dry application of SM1 nor does it include data for the wet application of SM1 (neat only)..

SampleMatrix™ Application Method

Data was gathered to determine whether the time delay in applying SampleMatrix™ to the saliva swab affected the recovery of DNA. The dry application of both formulations of SampleMatrix™ results in higher DNA recovery relative to comparable samples prepared using the wet application method. Since there was no data for Chelex dry SM1, it was not included in this graph.

The extraction method was also compared according to wet or dry application of SM1 (SM1) or SM2 (SM2). Samples extracted using the Siegen produced the most consistent results as evident in the standard deviation values. The trend appears to be that the standard deviation values decrease as dilution decreases regardless of extraction method, formulation, or application method (wet or dry).

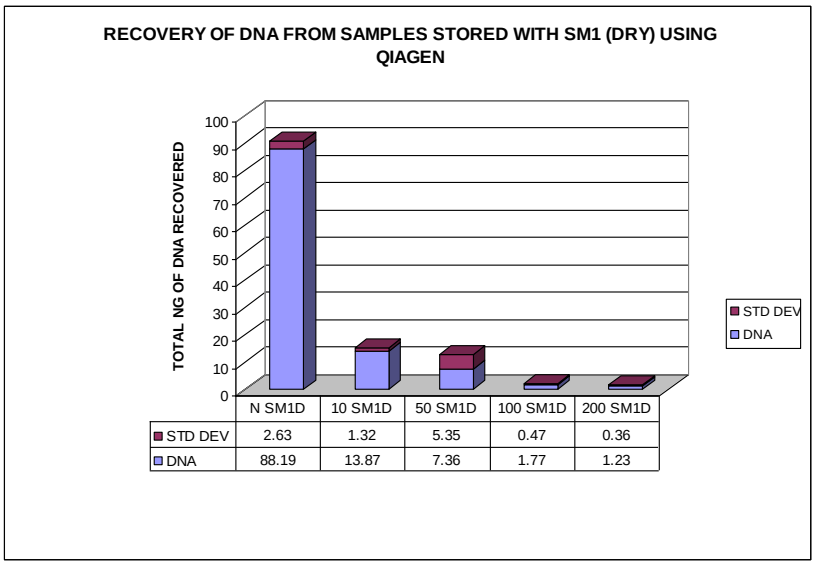


Fig. 127: Recovery of DNA of Samples Stored with SM1 (Dry) Using Qiagen Extraction

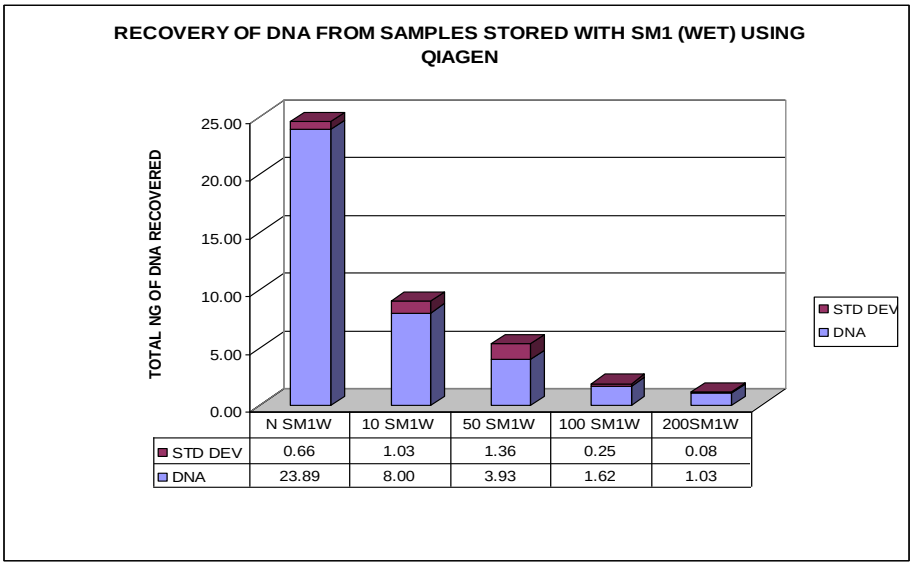


Fig. 128: Recovery of DNA from Samples Stored with SM1 (Wet) Using Qiagen Extraction.

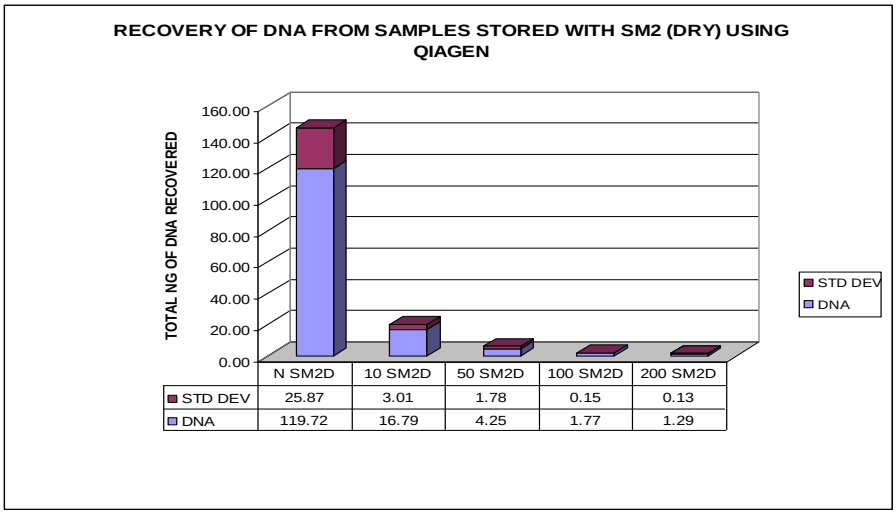


Fig 129: Recovery of DNA from Samples Stored with SM2 (Dry) Using Qiagen Extraction.

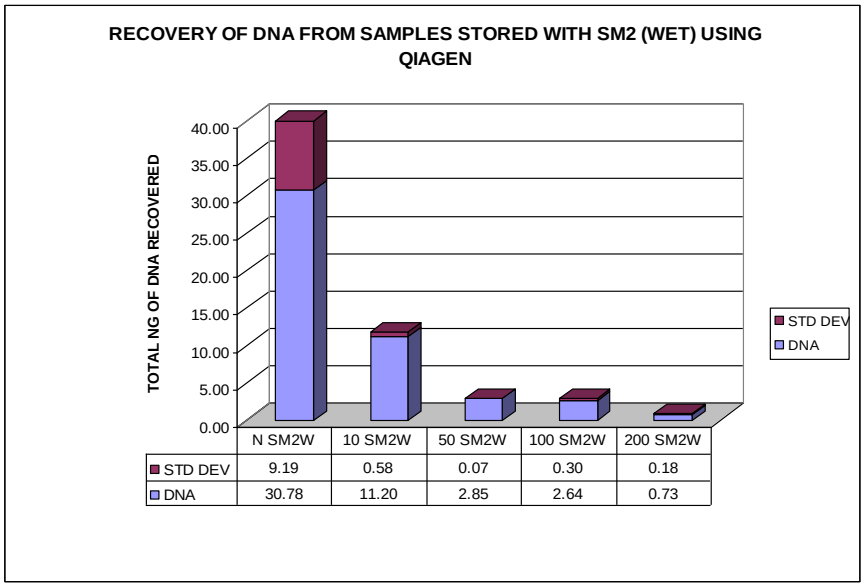


Fig. 130: Recovery of DNA from Samples Stored with SM2 (Wet) Using Qiagen Extraction.

ANOVA Statistical Analysis - SampleMatrix™ as Coating Agent for Saliva (17-24M)

The ANOVA analysis was performed on data that combined all of the saliva dilutions for each substrate and the specific extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

QIAGEN COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	20	70.4095	105.41430	23.57135	21.0741	119.7449	.00	329.60
RT	20	47.1850	73.13631	16.35378	12.9562	81.4138	1.34	241.55
SM1D	10	22.4820	35.02445	11.07570	-2.5730	47.5370	.97	90.05
SM1W	10	7.6920	8.93958	2.82694	1.2970	14.0870	.97	24.36
SM2D	10	28.7630	49.08437	15.52184	-6.3498	63.8758	1.20	138.01
SM2W	10	9.6380	12.16661	3.84742	.9345	18.3415	.60	37.27
Total	80	37.9705	70.32672	7.86277	22.3201	53.6209	.00	329.60

ANOVA ANALYSIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	43185.813	5	8637.163	1.839	.116
Within Groups	347536.181	74	4696.435		
Total	390721.993	79			

PCI COMBINING ALL DILUTIONS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
F	19	90.6321	146.96969	33.71716	19.7950	161.4692	2.61	448.00
RT	20	66.9005	121.72564	27.21868	9.9311	123.8699	1.33	424.32
SM1D	10	60.2160	118.28350	37.40453	-24.3989	144.8309	1.81	335.25
SM1W	10	25.2160	39.58805	12.51884	-3.1036	53.5356	1.26	122.43
SM2D	10	68.5650	128.04931	40.49275	-23.0360	160.1660	.55	389.30
SM2W	10	26.7560	53.28786	16.85110	-11.3638	64.8758	.24	174.47
Total	79	61.6146	114.68667	12.90326	35.9261	87.3030	.24	448.00

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	42459.554	5	8491.911	.630	.677
Within Groups	983476.900	73	13472.286		
Total	1025936.454	78			

T Test (Independent Samples) Statistical Analysis - SampleMatrix™ as Coating Agent for Saliva (6-24M)

The following t test analysis was performed on data that combined the mean recovery of DNA for both SampleMatrix™ formulations (including wet v dry application) as compared to the unprotected control with each extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison. The results are based on samples that were stored for a period of 6-24 months. Representative results are presented below:

CHELEX NEAT

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	12	95.3208	152.69426	44.07904
	NO SM F	5	189.7580	169.41941	75.76666

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.317	.582	-1.128	15	.277	-94.43717	83.74435	-272.93403	84.05970
	Equal variances not assumed			-1.077	6.879	.318	-94.43717	87.65585	-302.44961	113.57528

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	12	95.3208	152.69426	44.07904
	NO SM RT	6	315.0650	246.88449	100.79017

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	3.284	.089	-2.347	16	.032	-219.744	93.64416	-418.2609	-21.22741
	Equal variances not assumed			-1.998	6.98	.086	-219.744	110.00736	-480.0255	40.53712

CHELEX 1:50 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	14	2.8857	3.91573	1.04652
	NO SM F	6	4.5183	4.16641	1.70093

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.213	.650	-.839	18	.412	-1.63262	1.94543	-5.71981	2.45458
	Equal variances not assumed			-.817	9.006	.435	-1.63262	1.99709	-6.14992	2.88468

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	14	2.8857	3.91573	1.04652

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	14	2.8857	3.91573	1.04652
	NO SM RT	6	5.7250	4.62366	1.88760

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.161	.693	-1.411	18	.175	-2.83929	2.01259	-7.06758	1.38901
	Equal variances not assumed			-1.316	8.247	.224	-2.83929	2.15830	-7.79055	2.11198

CHELEX 1:100 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	14	1.9414	2.65327	.70912
	NO SM F	6	2.4700	2.16609	.88430

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.668	.425	-.429	18	.673	-.52857	1.23323	-3.11950	2.06236
	Equal variances not assumed			-.466	11.646	.650	-.52857	1.13351	-3.00663	1.94949

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	14	1.9414	2.65327	.70912
	NO SM RT	6	2.6083	2.53487	1.03486

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.023	.880	-.521	18	.608	-.66690	1.27888	-3.35372	2.01991
	Equal variances not assumed			-.532	9.954	.607	-.66690	1.25450	-3.46387	2.13006

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Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	15	64.8113	62.90538	16.24210
	NO SM F	6	229.6517	75.06327	30.64445

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.644	.432	-5.145	19	.00	-164.84033	32.03621	-231.89290	-97.78777
	Equal variances not assumed			-4.753	7.979	.001	-164.84033	34.68268	-244.85559	-84.82508

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	15	64.8113	62.90538	16.24210
	NO SM RT	6	130.0433	90.50027	36.94658

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	2.924	.104	-1.896	19	.073	-65.23200	34.39846	-137.22881	6.76481
	Equal variances not assumed			-1.616	7.026	.150	-65.23200	40.35908	-160.59561	30.13161

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Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	22.1569	29.46226	7.36557
	NO SM F	6	109.9483	87.43307	35.69440

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	15.21	.001	-3.623	20	.002	-87.79146	24.23139	-138.33725	-37.24566
	Equal variances not assumed			-2.409	5.432	.057	-87.79146	36.44642	-179.28454	3.70162

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	22.1569	29.46226	7.36557
	NO SM RT	6	54.9083	67.28916	27.47068

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	3.275	.085	-1.620	20	.121	-32.75146	20.21383	-74.91676	9.41384
	Equal variances not assumed			-1.152	5.735	.295	-32.75146	28.44099	-103.13145	37.62853

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Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	3.0794	2.59037	.64759
	NO SM F	6	10.3450	6.83524	2.79048

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	17.212	.000	-3.713	20	.001	-7.26563	1.95703	-11.34793	-3.18332
	Equal variances not assumed			-2.536	5.548	.047	-7.26563	2.86464	-14.41602	-.11523

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	3.0794	2.59037	.64759
	NO SM RT	6	8.0567	4.96011	2.02496

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	4.591	.045	-3.109	20	.006	-4.97729	1.60088	-8.31667	-1.63792
	Equal variances not assumed			-2.341	6.054	.057	-4.97729	2.12599	-10.16818	.21359

QIAGEN 1:100 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	2.0775	.72854	.18213
	NO SM F	6	7.1367	4.70538	1.92096

SIG DIFFERENCE \AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	34.975	.000	-4.339	20	.000	-5.05917	1.16606	-7.49153	-2.62681
	Equal variances not assumed			-2.622	5.090	.046	-5.05917	1.92958	-9.99300	-.12533

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	2.0775	.72854	.18213
	NO SM RT	6	5.0217	3.42865	1.39974

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	37.426	.000	-3.367	20	.003	-2.94417	.87449	-4.76831	-1.12002
	Equal variances not assumed			-2.086	5.170	.090	-2.94417	1.41154	-6.53700	.64867

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Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	153.3850	174.75350	43.68838
	NO SM F	6	257.9483	168.41879	68.75668

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.014	.907	-1.261	20	.222	-104.5633	82.90914	-277.50877	68.3821
	Equal variances not assumed			-1.284	9.345	.230	-104.5633	81.46260	-287.81358	78.6869

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	153.3850	174.75350	43.68838
	NO SM RT	6	216.6433	137.29302	56.04964

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.305	.587	-.795	20	.436	-63.25833	79.55354	-229.20411	102.68745
	Equal variances not assumed			-.890	11.51	.392	-63.25833	71.06502	-218.83698	92.32031

PCI 1:10 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	19.6231	12.27917	3.06979
	NO SM F	6	43.5367	18.14573	7.40796

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	2.580	.124	-3.574	20	.002	-23.91354	6.69173	-37.87225	-9.95484
	Equal variances not assumed			-2.982	6.798	.021	-23.91354	8.01882	-42.98997	-4.83711

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	19.6231	12.27917	3.06979
	NO SM RT	6	22.3267	15.17634	6.19572

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.415	.527	-.432	20	.670	-2.70354	6.25384	-15.74881	10.34173
	Equal variances not assumed			-.391	7.603	.707	-2.70354	6.91452	-18.79434	13.38726

PCI 1:50 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	3.7088	3.45394	.86349
	NO SM F	6	7.7867	5.28357	2.15701

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	3.014	.098	-2.135	20	.045	-4.07792	1.91044	-8.06302	-.09281
	Equal variances not assumed			-1.755	6.674	.125	-4.07792	2.32342	-9.62684	1.47101

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	3.7088	3.45394	.86349
	NO SM RT	6	5.3517	5.08484	2.07588

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	1.981	.175	-.874	20	.392	-1.64292	1.87929	-5.56305	2.27722
	Equal variances not assumed			-.731	6.812	.489	-1.64292	2.24831	-6.98921	3.70338

PCI 1:100 DILUTION

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	1.6281	1.67132	.41783
	NO SM F	6	3.0883	3.45064	1.40872

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	13.264	.002	-1.35	20	.191	-1.46021	1.07808	-3.70905	.78863
	Equal variances not assumed			-.994	5.9	.359	-1.46021	1.46938	-5.06998	2.14956

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	16	1.6281	1.67132	.41783
	NO SM RT	6	2.1050	1.87597	.76586

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.052	.823	-.578	20	.570	-.47687	.82566	-2.19918	1.24543
	Equal variances not assumed			-.547	8.178	.599	-.47687	.87243	-2.48110	1.52735

CHAPTER 6: RESULTS OF ENVIRONMENTAL INSULT STUDIES

I. ACCELERATED AGING @ 50°C STUDIES (N = 810)

The objective of the accelerated aging study was to evaluate the total amount of DNA recovered from different biological fluid samples, deposited on a cotton swab, and exposed to accelerated aging conditions for 1-19 weeks. The reported results were based on triplicate samplings for each storage condition and are representative of the mean obtained. Separate negative controls were tested simultaneously for each dilution and storage period. For performing the ANOVA analysis, the dilution series were collapsed into a single category in order to compare the mean recovery for treated v untreated samples.

Incubating Time Frame

The oven incubation time for the samples was designated as 1 - 19 weeks; however, since there was a time lapse prior to and after incubation, it was necessary to take this time into account in determining the age of the samples. The accelerated aging at 50ºC was first converted to the corresponding age at room temperature using the non-isothermal accelerated aging model The total age of an individual sample was formulated by summing the time lapse prior to and after incubation, and the corresponding age from accelerated aging (Table 36).

Table 36: Total Aging Time for all Blood, Semen, and Saliva Samples

Sample	Time prior to incubation	Oven incubation @ 50 °C	Equivalent time @ room temp.	Time after incubation	Total Aging (day)
BLOOD					
BL 1W	14	7	49	7	70
BL 2W	0	14	98	5	103
BL 4W	0	28	195	20	215
BL 8W	0	56	390	17	407
BL 12W	0	84	585	10	595
BL 19W	0	228	926	4	930
SEMEN					
SE 1W	14	7	49	10	73
SE 2W	0	14	98	6	104
SE 4W	0	28	195	19	214
SE 8W	0	56	390	34	424
SE 12W	0	84	585	40	625
SE 19W	0	228	926	10	936
SALIVA					
SA 1W	0	7	49	13	62
SA 2W	0	14	98	7	105
SA 4W	0	28	195	14	209
SA 8W	0	55	383	35	418
SA 12W	0	84	585	34	619
SA 19W	0	231	940	1	941

Performance of SampleMatrix™ under Accelerated Aging Conditions

Figures 131-136 show the total DNA recovered for different blood dilution per time incubated at 50°C. The results indicate that samples protected with SM1 exhibit the highest mean DNA recovery across all three biological fluids. This also holds true regardless of the incubation time. A statistical analysis of the data indicates that the differences in the mean recovery are not significant (ANOVA).

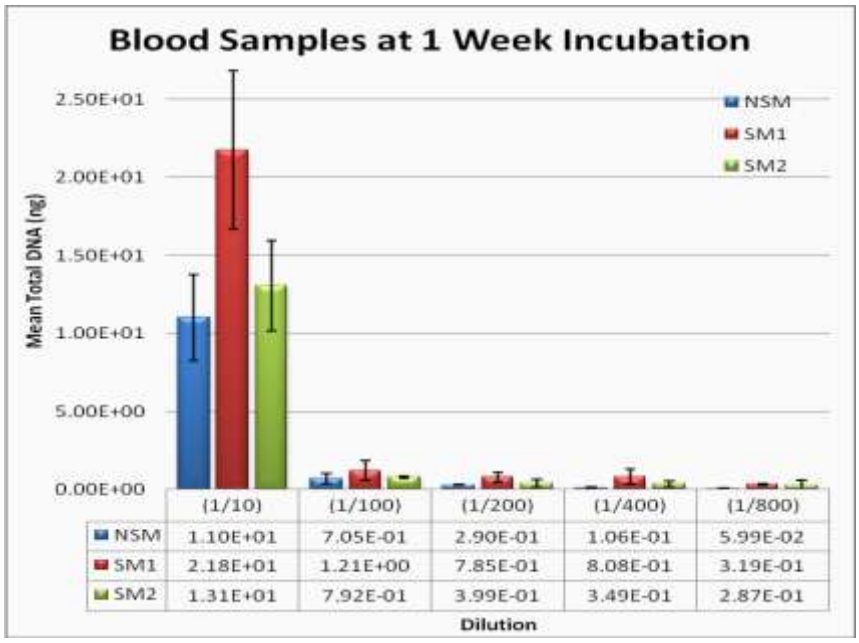

Fig. 131: Blood Sample Different Dilutions 1-Week Incubation

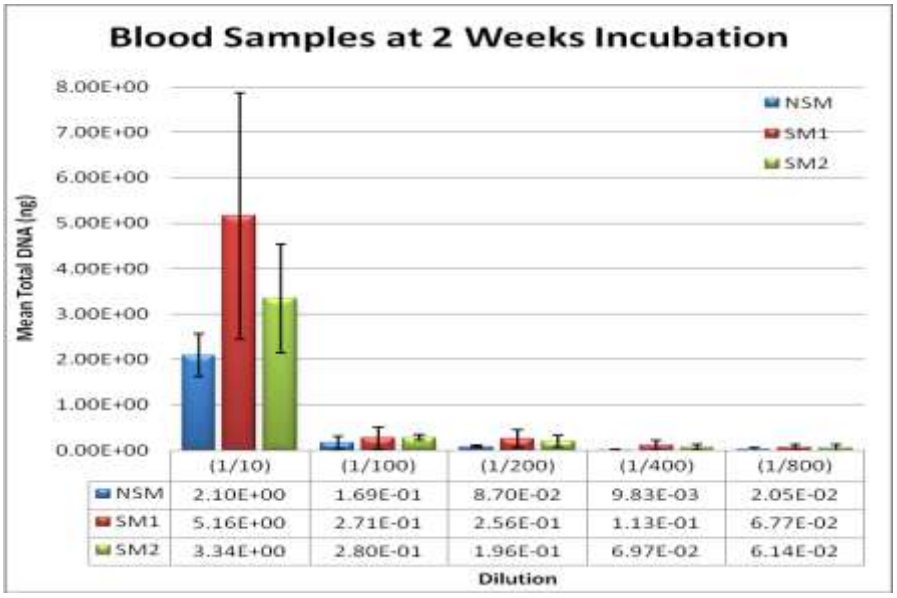

Fig. 132: Blood Sample Different Dilutions 2 Week Incubation

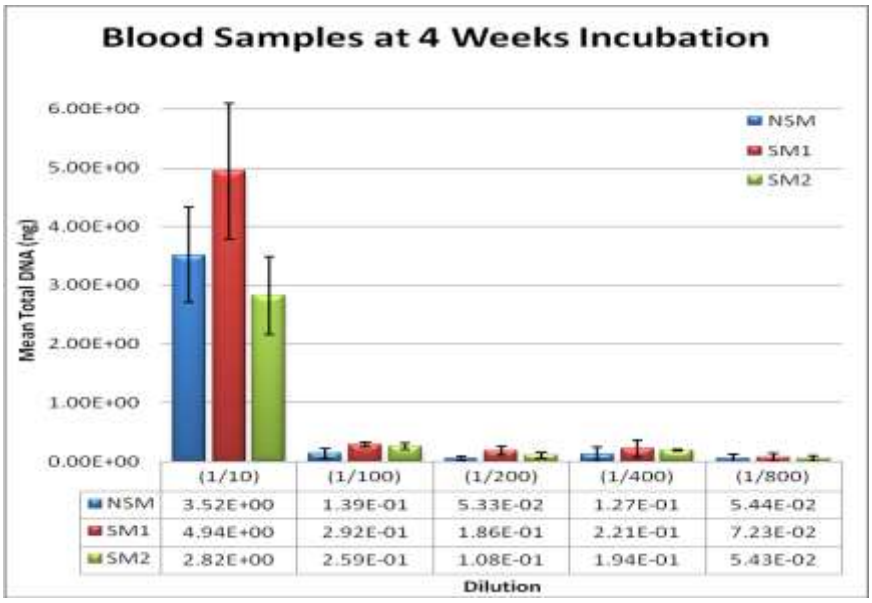

Fig. 133: Blood Samples Different Dilutions 4 Week Incubation

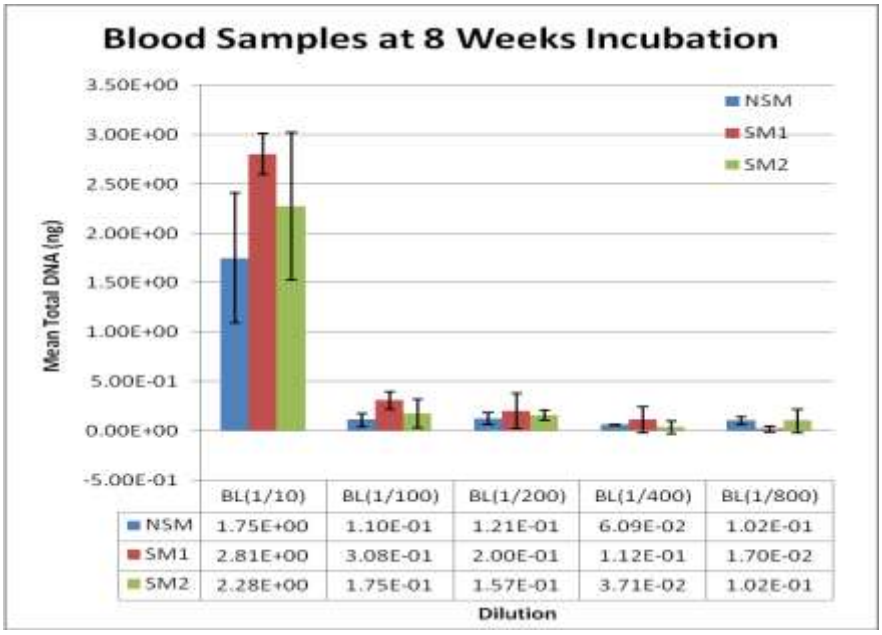

Fig. 134: Blood Samples Different Dilutions 8 Week Incubation

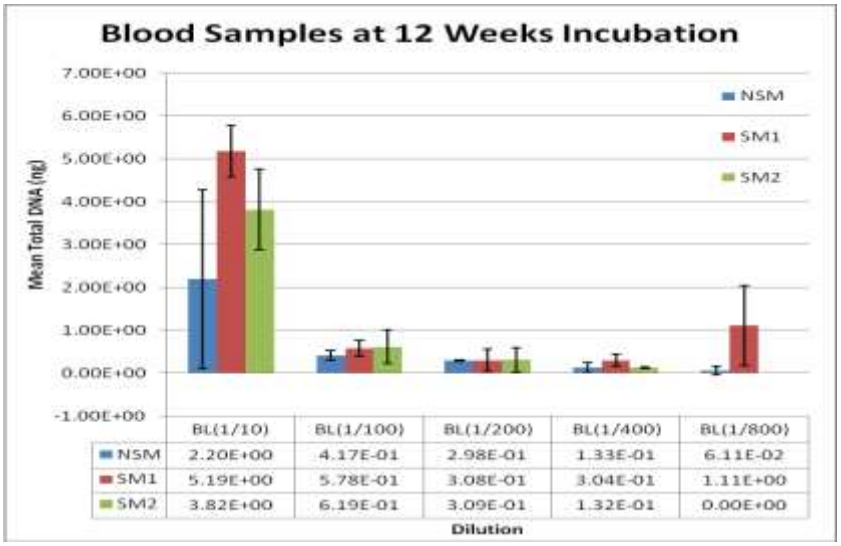

Fig. 135: Blood Samples Different Dilutions 12 Week Incubation

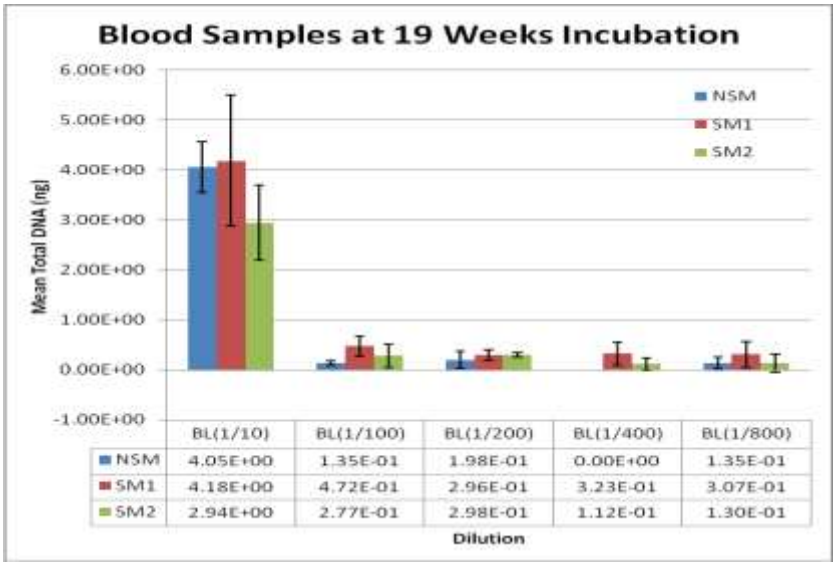

Fig. 136: Blood Samples Different Dilutions 19 Week Incubation

Accelerated Aging of Samples

To observe the accelerated aging profile of the sample, the incubation time was first translated to storage time at room temperature using the pharmaceutical accelerated aging model. The method in this experiment was non-isothermal; however, the 10°C rule was used to calculate the equivalent age of sample at room temperature due to the lack of information regarding the interaction between SampleMatrix™ and DNA in biological samples. The scientific community generally accepts that the ten-degree rule is valid at 50°C. The ten-degree rule describes the age of a sample as $t = t_0 \times 2\Delta T/10$, where $\Delta T = T - T_{ref}$; T_{ref} is a reference temperature at which the aging effect must be determined; t_0 is the incubation time; and T is the elevated temperature used to accelerate the aging effects. The age of the sample in this study can be determined by substituting respective numbers into the equation. For example, 1 week of incubation at 50 °C is equivalent to (1 week) x 2(50-22)/10 which is equivalent to 6.96 weeks (49 days). Figures 137-139 show the accelerated aging profile of blood samples at different dilutions.

The difference was noted when comparing the accelerated aging profiles of each biological fluid. For example, the blood samples (except 1:800 dilutions at approximately 600 days) gave a relatively high DNA recovery at the one-week incubation point (equivalent to 70 days at room temperature). However, after first week of incubation, DNA recovery drops dramatically. Whereas the amount of DNA recovered between two to 19 weeks of incubation is relatively stable. In semen samples, the amount of DNA recovery between one to 19 weeks shows fluctuations between 50-75%. Nonetheless, samples protected by SM1 consistently show a higher DNA recovery than samples protected by SM2 or the un-protected samples. The difference for DNA recovered between samples protected by SampleMatrix™ and unprotected samples increases as the dilution of semen increases.

The amount of DNA recovered in saliva samples of neat (no dilution) and 1:10 dilution is approximately similar between one and 19 weeks of incubation. The amount of DNA recovered in saliva samples protected by SM1 of neat and 1:10 dilution is consistently higher than unprotected samples and samples protected by SM2. DNA recovered from saliva samples of 1:50 and 1:100 dilutions is sometimes higher in samples protected by SM1 than samples protected by SM2. DNA recovered in protected samples of 1:50 and 1:100 dilutions were consistently higher than unprotected samples. No DNA was recovered after incubation for eight weeks from all samples (protected and unprotected) for the 1:200 dilution series.

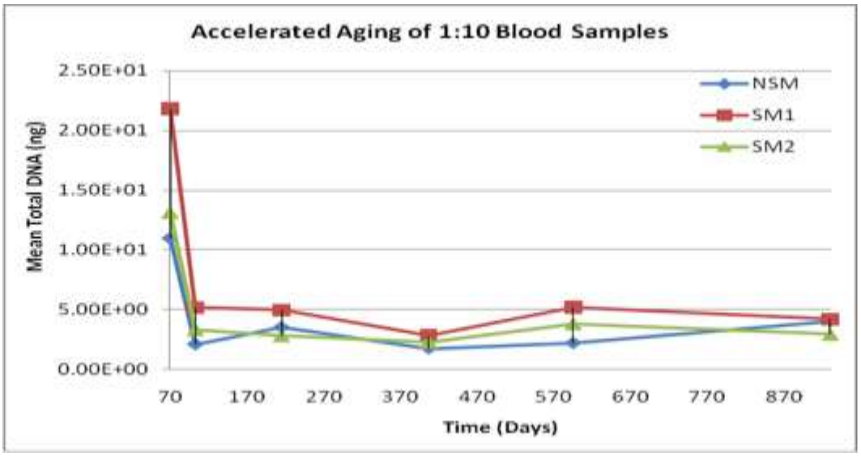

Fig. 137: Accelerated Aging Blood Samples at 1:10 Dilution DNA Quantity *v.* Projected Age.

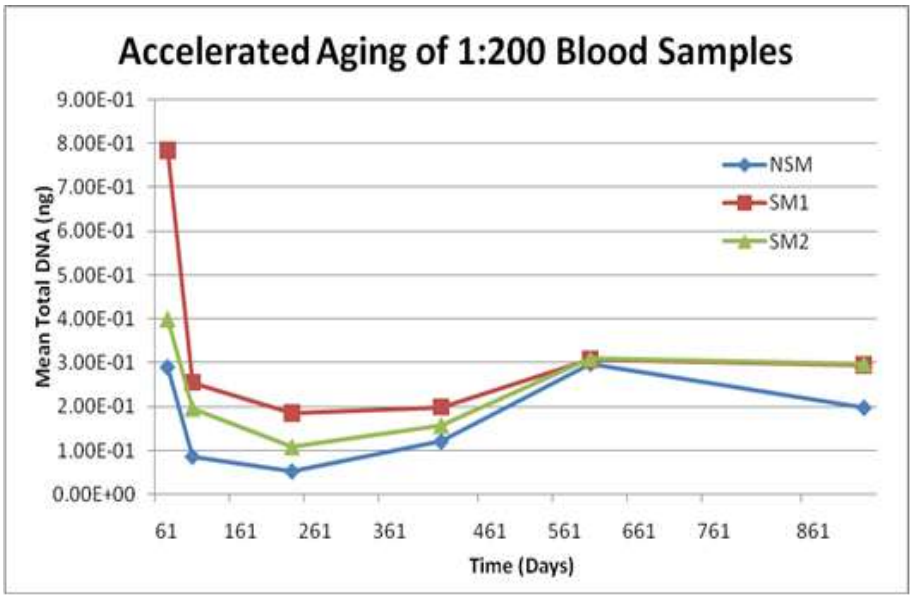

Fig. 138: Blood Samples at 1:200 Dilution DNA Amount *v.* Projected Age

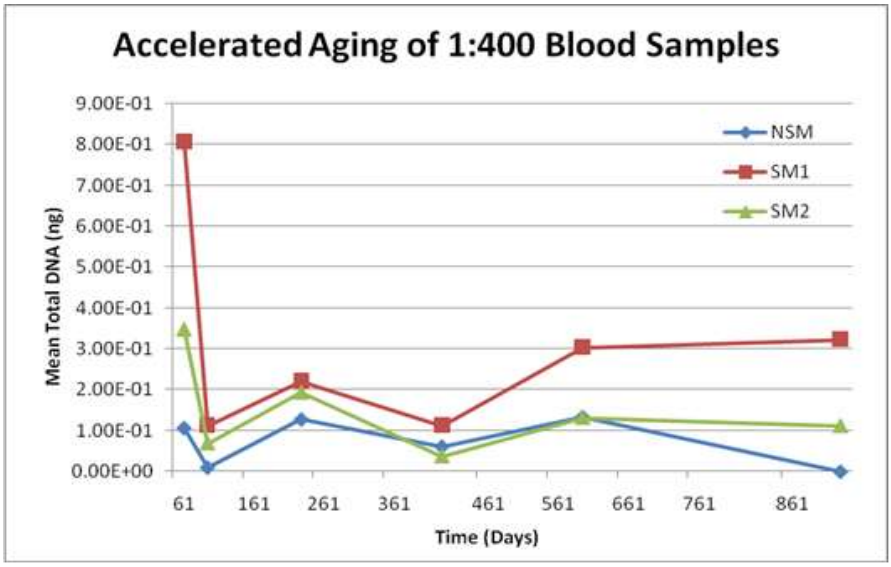

Fig. 139: Blood Samples at 1:400 Dilution DNA Amount *v.* Projected Age

ANOVA Statistical Analysis - SampleMatrix™ as Coating Agent in Accelerated Aging Studies

The ANOVA analysis was performed on data that combined all of the dilutions for each biological fluid (blood, saliva, semen) employing the Qiagen extraction chemistry in order to increase the number of samples included in the calculation of the mean. The storage condition reflecting the highest mean recovery is highlighted in yellow. Representative results are presented below:

BLOOD QALLDIL1WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	2.4377	4.57456	1.18115	-.0956	4.9711	.00	13.78
SM1	15	4.9647	8.90056	2.29811	.0357	9.8936	.26	26.40
SM2	15	2.9758	5.33503	1.37750	.0214	5.9302	.12	16.20
Total	45	3.4594	6.49069	.96757	1.5094	5.4094	.00	26.40

ANOVA: ACCELAGINGDNABLRECOVERQALLDIL1WEEK

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	53.151	2	26.576	.620	.543
Within Groups	1800.527	42	42.870		
Total	1853.678	44			

ACCELERATEDAGINGDNABLRECOVERQALLDIL2WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.4766	.86112	.22234	-.0003	.9534	.00	2.63
SM1	15	1.1721	2.30548	.59527	-.1046	2.4489	.00	8.26
SM2	15	.7901	1.40191	.36197	.0138	1.5665	.00	4.65
Total	45	.8129	1.62334	.24199	.3252	1.3006	.00	8.26

ANOVA:ACCELERATEDAGINGBLQALLDIL2WEEKS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.640	2	1.820	.681	.512
Within Groups	112.310	42	2.674		

ANOVA:ACCELERATEDAGINGBLQALLDIL2WEEKS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.640	2	1.820	.681	.512
Within Groups	112.310	42	2.674		
Total	115.950	44			

ACCELERATEDAGINGDNABLRECOVERQALLDIL4WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.7781	1.45175	.37484	-.0259	1.5820	.00	4.44
SM1	15	1.1428	2.01762	.52095	.0254	2.2601	.00	6.15
SM2	15	.6878	1.13620	.29337	.0586	1.3170	.00	3.57
Total	45	.8695	1.55440	.23172	.4025	1.3365	.00	6.15

ANOVA: ACCELERATEDAGINGDNABLRECOVERQALLDIL4WEEK

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.741	2	.870	.350	.707
Within Groups	104.571	42	2.490		
Total	106.311	44			

ACCELERATEDAGINGDNABLRECOVERQALLDIL12WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.6211	1.14392	.29536	-.0124	1.2545	.00	4.15
SM1	15	1.4985	1.98405	.51228	.3997	2.5972	.15	5.87
SM2	15	.9759	1.54067	.39780	.1227	1.8291	.00	4.88
Total	45	1.0318	1.59904	.23837	.5514	1.5122	.00	5.87

ANOVA:
ACCELERATEDAGINGDNABLRECOVERQALLDIL12WEEK

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.844	2	2.922	1.151	.326
Within Groups	106.661	42	2.540		

ANOVA:

ACCELERATEDAGINGDNABLRECOVERQALLDIL12WEEK

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.844	2	2.922	1.151	.326
Within Groups	106.661	42	2.540		
Total	112.505	44			

ACCELAGINGDNABLRECOVERQALLDIL1WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	2.4377	4.57456	1.18115	-.0956	4.9711	.00	13.78
SM1	15	4.9647	8.90056	2.29811	.0357	9.8936	.26	26.40
SM2	15	2.9758	5.33503	1.37750	.0214	5.9302	.12	16.20
Total	45	3.4594	6.49069	.96757	1.5094	5.4094	.00	26.40

ANOVA

ACCELAGINGDNABLRECOVERQALLDIL1WEEK

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	53.151	2	26.576	.620	.543
Within Groups	1800.527	42	42.870		
Total	1853.678	44			

ACCELERATEDAGINGDNARECOVERESEMENALLDIL1W

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	3.6219	7.87153	2.03242	-.7372	7.9810	.00	29.73
SM1	15	8.7214	15.50469	4.00329	.1352	17.3076	.09	44.40
SM2	15	8.2480	15.03675	3.88247	-.0791	16.5751	.01	42.80
Total	45	6.8638	13.17417	1.96389	2.9058	10.8217	.00	44.40

ANOVA

ACCELERATEDAGINGDNARECOVERESEMENALLDIL1W

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	238.147	2	119.073	.676	.514
Within Groups	7398.444	42	176.153		
Total	7636.591	44			

ACCELERATEDAGINGDNARECOVERESEMENALLDIL2W

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	3.0359	6.14805	1.58742	-.3688	6.4406	.00	19.04
SM1	15	4.1575	6.98793	1.80428	.2878	8.0273	.00	20.16
SM2	15	3.0700	5.71128	1.47465	-.0928	6.2328	.00	18.46
Total	45	3.4211	6.18225	.92160	1.5638	5.2785	.00	20.16

ANOVA

ACCELERATEDAGINGDNARECOVERESEMENALLDIL2W

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	12.211	2	6.105	.154	.858
Within Groups	1669.478	42	39.749		
Total	1681.689	44			

ACCELERATEDAGINGDNARECOVERESEMENALLDIL4W

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	1.9196	3.16948	.81836	.1644	3.6748	.00	9.32
SM1	15	7.5428	14.59565	3.76858	-.5400	15.6256	.06	55.10
SM2	15	5.5121	10.15415	2.62179	-.1111	11.1353	.00	28.80
Total	45	4.9915	10.45537	1.55859	1.8503	8.1326	.00	55.10

ANOVA

ACCELERATEDAGINGDNARECOVERESEMENALLDIL4W

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	243.253	2	121.627	1.119	.336
Within Groups	4566.593	42	108.728		
Total	4809.846	44			

ACCELERATEDAGINGDNARECOVERESEMENALLDIL8W

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	1.5791	2.52689	.65244	.1797	2.9784	.00	7.25
SM1	15	3.9415	6.45329	1.66623	.3678	7.5152	.36	23.40
SM2	15	2.1219	3.82518	.98766	.0036	4.2402	.07	13.20
Total	45	2.5475	4.58060	.68284	1.1713	3.9236	.00	23.40

ANOVA

ACCELERATEDAGINGDNARECOVERESEMENALLDIL8W

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.932	2	22.966	1.100	.342
Within Groups	877.270	42	20.887		
Total	923.202	44			

ACCELERATEDAGINGDNARECOVERESEMENALLDIL19W

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	2.4327	3.82651	.98800	.3136	4.5517	.00	10.70
SM1	15	5.2491	8.76304	2.26261	.3963	10.1019	.08	27.10
SM2	15	3.0427	4.58700	1.18436	.5025	5.5829	.11	12.80
Total	45	3.5748	6.10606	.91024	1.7404	5.4093	.00	27.10

ANOVA

ACCELERATEDAGINGDNARECOVERESEMENALLDIL19W

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65.863	2	32.932	.878	.423
Within Groups	1574.630	42	37.491		
Total	1640.493	44			

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL1WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.9927	1.81633	.46898	-.0132	1.9985	.00	4.91
SM1	15	1.8264	3.57166	.92220	-.1515	3.8043	.00	13.10
SM2	15	1.4430	2.51372	.64904	.0510	2.8351	.00	7.56
Total	45	1.4207	2.69034	.40105	.6124	2.2290	.00	13.10

ACCELERATED AGING SALIVA QIAGEN COMBINING ALL DILUTIONS 1WEEK

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.225	2	2.612	.350	.707
Within Groups	313.245	42	7.458		
Total	318.469	44			

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL2WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.8241	1.73696	.44848	-.1378	1.7860	.00	6.13
SM1	15	1.3698	2.21088	.57085	.1455	2.5942	.00	6.52
SM2	15	.6945	1.25648	.32442	-.0013	1.3903	.00	3.99
Total	45	.9628	1.76214	.26268	.4334	1.4922	.00	6.52

AVOVA: ACCELERATEDAGINGDNASALIVARECOVERQALLDIL2WEEK

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.853	2	1.927	.609	.548
Within Groups	132.773	42	3.161		
Total	136.626	44			

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL4WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.8813	1.61118	.41600	-.0110	1.7735	.00	4.20
SM1	15	2.6946	5.14939	1.32957	-.1570	5.5462	.00	15.00
SM2	15	1.0120	1.73967	.44918	.0486	1.9754	.00	5.77
Total	45	1.5293	3.30503	.49268	.5364	2.5223	.00	15.00

ANOVA

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL4WEEK

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30.681	2	15.340	1.432	.250
Within Groups	449.941	42	10.713		
Total	480.622	44			

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL8WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.5798	.97385	.25145	.0405	1.1191	.00	2.61
SM1	15	1.6761	2.83052	.73084	.1086	3.2436	.00	7.72
SM2	15	1.2009	2.15666	.55685	.0066	2.3952	.00	6.75
Total	45	1.1523	2.13002	.31752	.5123	1.7922	.00	7.72

ANOVA

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL8WEEK

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	9.068	2	4.534	.999	.377
Within Groups	190.559	42	4.537		
Total	199.628	44			

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL12WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.2185	.45990	.11875	-.0362	.4732	.00	1.62
SM1	15	8.7595	27.59487	7.12497	-6.5221	24.0410	.00	108.00
SM2	15	1.5676	3.43273	.88633	-.3334	3.4686	.00	13.30
Total	45	3.5152	16.13936	2.40591	-1.3336	8.3640	.00	108.00

ANOVA

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL12WEEK

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	632.459	2	316.229	1.227	.304
Within Groups	10828.609	42	257.824		
Total	11461.068	44			

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL19WEEK

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NSM	15	.3341	.60080	.15513	.0013	.6668	.00	1.61
SM1	15	1.1463	2.28125	.58902	-.1170	2.4096	.00	7.30
SM2	15	.6465	1.11203	.28712	.0307	1.2624	.00	3.22
Total	45	.7090	1.50951	.22502	.2555	1.1625	.00	7.30

ANOVA

ACCELERATEDAGINGDNASALIVARECOVERQALLDIL19WEEK

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.036	2	2.518	1.111	.339
Within Groups	95.223	42	2.267		
Total	100.259	44			

II. ELEVATED TEMPERATURE (55°C) AND HUMIDITY (60%) STUDIES (N = 324)

The three SampleMatrix™ formulations that were investigated in this study were SM1, SM2, and a lysis formulation. The mean and standard deviations of the total DNA recovered for the triplicate samples stored under three conditions were calculated (Table 37). The reported results were based on triplicate samplings for each storage condition and are representative of the mean obtained. Separate negative controls were tested simultaneously for each dilution and storage period. For the purpose of performing the t test analysis, the three SampleMatrix™ formulations were collapsed into a single category in order to compare the mean recovery for treated v untreated samples.

Table 37**:** Mean and SD of Recovered DNA for All Samples at Four Protection Conditions

STORAGE CONDITION	**AVG DNA (ng)**			
	UNP	**SM1**	**SM2**	**LYS**
FROZEN	257.66	275.18	170.66	452.75
55°C, 60% HUMIDITY	133.23	172.05	172.36	390.90
ROOM TEMP	192.20	296.81	318.45	456.32
AVERAGE	194.36	248.01	220.49	433.33
STORAGE CONDITION	**STD DEV (ng)**			
	UNP	**SM1**	**SM2**	**LYS**
FROZEN	139.15	93.37	76.64	298.15
55°C, 60% HUMIDITY	191.72	289.35	54.69	192.60
ROOM TEMP	61.48	91.18	91.94	117.61
AVERAGE	130.78	157.97	74.43	202.79

From the calculated data from all samples, it appears that the standard deviation values for all three SampleMatrix™ formulations and the unprotected samples do not exceed their respective averages, indicating that the data is fairly clustered. However, the standard deviation values for the unprotected samples and SM1 coated samples at 55°C with 60% humidity do exceed their respective averages, indicating that the data for these samples is widespread. Therefore, when comparing the total DNA recovered from samples stored at room temperature to samples stored at elevated temperature and humidity, the high standard deviation values of these two data sets should be considered. The results indicate that unprotected samples stored frozen resulted in a greater recovery of DNA than unprotected samples stored at elevated temperature and humidity, but gave approximately the same yields as samples stored at room temperature. According to this graph, the lysis formulation affords comparable protection at room temperature and elevated temperature and humidity. However, protection with SM1 and SM2 resulted in increased recovery at room temperature relative to equivalent samples stored at elevated temperature and humidity.

All three SampleMatrix™ formulations allowed for increased protection at elevated temperature and humidity as compared to unprotected samples when considering the total amount of DNA recovered. The data also show an increase in the total amount of DNA recovered for the samples at room temperature as compared to those exposed to elevated temperature and humidity. A statistical analysis of the data indicates that the differences in the

mean yield across the majority of storage conditions are not statistically significant. This holds true regardless of the dilution factor and storage period. One exception was noted: the 1:800 dilution samples stored for 2 weeks exhibited a higher mean recovery that was statistically significant in favor of unprotected samples.

Performance of SampleMatrix™ Formulations

The average amount of recovered DNA for the two non-lysis formulations (SM1 and SM2) showed no difference at the elevated temperature and humidity condition when considering all of the samples analyzed. However, these results indicate that the SM2 formulation resulted in a marginal increase for DNA at room temperature as compared to SM1. Additionally, the lysis formulation resulted in more than double the amount of recoverable DNA at elevated conditions, and only slightly less than double for the samples incubated at room temperature, as compared to the SM1 and SM2 SampleMatrix™ formulations. Generally, the SampleMatrix™ formulations demonstrated greater protective properties for the neat samples as compared with diluted samples stored under the same conditions.

T-TEST Statistical Analysis (Independent Samples) - SampleMatrix™ as Coating Agent at Elevated Temperature and Humidity

The following t test analysis was performed on data that combined the mean recovery of DNA for all three SampleMatrix™ formulations (SM1, SM2, and lysis buffers) as compared to the unprotected control with each extraction chemistry. The Levene's Test for Equality of Variances was performed and the result is highlighted in yellow as applicable to a given comparison. The results are based on samples that were stored for a period of 1 – 4. Representative results are presented below:

NEAT QIAGEN 1WEEK

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	658.5589	369.31772	123.10591
	NO SM RT	3	784.3000	56.68340	32.72617

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	7.444	.021	-.569	10	.582	-125.74	220.86604	-617.86131	366.37908

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	7.444	.021	-.569	10	.582	-125.74	220.86604	-617.86131	366.37908
	Equal variances not assumed			-.987	8.991	.349	-125.74	127.38158	-413.94197	162.45975

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	658.5589	369.31772	123.10591
	NO SM F	3	1012.2120	462.05445	266.76726

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.029	.869	-1.36	10	.203	-353.65	259.75657	-932.4268	225.12060
	Equal variances not assumed			-1.204	2.910	.317	-353.653	293.80238	-1305.325	598.01920

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM HUM 60% AND 55 DEG	9	631.5385	368.50786	122.83595
	NO SM HUM 60% AND 55 DEG	3	548.8633	22.96369	13.25809

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	4.353	.064	.376	10	.715	82.67519	219.84226	-407.16390	572.51428
	Equal variances not assumed			.669	8.183	.522	82.67519	123.54938	-201.12473	366.47510

1:400 DILUTION QIAGEN 1WEEK

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	1.7756	1.02263	.34088
	NO SM RT	3	1.2,000	.35595	.20551

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	3.784	.080	.930	10	.374	.57556	.61895	-.80354	1.95465
	Equal variances not assumed			1.446	9.730	.180	.57556	.39803	-.31466	1.46577

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	1.7756	1.02263	.34088
	NO SM F	3	3.6233	1.64968	.95244

SIG DIFERENCE @ 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.583	.463	-2.359	10	.040	-1.84778	.78341	-3.59333	-.10222
	Equal variances not assumed			-1.827	2.535	.182	-1.84778	1.01160	-5.42865	1.73310

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM HUM 60% AND 55 DEG	9	3.9433	2.40097	.80032
	NO SM HUM 60% AND 55 DEG	3	2.1767	.64010	.36956

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	7.041	.024	1.223	10	.249	1.76667	1.44432	-1.45149	4.98482
	Equal variances not assumed			2.004	9.963	.073	1.76667	.88153	-.19848	3.73182

1:800 DILUTION QIAGEN 1WEEK

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	8	2.6175	.70222	.24827
	NO SM RT	3	2.8967	.57492	.33193

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.219	.651	-.610	9	.557	-.27917	.45766	-1.31446	.75612
	Equal variances not assumed			-.673	4.465	.534	-.27917	.41451	-1.38430	.82597

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	8	2.6175	.70222	.24827
	NO SM F	3	4.7333	1.61299	.93126

SIG DIFFERENCE AT 95% CONFIDENCE LEVEL

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	2.443	.152	-3.187	9	.011	-2.11583	.66391	-3.61770	-.61397
	Equal variances not assumed			-2.195	2.29	.143	-2.11583	.96379	-5.79653	1.56486

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM HUM 60% AND 55 DEG	9	2.1878	1.08806	.36269
	NO SM HUM 60% AND 55 DEG	3	3.3200	1.32661	.76592

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.252	.626	-1.490	10	.167	-1.13222	.75985	-2.82527	.56082
	Equal variances not assumed			-1.336	2.960	.275	-1.13222	.84745	-3.84978	1.58534

NEAT QIAGEN 2 WEEKS

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	744.5644	307.95486	102.65162
	NO SM RT	3	742.8200	283.13827	163.46995

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	1.087	.322	.009	10	.993	1.744	202.1027	-448.56851	452.05740
	Equal variances not assumed			.009	3.743	.993	1.744	193.0279	-549.02881	552.51770

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	744.5644	307.95486	102.6516
	NO SM F	3	578.0167	274.36506	158.4047

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	1.112	.316	.828	10	.427	166.54778	201.02426	-281.36218	614.45774
	Equal variances not assumed			.882	3.862	.429	166.54778	188.75756	-364.98336	698.07892

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM HUM 60% AND 55 DEG	9	1042.2756	786.27204	262.09068
	NO SM 60% HUM AND 55 DEG	3	556.0200	198.69796	114.71832

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.823	.386	1.029	10	.328	486.25556	472.56988	-566.69575	1539.20687
	Equal variances not assumed			1.700	9.905	.120	486.25556	286.09757	-152.04115	1124.55226

1:400 DILUTION QIAGEN 2 WEEKS

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	2.5233	1.66749	.55583
	NO SM RT	3	2.2067	.11930	.06888

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	2.016	.186	.318	10	.757	.31667	.99494	-1.90019	2.53352
	Equal variances not assumed			.565	8.240	.587	.31667	.56008	-.96837	1.60170

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	2.5233	1.66749	.55583
	NO SM F	3	1.3933	.20502	.11837

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	1.830	.206	1.134	10	.283	1.13000	.99618	-1.08962	3.34962
	Equal variances not assumed			1.988	8.671	.079	1.13000	.56829	-.16305	2.42305

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM HUM 60% AND 55 DEG	9	2.2967	.69471	.23157
	NO SM HUM 60% AND 55 DEG	3	2.8000	.34220	.19757

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	1.275	.285	-1.180	10	.265	-.50333	.42662	-1.45391	.44725
	Equal variances not assumed			-1.654	7.657	.139	-.50333	.30440	-1.21079	.20412

1:800 DILUTION QIAGEN 2 WEEKS

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	3.0722	1.16308	.38769
	NO SM RT	3	5.1167	1.23581	.71350

SIG DIFFERENCE @ 95% CONFIDENCE LEVEL		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.013	.910	-2.603	10	.026	-2.04444	.78532	-3.79425	-.29464
	Equal variances not assumed			-2.518	3.284	.079	-2.04444	.81202	-4.50681	.41792

Group Statistics

	PROTECTION	N	Mean	Std. Deviation	Std. Error Mean
DNAAMT	SM RT	9	3.2011	1.11122	.37041
	NO SM F	3	2.1100	.80889	.46701

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
DNAAMT	Equal variances assumed	.073	.792	1.547	10	.153	1.09111	.70513	-.48001	2.66223
	Equal variances not assumed			1.831	4.830	.129	1.09111	.59607	-.45751	2.63973

CHAPTER 7: RESULTS OF CELL MORPHOLOGY STUDIES

The purpose of these studies was to establish the viability of SM3 in preserving whole blood samples stored at room temperature. However, in handling SM3 protected blood samples it became apparent early on that a protocol to define the handling procedures was required.

I. HANDLING ISSUES

Application of Handling Data and Results

After the study began, it became apparent that SM3 itself, in addition to SM3-whole blood mixtures, had some properties that required that attention be paid to handling techniques. Areas in which experimentation was required included varying of the initial SM3-blood ratio, varying of the rehydration period for SM3-blood mixtures, and pipetting techniques used to minimize frothing. Issues were also encountered in the staining of SM3-treated blood samples. Each of these factors was accounted for individually. In order to reduce the number of permutations, some factors (pipetting techniques, preservation mechanism of SM3, compatibility with staining processes) were optimized prior to the collection experimental data.

Properties of SM3-Treated Whole Blood

When exposed to air, small volumes of SM3 ($\leq$50 µL) dry to a tacky consistency within 24 hours. SM3 is somewhat viscous and has a strong tendency to froth when agitated repeatedly via pipette, forming thick-layered air pockets that take up to several hours to clear. The use of a low volume (~50µL) pipette with a narrow tip, combined with the use of slow pipetting action, proved to be a reliable technique for mixing fresh whole blood with SM3 while minimizing frothing. Even with initial agitation via pipette, whole blood tends to emulsify within SM3 when combined at the suggested 1:2 mixture ratio.

Mixture Ratio Assessment

Based upon the difficulties experienced in obtaining complete rehydration of blood / SM3 mixtures, it was hypothesized that decreasing the initial SM3: Blood ratio would facilitate complete rehydration.

Rehydration Interval

Rehydration of the dried 1:2 mixture was notably difficult; samples rehydrated for three hours showed no apparent difference in terms of the ease of breaking up emulsification than samples that were rehydrated for shorter periods. The use of a medium-volume (~200 µL) pipette with an extra wide tip, combined with the use of repeated pipetting action and mechanical probing of the emulsification, proved to be the most reliable technique for rehydrating whole blood/SM3 mixtures. It was therefore hypothesized that increasing the amount of time that the rehydrated sample was allowed to sit would facilitate more thorough rehydration.

Preservation Properties of SM3

As it has been documented that blood in protected environments may be preserved at room temperature for significantly longer than when unprotected, it became necessary to demonstrate that dried SM3 was not merely hermetically isolating the emulsified droplet. It was therefore hypothesized that SM3 was permeating the emulsification. A scalpel was used to remove the

entire dried SM3 / blood emulsification from the micro-titer plate wells that were stored for two days (Figure 140). The SM3 treated blood sample was then sectioned through the center in order to expose the central portion of the emulsification to air; the sample was left exposed in this manner for seven days (Figure 141). Upon rehydration in physiological saline, it was noted that the exposed blood core was considerably easier to hydrate than the surrounding SM3. The RBC and WBC concentrations were comparable to those of non-sectioned samples that had aged six and eight days, respectively. Further, the RBC and WBC concentrations from samples protected with SM3 were significantly greater than observed for untreated neat blood when aged no more than seven days.

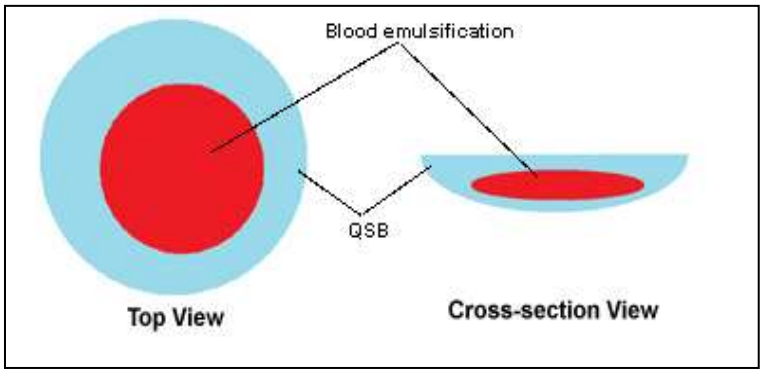

Fig. 140: Dried SM3-Blood Emulsification

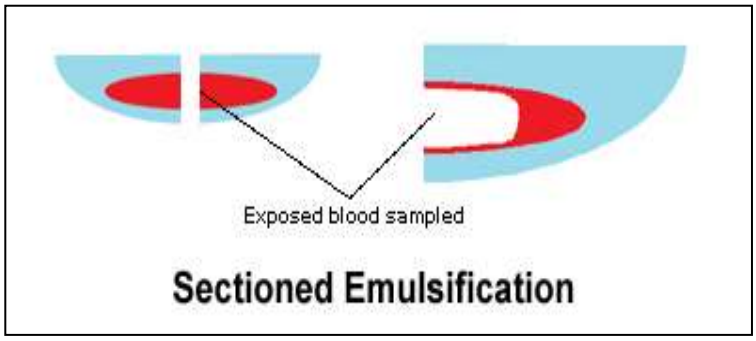

Fig. 141: Method of Cross-Section Sampling

Homogeneity of SM3-Blood Mixtures

The hypothesis that mechanical agitation upon depositing blood in SM3 would provide a more homogeneous mixture and thereby increase the effectiveness of SM3 in preserving blood was also tested. SM3/whole blood mixtures were mechanically agitated via pipette and compared to equivalent SM3/whole blood mixtures for which no mechanical mixing was employed. Samples were compared microscopically and qualitatively rated with respect to RBC and WBC morphology count and morphological cell integrity over the course of 104 days; results are presented in Tables 38-39 and Figures 142-143. While it was observed that WBC tended to degrade more rapidly than RBC, the mechanical agitation of samples did not lead to any notable increase in abundance of either RBC or WBC. In some instances, mixed samples actually showed a greater rate of morphological degradation than unmixed samples; the difference was slight for RBC, but more apparent for WBC.

Table 38: Qualitative RBC Viability over Time in Mixed vs. Unmixed Samples*

	Mixed		Unmixed	
# Days	Abundance	Morphology	Abundance	Morphology
0	10	10	10	10
2	9.75	10	10	10
13	9	10	10	10
17	9	10	8	10
24	9	10	8	10
34	8	10	8	10
49	7	10	7	10
84	6	9	6	9

* 1 = Low / Poor; 10 = High / Good

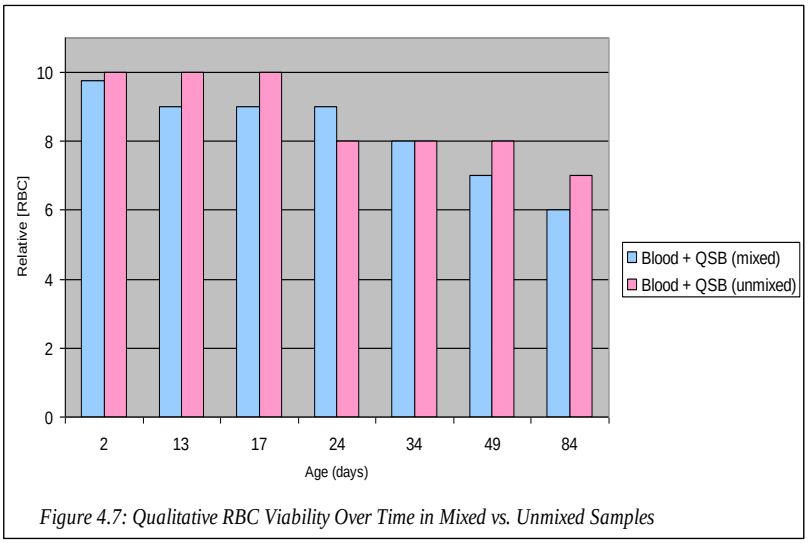

Figure 4.7: Qualitative RBC Viability Over Time in Mixed vs. Unmixed Samples

Fig. 142: Qualitative RBC Viability in Mixed v. Unmixed Samples as Function of Tim

Table 39: Qualitative WBC Viability over Time in Mixed vs. Unmixed Samples*

	Mixed		Unmixed	
# Days	Abundance	Morphology	Abundance	Morphology
0	10	10	10	10
2	9.25	10	9.75	10
13	8	10	8	10
17	8	10	8	10
24	5	10	5	10
34	4	10	4	10
49	0	--	0	--
84	0	--	0	--

* 1 = Low / Poor; 10 = High / Good

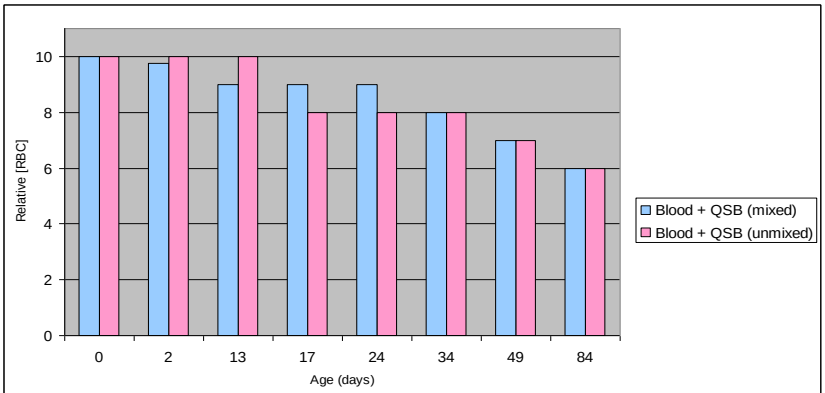

Figure 4.8: Qualitative WBC Viability Over Time in Mixed vs. Unmixed Samples

Fig. 143: Qualitative WBC Viability in Mixed v. Unmixed Samples as Function of Time

Staining of SM3-Treated Specimens

10µL aliquots of each sample were stained per the H and E Staining Protocol. These initial samples presented what appeared to be a very high density of stained cells, which obscured cell morphological characteristics of interest. Two additional 10µL aliquots were diluted to 1:10 and 1:100, respectively. Despite diluting the samples several-fold, the cell morphology was still obscured by a blue tint, which appeared to the staining of the SM3 matrix.

II. QUALITATIVE MORPHOLOGY

RBC Viability

Red blood cells aged over the course of 230 days were examined microscopically for both abundance and morphological integrity, specifically the maintenance of the unique disc-shaped morphology as well as the characteristic rouleaux pattern stack formations (Figure 144). These observations are summarized in Table 40 and Figure 146; both abundance and maintenance of morphology were assessed subjectively relative to a fresh whole blood sample and rated on a scale of 1–10. In general, untreated whole blood showed a steady degradation of RBC starting within two days of environmental exposure and progressed to near full degradation within a few days. Although the preservation period can be increased somewhat by sealing the well plate and limiting the exposure to open air, the morphology of red blood cells in untreated samples was sustained for only few weeks at best before complete degradation. Degradation was gradual, and cells appear “deflated” or adopt a folded appearance. Eventual rupturing of the membrane resulted in characteristic RBC debris (Figure 145). While replicate slides were created and observed for each time storage period, RBC characteristics proved to be generally consistent for all samples collected on a single day. Thus, results reported in Table 40 represent a combined assessment of all samples for a given day.

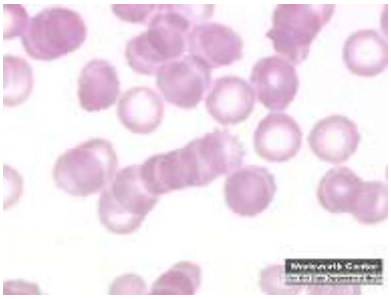

Figure 144: Healthy RBC w/ Stacking (69)

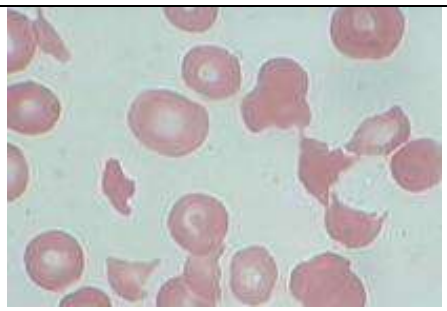

Figure 145: Damaged RBC w/ Debris (70)

Table 40: Summary of RBC Abundance with Aging (qualitative)*

	Whole Blood RBC		SM3-treated Blood RBC	
#	Abundance	Morphology	Abundance	Morphology
0	10	10	10	10
2	8.75	6	10	10
13	2	1	9.5	10
17	1	1	8.5	10
24	--	--	8.5	10
34	0	0	8	10
49	--	--	7	10
84	--	--	6	9
230	0	0	4.5	5

* 1 = Low / Poor; 10 = High / Good

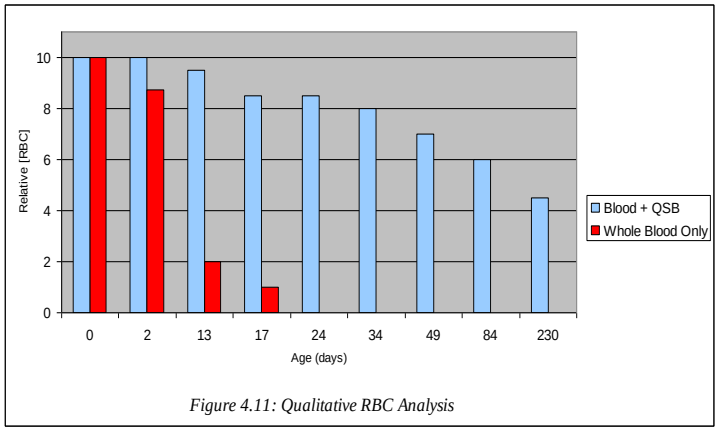

Figure 4.11: Qualitative RBC Analysis

Fig. 146: Qualitative RBC Viability over Time

White Blood Cell Viability

White blood cells aged over the course of 230 days were examined microscopically for abundance and morphological integrity as well as the survival of intact nuclei (Figure 147), which are the primary source of DNA obtained from whole blood samples. Observations are summarized in Table 41; both abundance and maintenance of morphology were assessed relative to a fresh whole blood sample and rated on a scale of 1–10. In general, the degradation of WBC in untreated whole blood coincided with the degradation of RBC, which started within two days of environmental exposure and progressed to near full degradation in approximately two weeks. As was the case with RBC, the WBC preservation period could be increased by sealing the well plate. WBC degradation was also gradual; degrading cells took on the appearance of sickle-cell-affected leukocytes (Figure 148), with an increase of cellular debris and "ghost cells" containing no nuclei. Results reported in Table 41 represent a combined assessment of all samples for a given day.

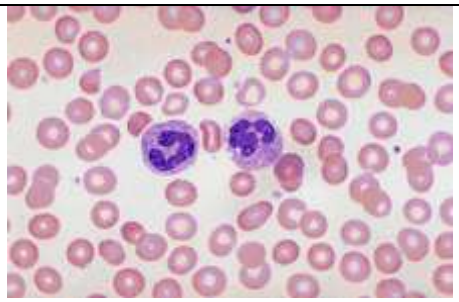

Figure 147: Two normal WBC shown among RBC(67)

Figure 148: Sickle-cell affected WBC (68)

Table 41: Summary of WBC Abundance with Aging (qualitative)*

	Whole Blood WBC		SM3-treated Blood WBC	
# Days Aged	Abundance	Morphology	Abundance	Morphology
0	10	10	10	10
2	8.75	6	9.5	10
13	2	1	8	10
17	1	1	8	10
24	--	--	5	10
34	0	0	4	10
49	--	--	0	--
84	--	--	0	--
230	0	0	1	5

* 1 = Low / Poor; 10 = High / Good

Free Nuclei Viability

In conjunction with the study of RBC and WBC aged over the course of 230 days, the degree of free nuclei was also examined microscopically. Intact nuclei, which can be observed under phase contrast as dark blue kernels within WBC and free in the hematocrit following WBC degradation, are the primary source of genetic material obtained from forensic whole blood samples. Observations are summarized in Table 42; the morphology of the nuclei was not taken into consideration, as long as they were intact. As with the blood cells, the abundance was assessed subjectively relative to the fresh whole blood sample and rated on a scale of 1 – 10. In general, the degradation of WBC in untreated whole blood directly correlates with the increased presence of free nuclei. Results reported in Table 42 and Figure 149 represents a combined assessment of all samples for a given day.

Table 42: Summary of Free Nuclei Abundance with Aging (qualitative)*

# Days Aged	Whole Blood Nuclei	SM3-treated Nuclei
0	1	1
2	2	1
13	7	1
17	8	2
24	8	5
34	8	7
49	8	8
84	7	8
230	1	8

* 1 = Low / Poor; 10 = High / Good

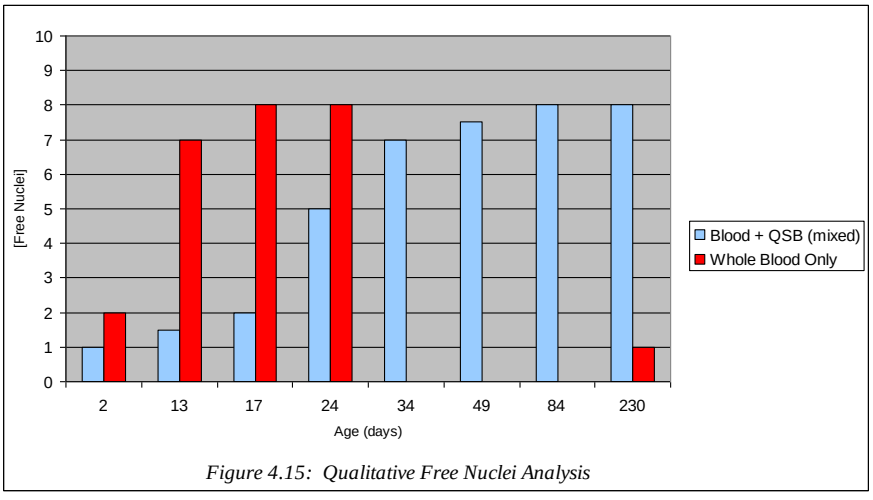

Figure 4.15: Qualitative Free Nuclei Analysis

Fig. 149: Qualitative Free Nuclei Viability over Time

CHAPTER 8: RESULTS OF DNA GENOTYPING ANALYSIS

I. SHORT-TERM STUDIES (6 MONTH STORAGE)

A. COATING AGENT STUDY

The target input of the sample extracts was adjusted to a DNA concentration of 1.5ng/20 µL for STR analysis. A of total 275 samples met the 1.5ng threshold and were genotyped. These samples comprised of 94 samples protected with SM1 and 91 samples protected with SM2 while 45 frozen and 45 room temperature stored samples were genotyped. The results are presented by comparing the number of full (CODIS 13 core loci) or partial (missing at least one allele from the full profile) DNA profiles with respect to dilutions and extraction chemistries at each storage condition. In all instances, full or partial DNA profiles were recovered for each of the three body fluids analyzed. However, for blood samples, there were instances in which the number of full DNA profiles recovered was greater relative to other extraction chemistries. In general, when taking into consideration the extraction method, the number of profiles obtained with the SampleMatrix™ is often equivalent to the samples stored unprotected. There appears to be evidence that the performance of SampleMatrix™ protected samples outperform room temperature and frozen samples in several instances; this seems to holds true regardless of the body fluid compared (Figures 151-153) or dilution factor (Figures 154-156). As a general trend, SM1 tends to outperform SM2; however, this distinction is not as apparent when the organic extraction is performed. Further, the superior nature of SM1 is not as evident with the saliva samples as compared with blood or semen. Finally, when considering the results obtained for the application of SampleMatrix™ to a dried versus wet sample, there appears to be a marginal benefit when SampleMatrix™ is applied to a dried body fluid. While this generally holds true for the blood and semen samples that are subjected to the chelex or Qiagen chemistries, both applications appear to be equivalent for the organic extraction. In contrast, the results indicate that the wet application approach is better served for saliva samples.

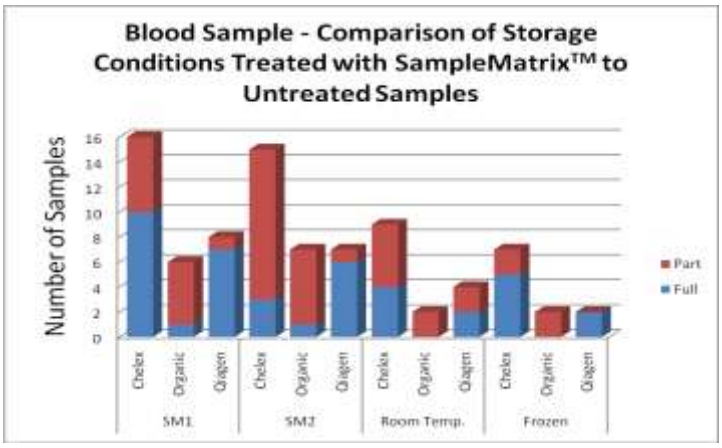

Fig. 151: Comparison of Full and Partial DNA Profiles for Blood Samples Untreated *vs*. Treated with SampleMatrix™ for Each Storage Condition.

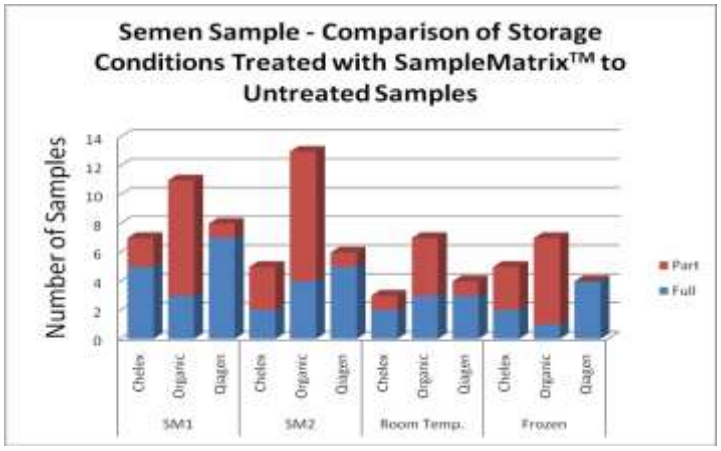

Fig. 152: Comparison of Full and Partial DNA Profiles for Semen Samples Untreated *vs.* Treated with SampleMatrix™ for Each Storage Condition.

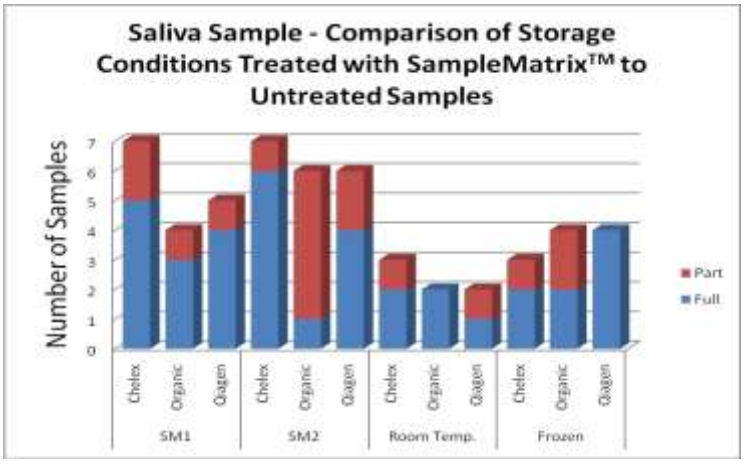

Fig. 153: Comparison of DNA Profiles for Saliva Samples Untreated *vs*. Treated with SampleMatrix™ for Each Storage Condition

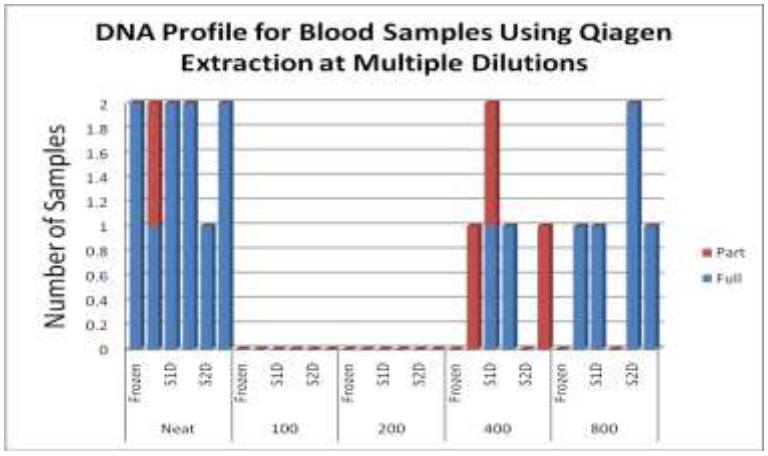

Fig. 154: Quality of Full and Partial DNA Profile Recovered for Blood Samples Using Qiagen Extraction

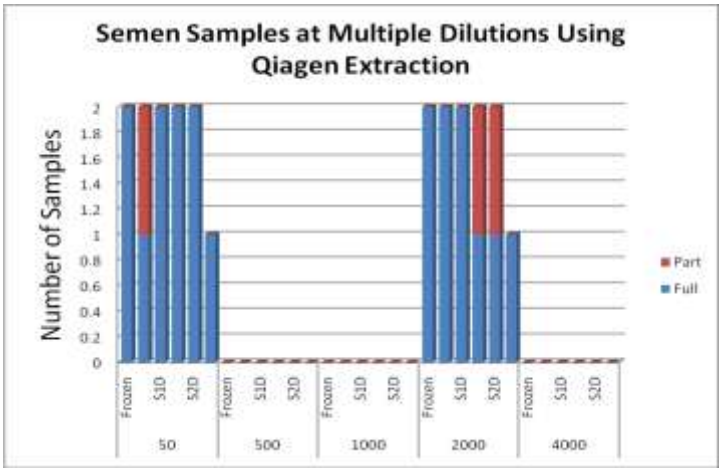

Fig. 155: Quality Full and Partial DNA Profile Recovered for Semen Samples Using Qiagen Extraction at Multiple Dilutions.

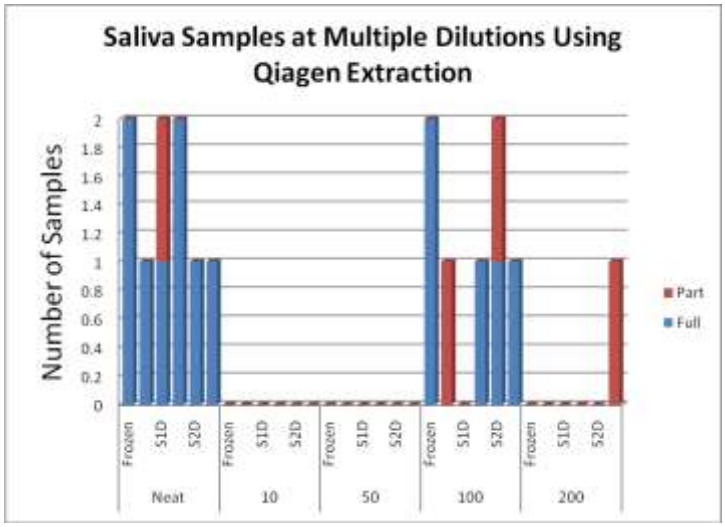

Fig. 156: Quality Full and Partial DNA Profile Recovered for Saliva Samples Using Qiagen Extraction at Multiple Dilutions.

B. WETTING AGENT STUDY

Again, the target input of the sample extracts was adjusted to a DNA concentration of 1.5ng/20 µL for STR analysis. A of total 322 samples met the 1.5ng threshold and were genotyped. Within this study, 107 and 112 samples that were wetted with SM1 and SM2 were genotyped, respectively; in comparison, 103 samples where water served as the wetting agent were typed.
The data indicate that blood samples stored with SampleMatrix™ formulation SM1 result in higher quality DNA profiles as compared with SM2. Further, the SampleMatrix™ formulation SM2 resulted in higher quality DNA profiles relative to the samples that were stored in frozen conditions. In relation to semen and saliva samples, the quality the DNA profiles protected with SampleMatrix™ formulation SM1 were generally better than the unprotected frozen samples. However, the frozen control samples did appear to outperform the samples that were protected with SampleMatrix™ formulation SM2. Figures 157-159 present the data for blood broken down by different storage condition/wetting agents and dilution for each the Qiagen chemistry.

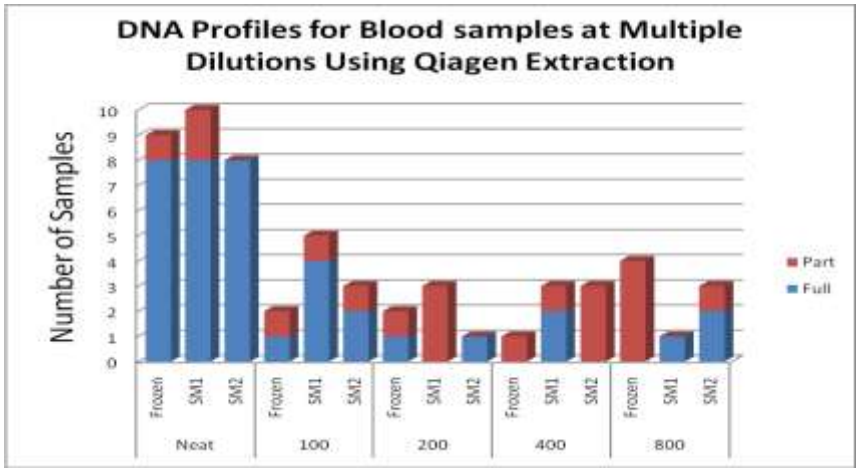

Fig. 157: Quality DNA Profiles Recovered for Blood Samples Using Qiagen Extraction at Multiple Dilutions.

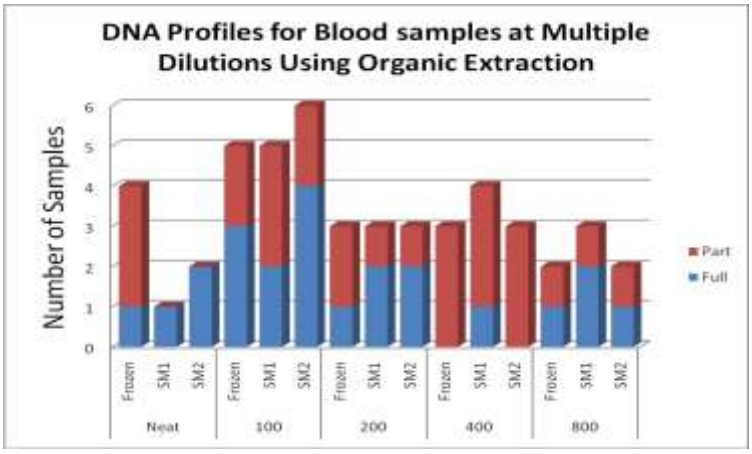

Fig. 158: Quality DNA Profiles Recovered for Blood Samples Using Organic Extraction at Multiple Dilutions.

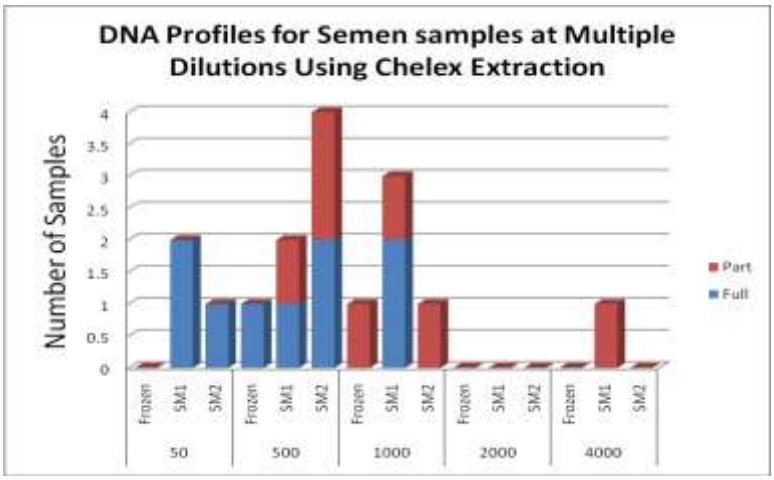

Fig. 159: Quality DNA Profiles Recovered for Semen Samples Using Chelex Extraction at Multiple Dilutions.

In terms of the relative effectiveness of the three wetting agents with respect to the DNA profile obtained, it appears that Sample Matrix™ provides comparable results to water. There are marginal differences that seem to be substrate-dependent. For example, water seems to be marginally more effective in terms of recovering blood from glass (although only at the lower dilutions) whereas Sample Matrix™ is marginally more effective in terms of recovering blood from wood and carpet. There is some variation on these results based on the extraction chemistry; generally, the Qiagen the extraction method resulted in higher quality DNA profiles relative to the organic method. Figures 160-162 illustrate the results obtained with the Qiagen extraction for several dilutions of blood while figures 163-164 show the data obtained for the extraction of saliva with Chelex.

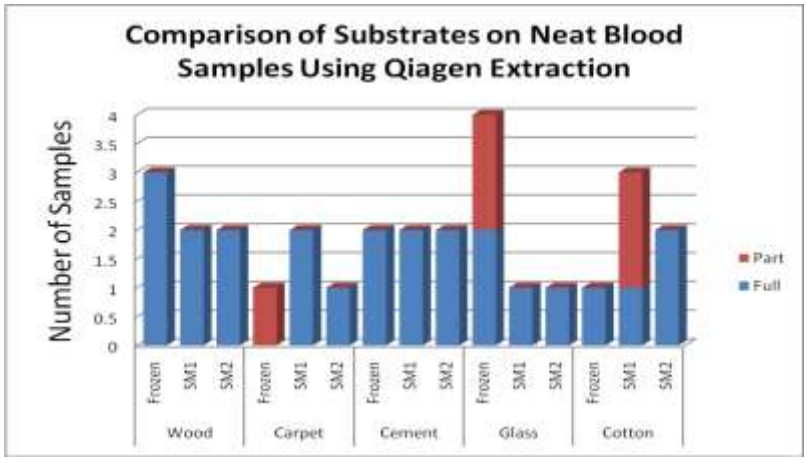

Fig. 160: Quality of DNA Profiles for Neat Blood Samples Using Qiagen Extraction with Different Substrates and Storage Conditions.

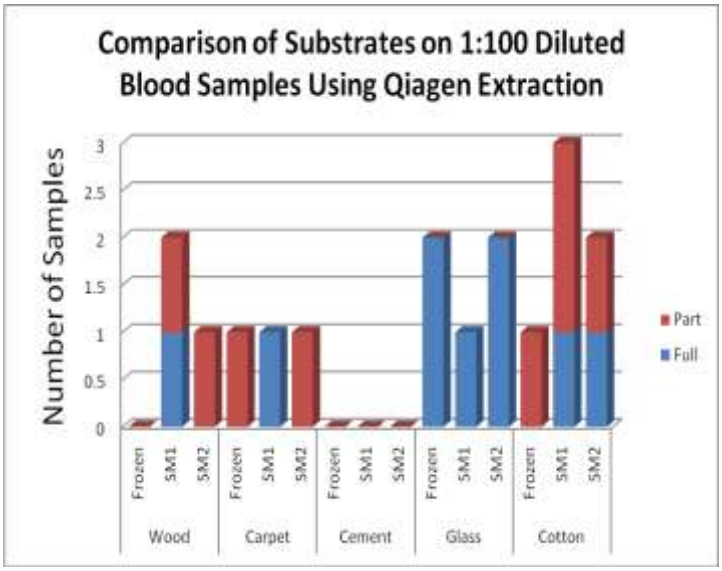

Fig. 161: Quality of DNA Profiles for 1:100 Diluted Blood Samples Using Qiagen Extraction with Different Substrates and Storage Conditions.

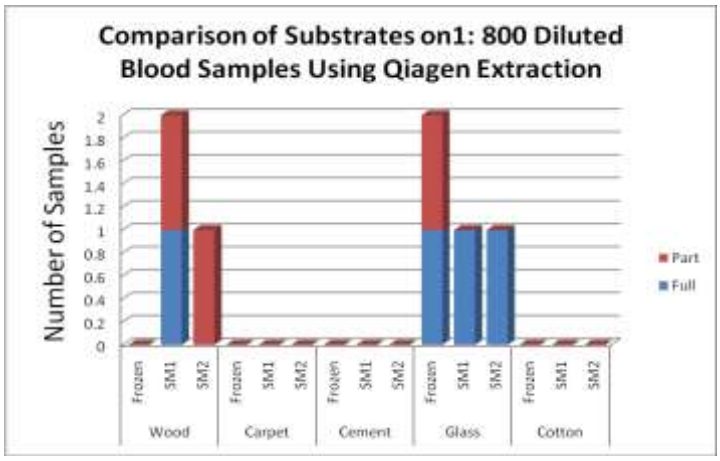

Fig. 162: Quality of DNA Profiles for 1:800 Diluted Blood Samples Using Qiagen Extraction with Different Substrates and Storage Conditions.

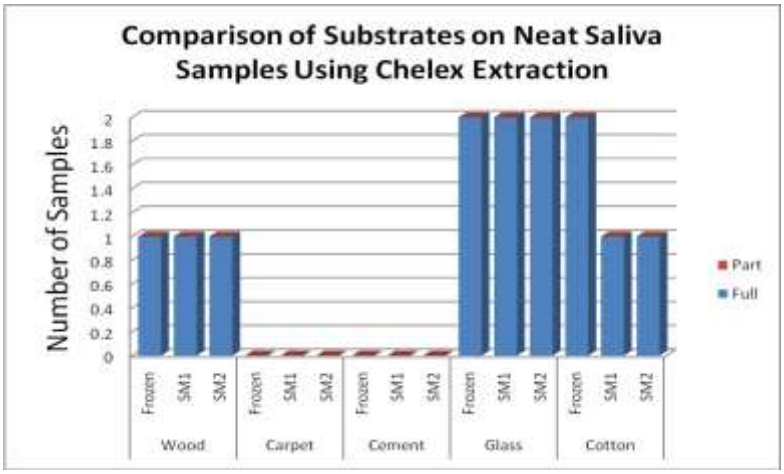

Fig. 163: Quality of DNA Profiles for Neat Saliva Samples Using Chelex Extraction with Different Substrates and Storage Conditions.

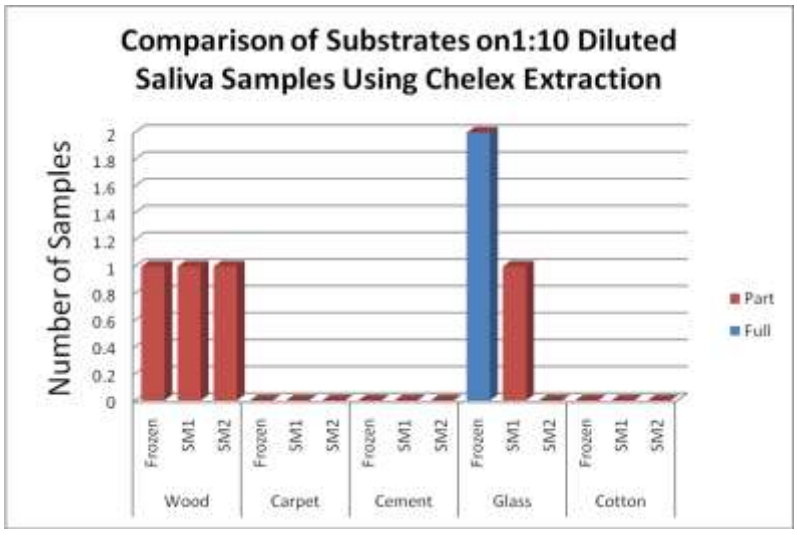

Fig. 164: Quality of DNA Profiles for 1:10 Diluted Saliva Samples Using Chelex Extraction with Different Substrates and Storage Conditions.

II. LONGER TERM STUDIES (17-24 MONTH STORAGE)

COATING AGENT STUDY

Storage Conditions

The first question addressed in this study was a comparison of the quality of the DNA profiles from samples protected with SampleMatrix™ relative to equivalent samples that were stored either unprotected at room temperature or stored under frozen conditions (Figure 166). Sixty-three profiles were genotyped from samples protected with SampleMatrix™ and stored at room temperature. Of the 63 profiles, 22% of the samples gave a full profile, and 29% gave a partial profile that was greater than 75% of the full profile. Two of the samples protected with SampleMatrix™ did not result in a profile. These two samples were 1) a 1/800 dilution of blood that was organically extracted and protected with SM1 applied to a dried sample, and 2) a 1/50 dilution of semen that was organically extracted and protected with SM1 applied to a wet sample. Based on the real-time data and retentate volumes, both of these samples were determined to contain a total DNA value greater than 0.075ng/µL. Thirty-eight unprotected samples that were stored at room temperature were also genotyped (Figure 167). 16% of the room temperature samples gave full profiles, and 35% provided partial profiles greater than 75% of the full profile. All of the unprotected samples resulted in at least a partial profile. Finally, 35 total frozen control samples were genotyped for comparison with the room temperature samples (Figure 168). 23% of the frozen samples gave full profiles and 34% gave partial profiles greater than 75% of the full profile (Figure 169). All of the frozen samples resulted in at least a partial profile. Regardless of the storage condition, the majority of samples presented profiles representing less than 75% of the alleles for a full profile. 46% of the SampleMatrix™ protected samples, 49% of the unprotected room temperature samples and 43% of the frozen samples fell into this category.

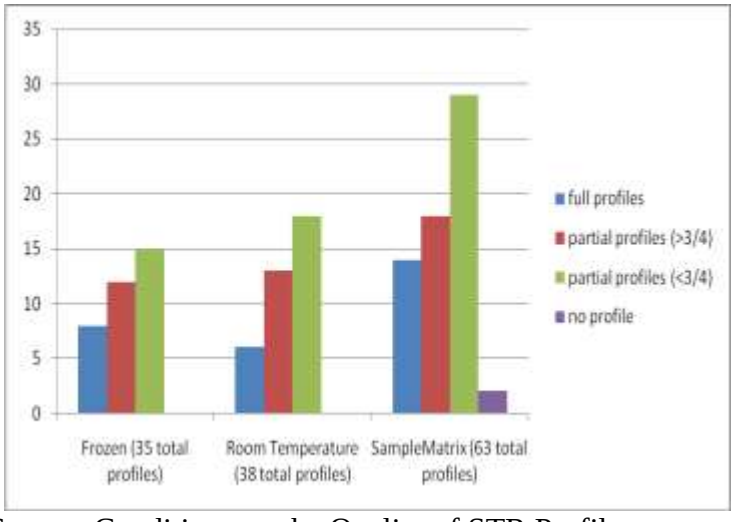

Fig. 166: Effect of Storage Conditions on the Quality of STR Profiles

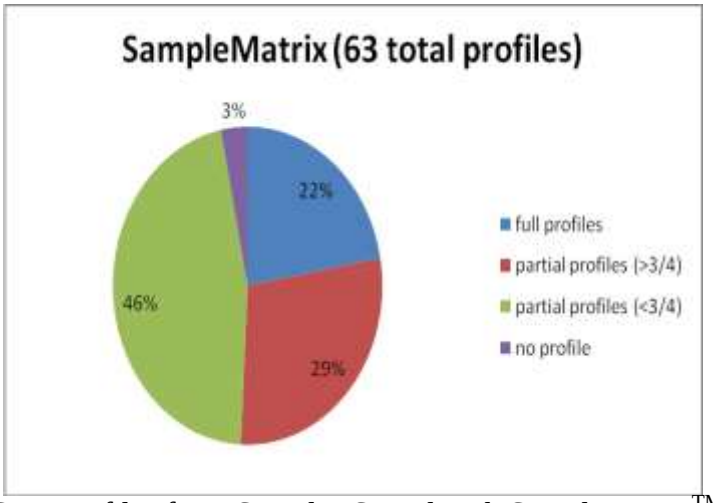

Fig. 167: Quality of STR Profiles from Samples Stored with SampleMatrix™

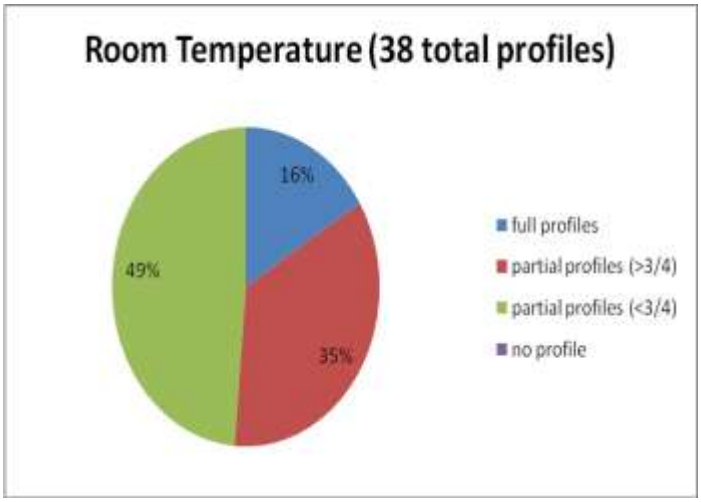

Fig. 168: The Quality of STR Profiles from Samples Stored at Room Temperature

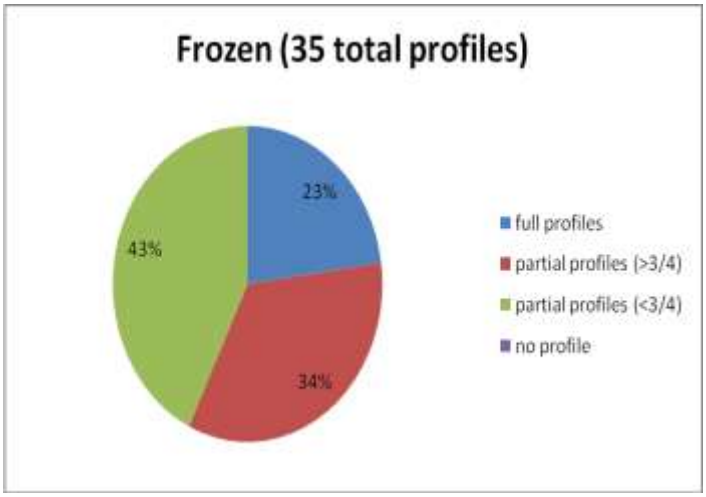

Fig. 169: The Quality STR Profiles from Samples Stored under Frozen Storage Conditions

CODIS Eligibility

A different approach to address the quality of the STR profile recovered from the samples was also considered in this study by designating the profile in accordance with CODIS eligibility. The Combined DNA Index System (CODIS) operates the local, State, and national databases of DNA profiles. These profiles consist of, but are not limited to, DNA profiles collected from convicted offenders, unsolved crime scene evidence, and mission persons. Each state has different criteria that must be met before a suspect profile can be entered into the convicted offender database. When a profile is obtained from an item of evidence associated with an unsolved crime, the profile is first compared with profiles retained in the database at the local level. If a profile match is not made at the local level, the profile is searched against the database at the state level. A profile is only searched through the national database when a match is not made at either of the previous levels. A match made through CODIS can link crime scenes together in order to identify serial offenders or can identify a suspect in an otherwise unsolved crime. The Combined DNA Index System comprises 13 core STR Loci. For a profile to be eligible for entry into CODIS at the state level, the profile must have representation at seven loci. For eligibility at a national level, there must be representation at a minimum of ten loci. The purity of a profile also plays a role in the eligibility of a profile for CODIS. The profile must be: 1) single source (from only one individual), 2) a major donor is obvious, or 3) there are no more than four alleles at four different loci.

Based on CODIS eligibility, 77% of the frozen samples, 51% of the room temperature samples, and 61% of the SampleMatrix™ protected samples were eligible for NDIS (Figure 280). Samples with at least one allele present at seven or more loci are eligible for CODIS at the State level. Samples were considered SDIS eligible if seven or more loci were represented in the profile but less than 10; 20% of the frozen samples, 11% of the room temperature, and 26% of the SampleMatrix™ protected samples were eligible for SDIS. Of the samples that were stored frozen, only one profile from was not eligible for either of the CODIS databases. In comparison, 38% of the unprotected room temperature stored samples were not eligible, and 13% of samples protected with SampleMatrix™ were not eligible for either database (Figure 170).

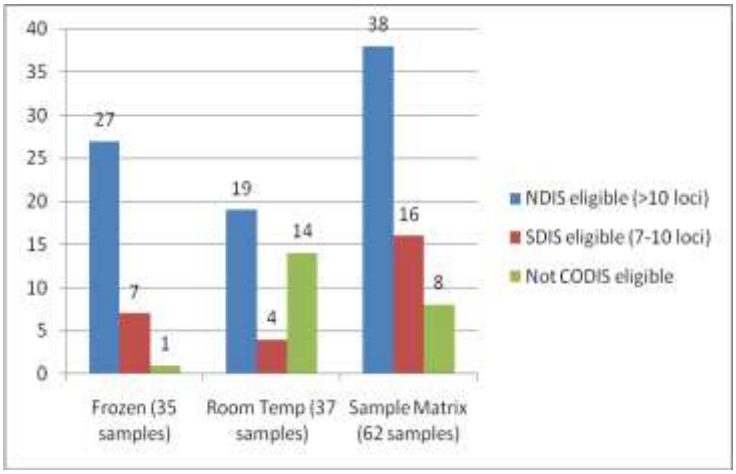

Fig. 170: The Effects of Storage Condition on the Eligibility of STR Profiles for CODIS Body Fluid Comparison

SampleMatrix™ Formulation

The second question addressed in this study is whether the particular SampleMatrix™ formulation affects the quality of DNA recovered for STR profiling (Figure 171). When the sample was protected using SM1, 25% of the samples gave a full profile, 38% of the samples gave partial profiles greater than 75% of a full profile, 21% of the samples gave partial profiles less than 75% of a full profile while two samples gave no profile (Figure 172). When the sample was protected using SM2, 20% gave full profiles, 19% gave partial profiles greater than 75% of the full profile, and 61% gave partial profiles less than 75% of the full profile (Figure 173). All of the samples resulted in at least a partial profile when SM2 was used as a protecting agent.

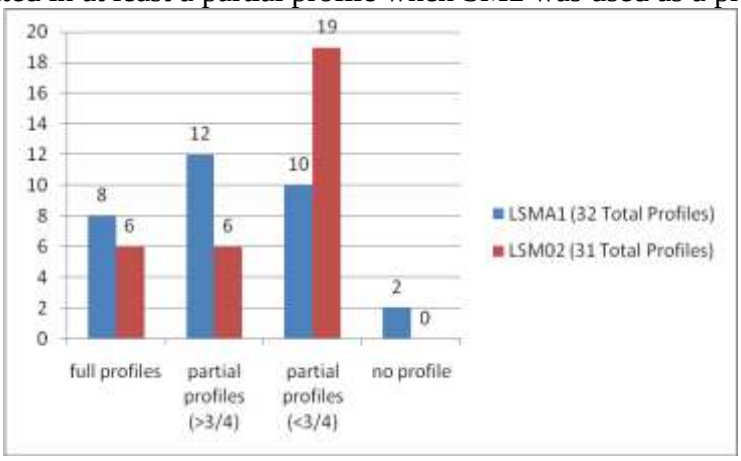

Fig. 171: Comparison of STR Profiles from Samples Protected Using two Different Formulations of SampleMatrix™

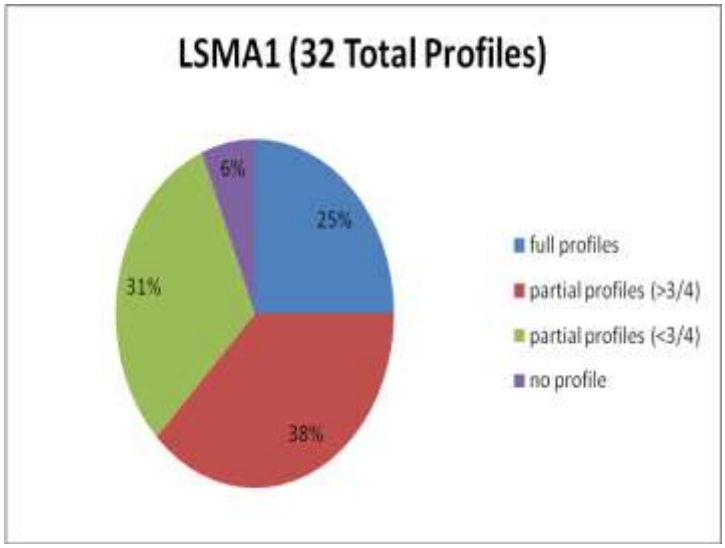

Fig. 172: The Quality of STR Profiles when Protected Using SM1

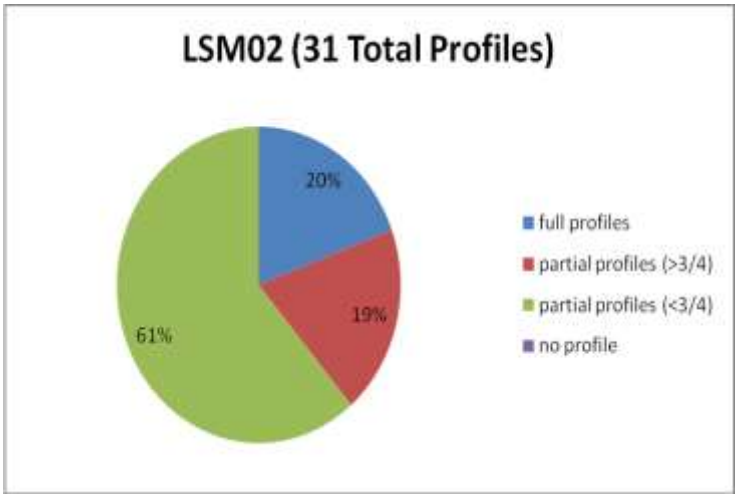

Fig. 173: The Quality of STR Profiles when Protected Using SM2

When analyzing the impact of the SampleMatrix™ formulation for CODIS eligibility it was observed that the majority of samples are NDIS eligible regardless of the formulation applied as a protective agent (Figure 174). Of the samples protected with SM1, 69% of the samples were NDIS eligible, 19% are eligible for SDIS, and only four samples (12%) were not eligible for either database (Figure 175). Similarly, when samples were protected with SM2, 55% were eligible for NDIS, 32% were eligible for SDIS and similarly only 13% were not eligible for either database (Figure 176).

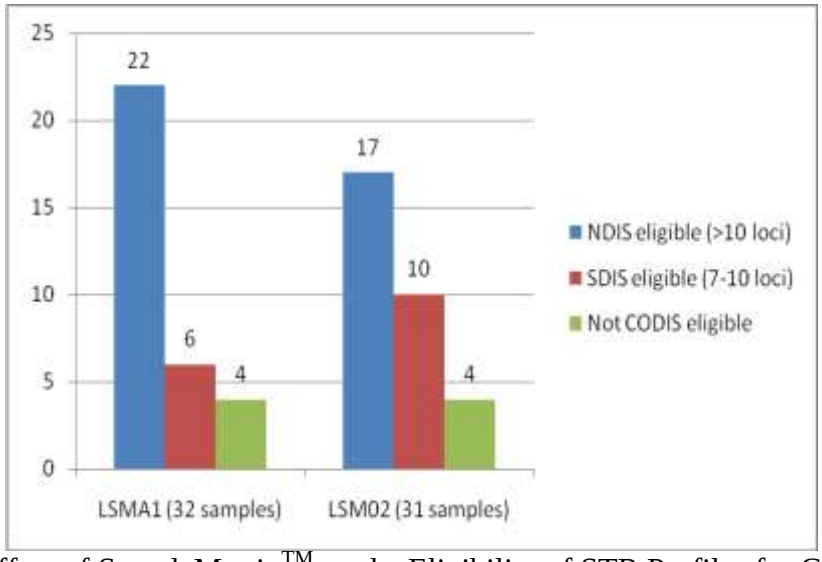

Fig. 174: The Effect of SampleMatrix™ on the Eligibility of STR Profiles for CODIS

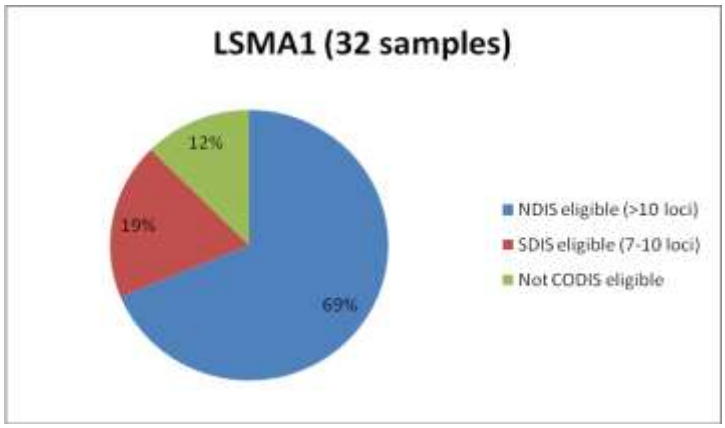

Fig. 175: The Effect of SM1 on the Eligibility of STR Profiles for CODIS

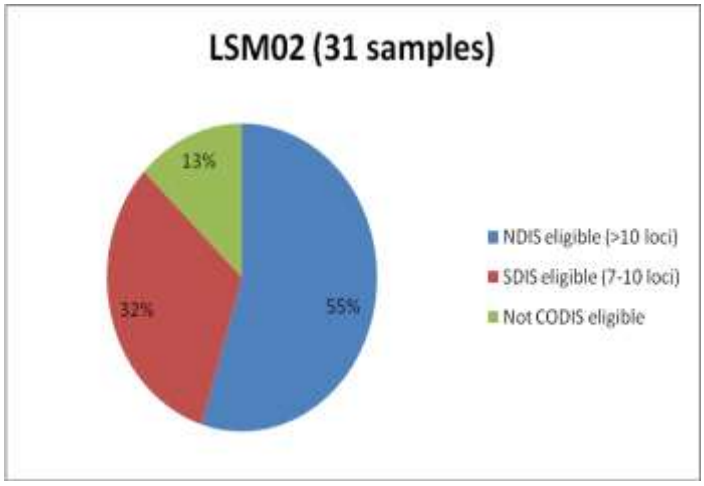

Fig. 176: The Effect of SM2 on the Eligibility of STR Profiles for CODIS

Application Method

The third question addressed in this study related to the application method (wet vs. dry) in order to determine if one method is more effective in protecting DNA for optimal STR analysis (Figure 177). When using the dry application technique, 26% of the samples provided a full profile, 26% of the samples provided a partial profile with greater than 75% of the full profile and 45% of the samples gave profiles less than 75% of the full profile (Figure 178). When using the wet application technique, 19% of the samples provided full profiles, 31% of the samples gave profiles greater than 75% of the full profile and 47% of the samples gave partial profiles less than 75% of the full profile (Figure 179). For both wet and dry application techniques there was one sample that did not give a profile, blood diluted 1/800 using SM01 and semen diluted 1/50 using SM01, respectively.

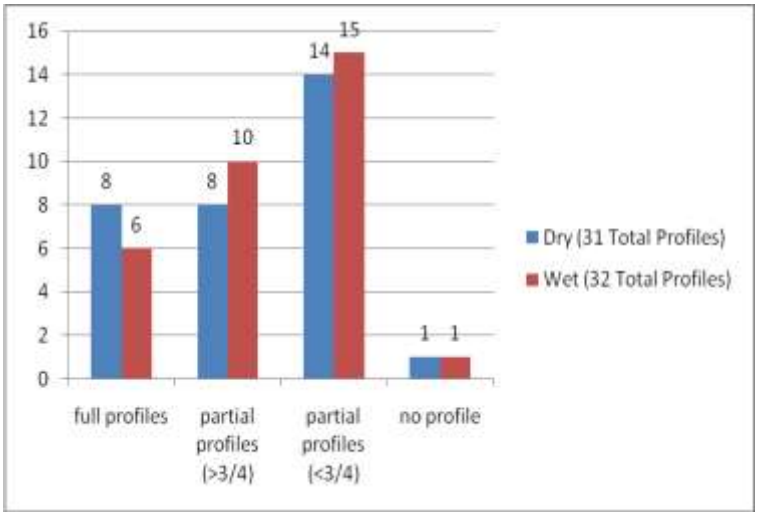

Fig. 177: Comparison of STR Profiles Samples Different Application Techniques

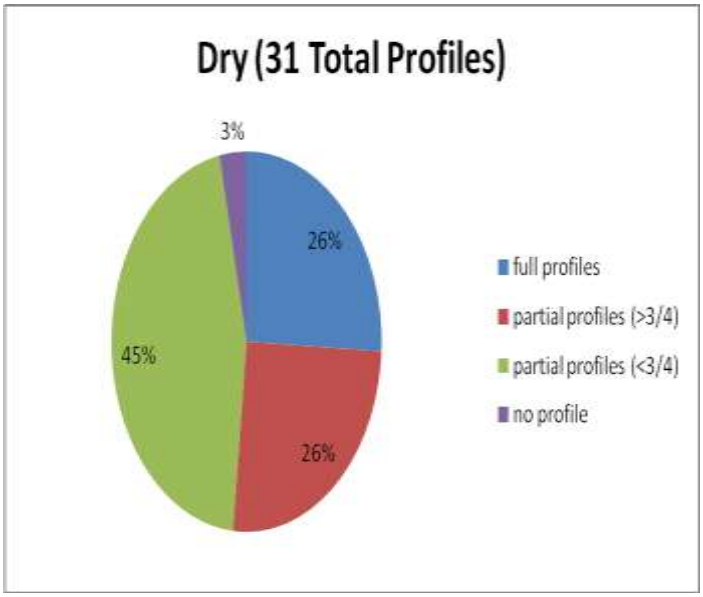

Fig. 178: The Quality of STR Profiles when Assuming a Dry Application Technique

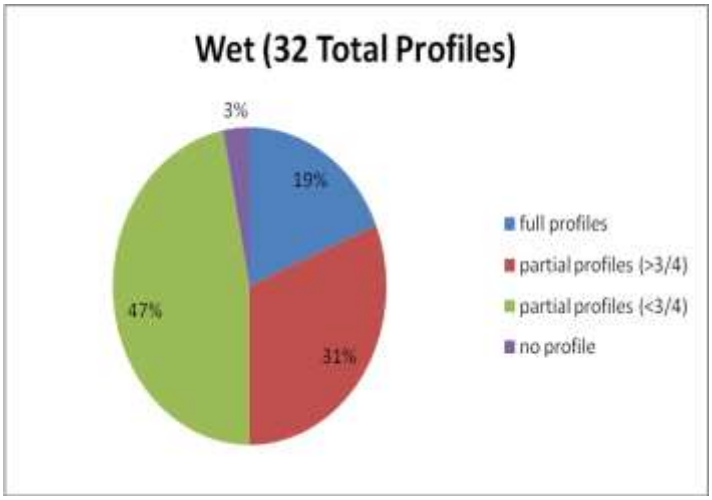

Fig. 179: The Quality of STR Profiles when Assuming a Wet Application Technique

The majority of samples that were applied using the wet or the dry methods were eligible for NDIS (Figure 180) 62% of the samples where SampleMatrix™ was applied to a wet sample were eligible for NDIS, and 61% of samples where SampleMatrix™ was applied to a dry sample were also NDIS eligible. 22%, of the wet application, samples and 29% of the dry application samples were eligible for SDIS, and only five wet and three dry application samples were not eligible for either database. Of those samples not eligible for CODIS, four of the five wet application samples were saliva in origin. Of the samples not eligible for CODIS when using dry application techniques there was no apparent consistency in terms of the source of the body fluid.

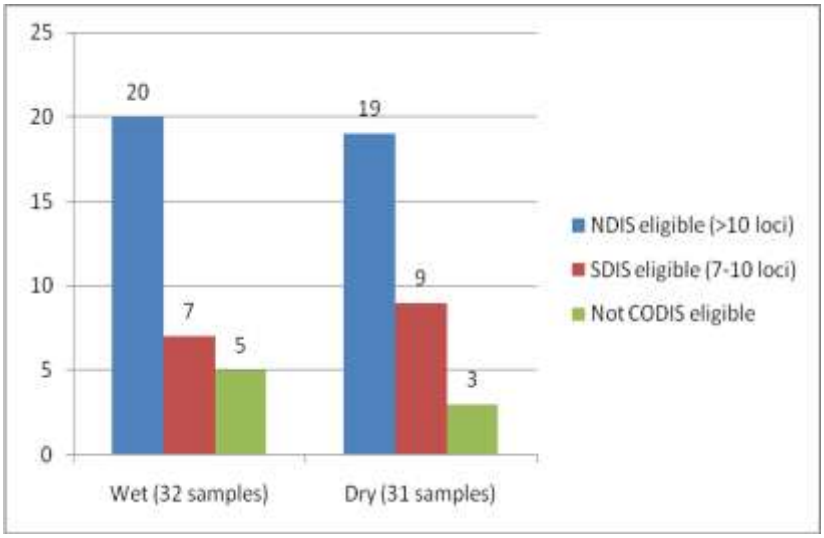

Fig. 180: Effects of Application Method of the Eligibility of STR Profiles for CODIS

Genotyping Results

The results of a select number of STR analyses appear to support the assumption that the two SampleMatrix™ formulations do not interfere with the genotyping analysis (Figures 181-196).

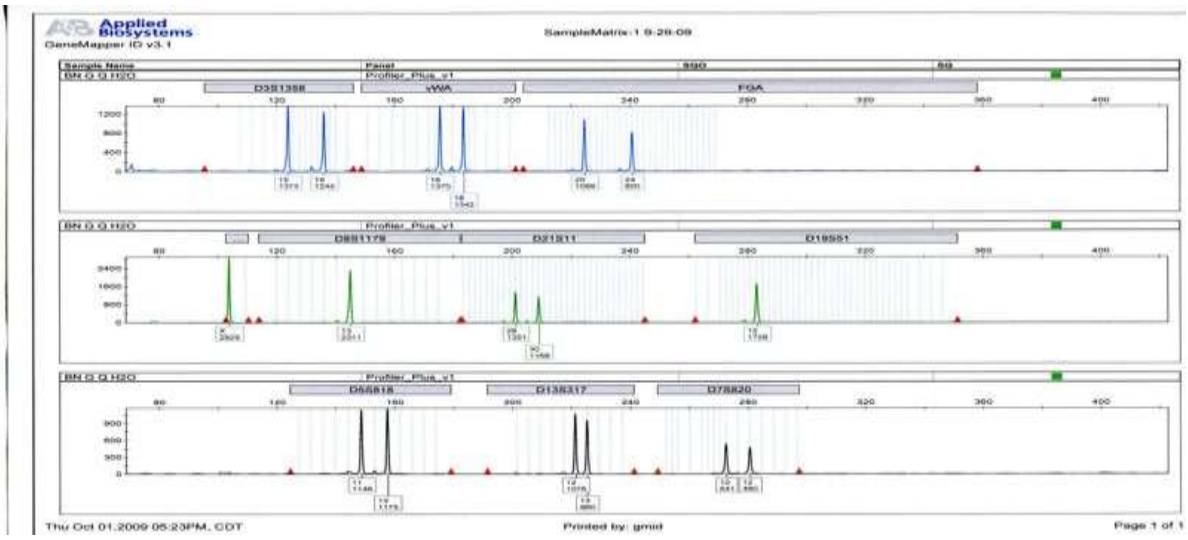

Fig. 181: Neat Blood Recovered with H_2O (Qiagen)

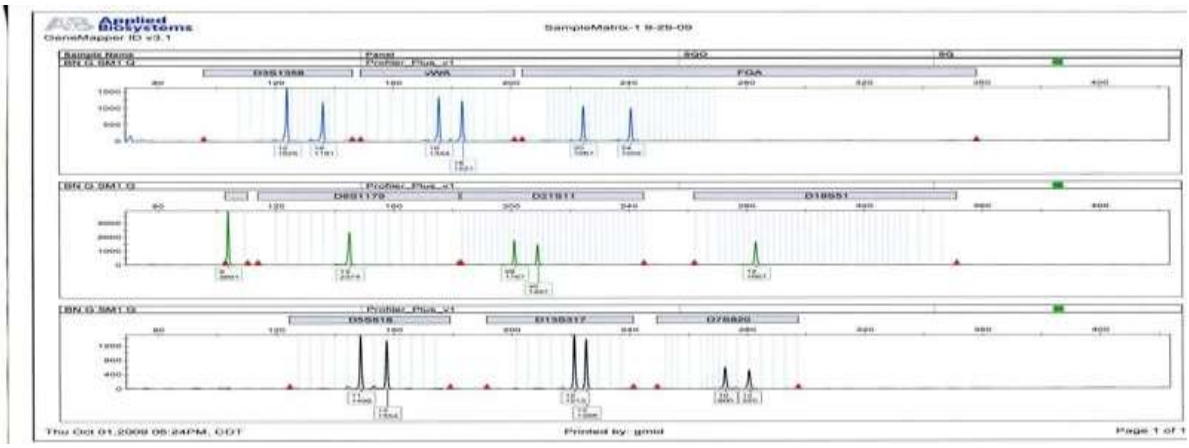

Fig. 182: Neat Blood Recovered with SM1 (Qiagen)

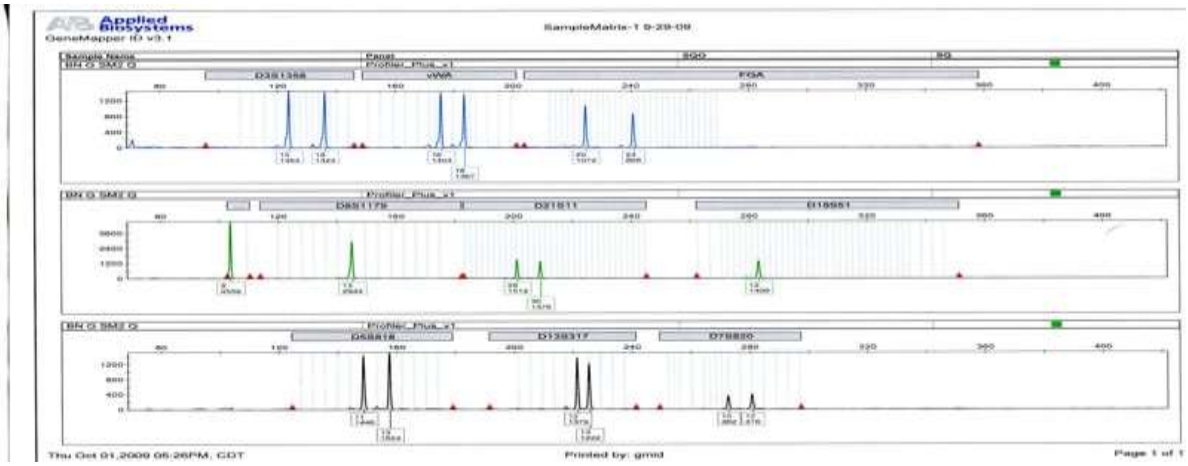

Fig. 183: Neat Blood Recovered with SM2 (Qiagen)

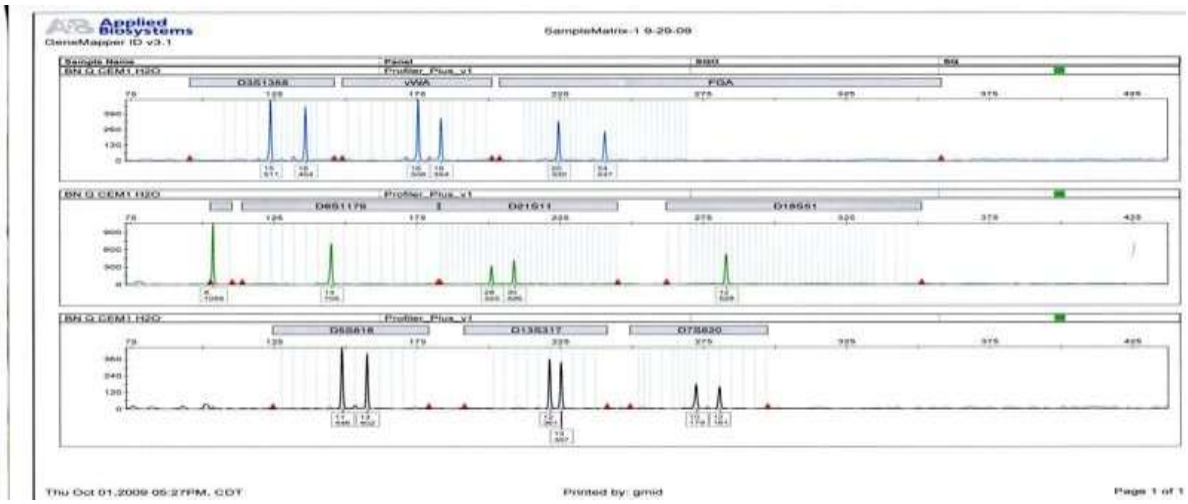

Fig. 184: Neat Blood Recovered from Cement with H_2O (Qiagen)

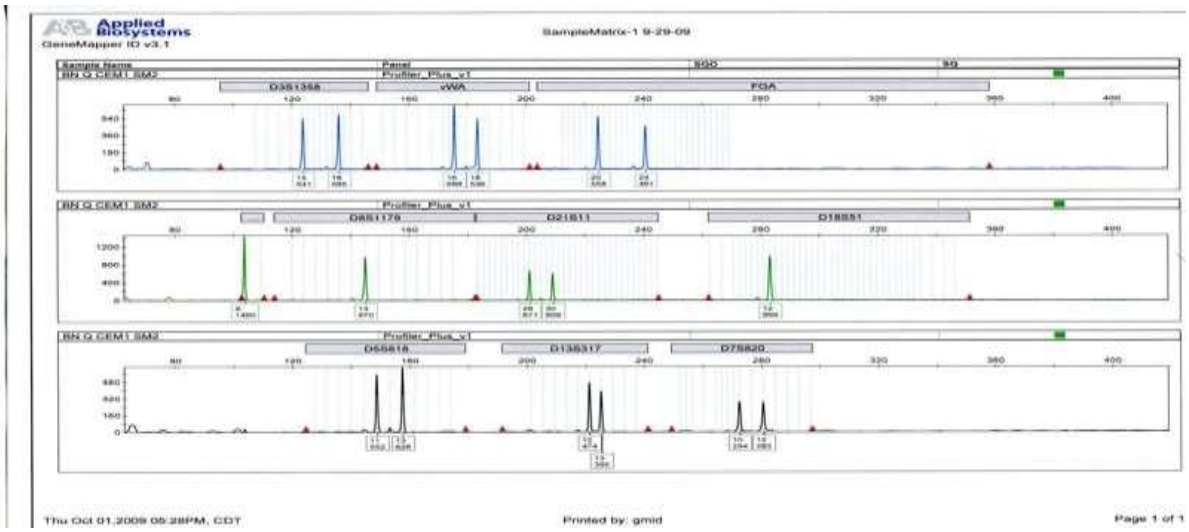

Fig. 185: Neat Blood Recovered from Cement with SM2 (Qiagen)

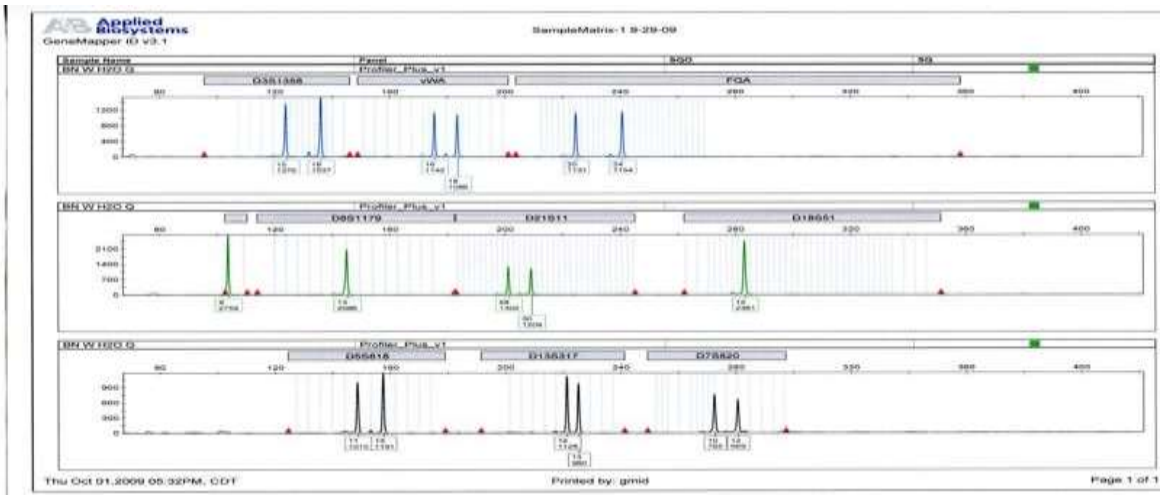

Fig. 186: Neat Blood Recovered from Wood with H_2O (Qiagen)

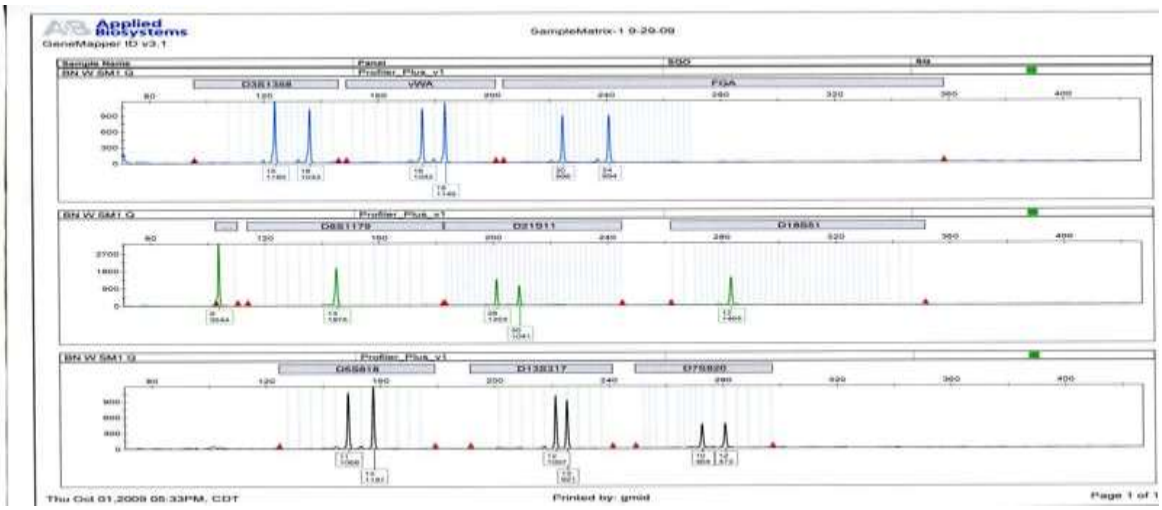

Fig. 187: Neat Blood Recovered from Wood with SM1 (Qiagen)

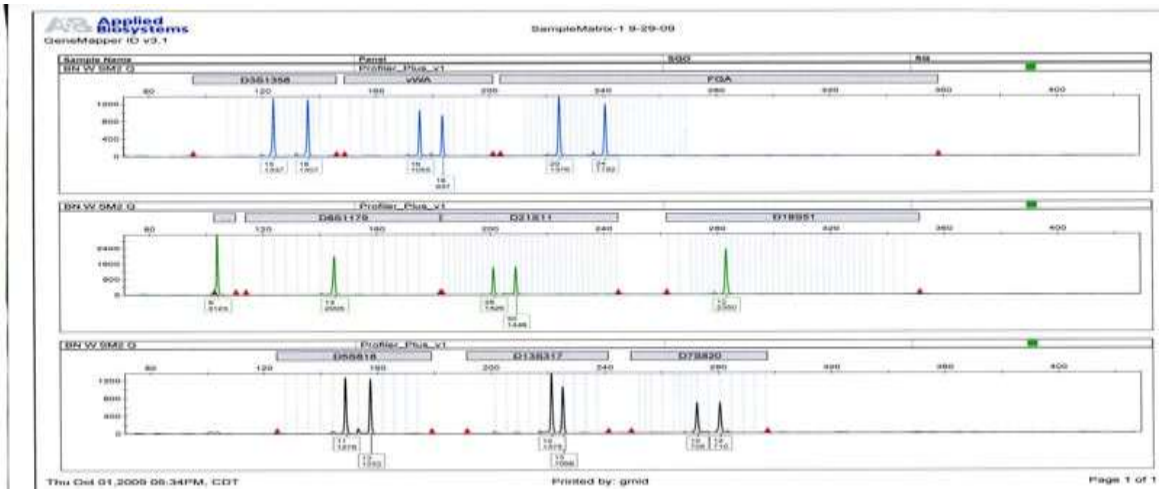

Fig. 188: Neat Blood Recovered from Wood with SM2 (Qiagen)

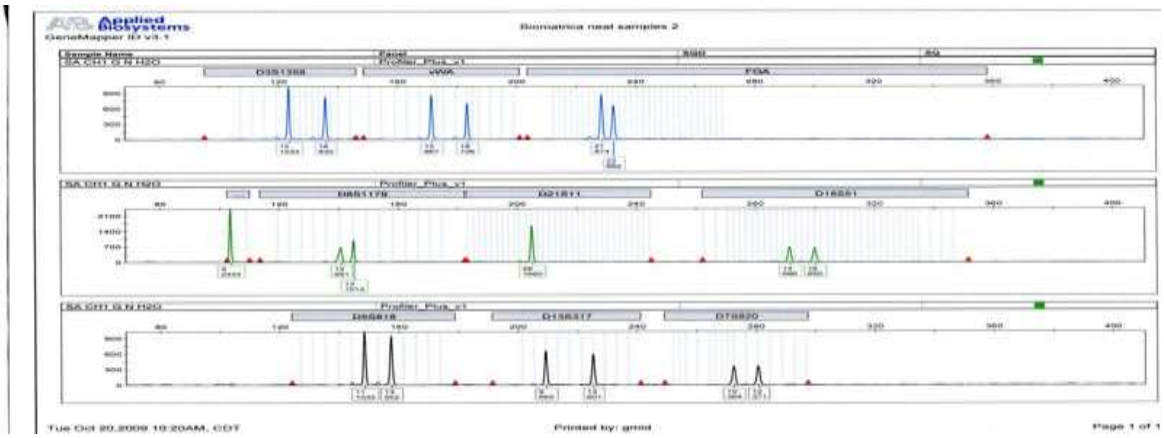

Fig. 189: Neat Saliva Recovered from Glass with H_2O (Chelex)

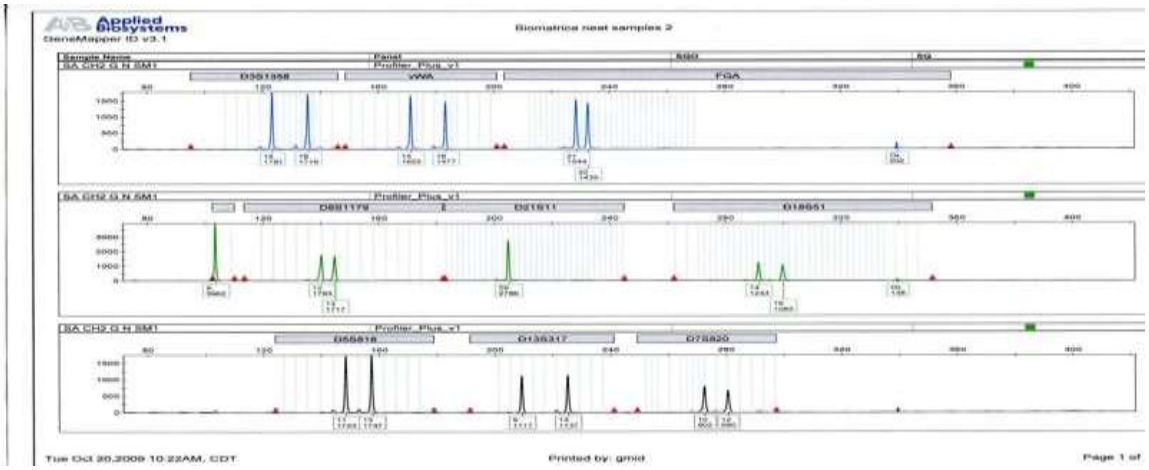

Fig. 190: Neat Saliva Recovered from Glass with SM1 (Chelex)

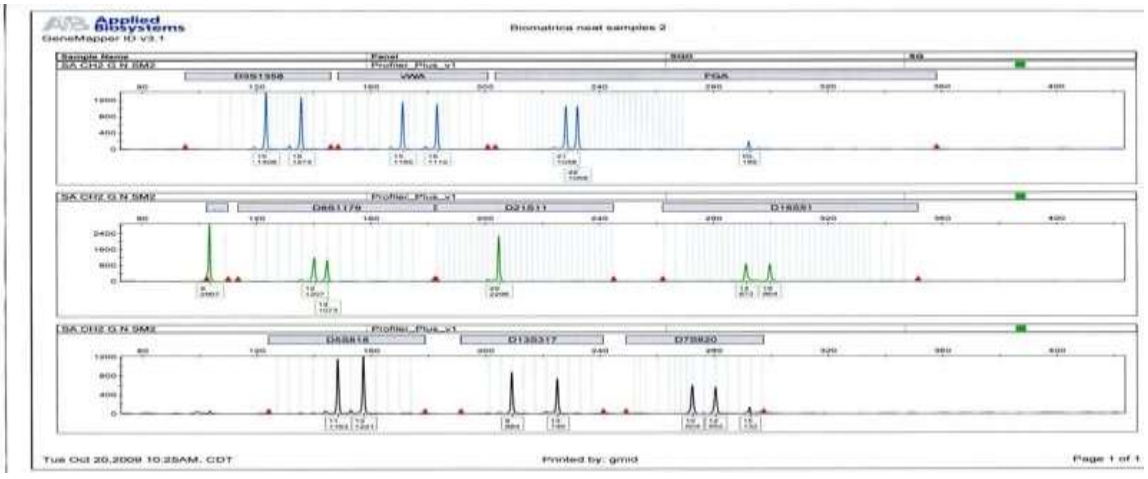

Fig. 191: Neat Saliva Recovered from Glass with SM2 (Chelex)

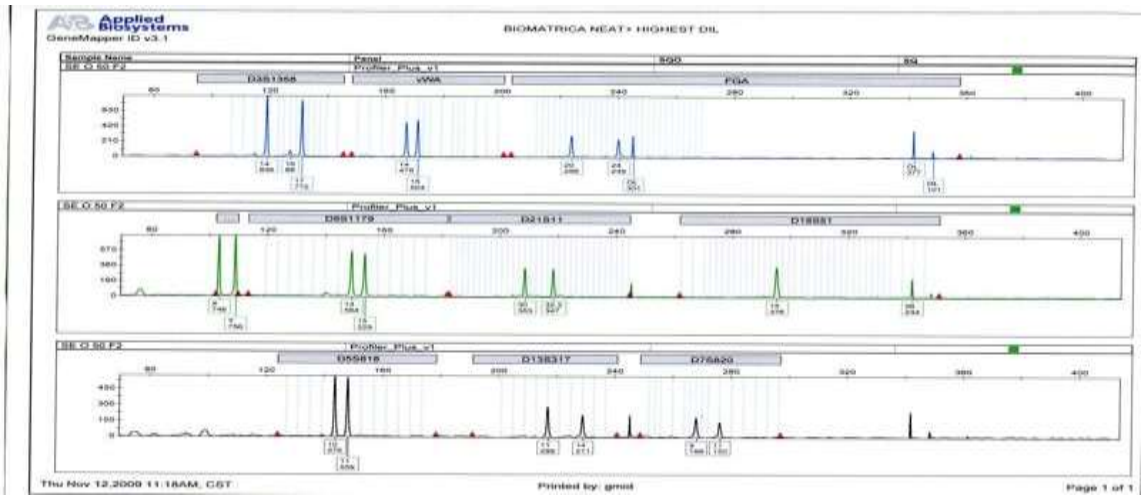

Fig. 192: Neat Saliva Recovered from Glass with SM2 (Chelex)

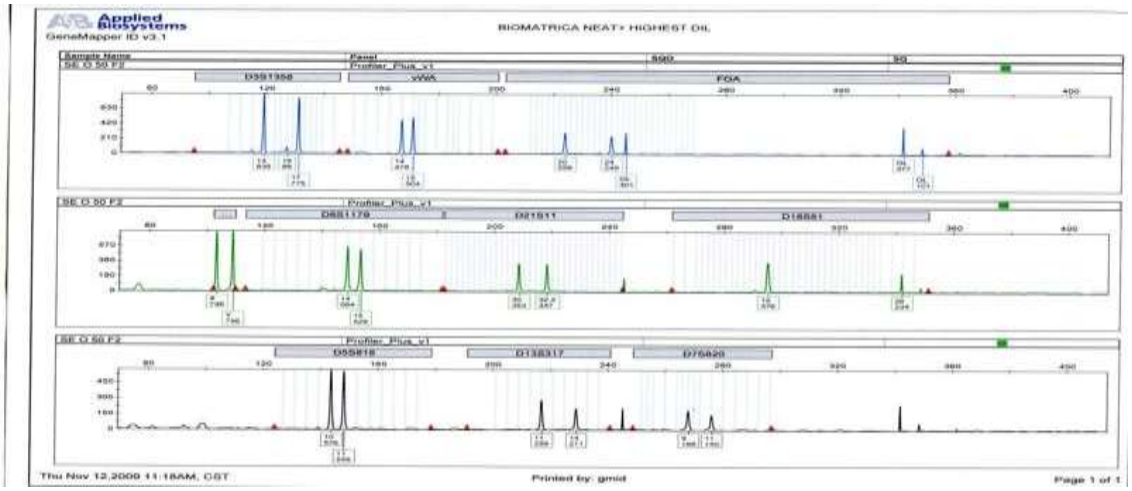

Fig. 193: 1:50 Semen Frozen 6 Month (PCI)

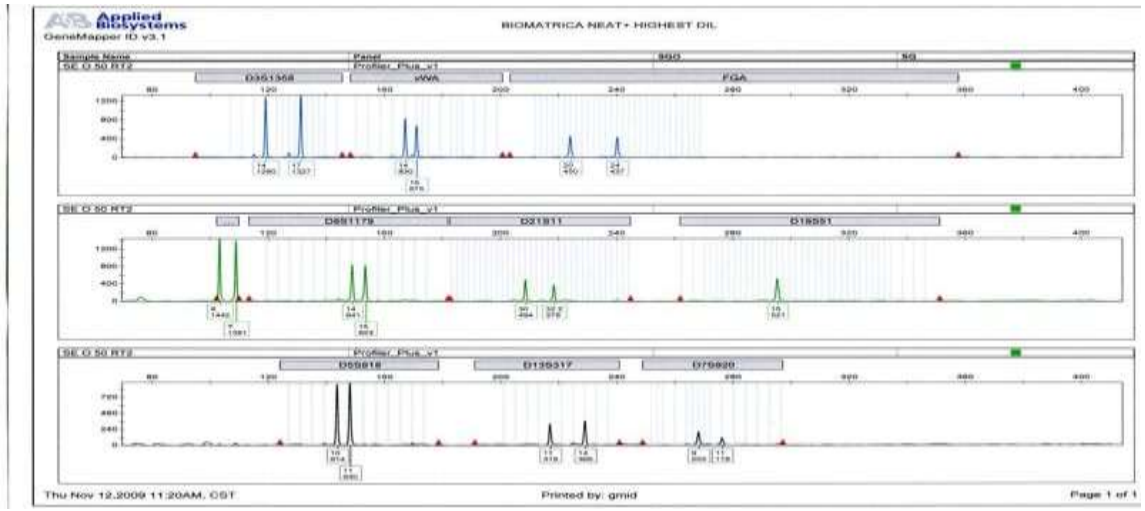

Fig. 194: 1:50 Semen RT 6 Month Storage (PCI)

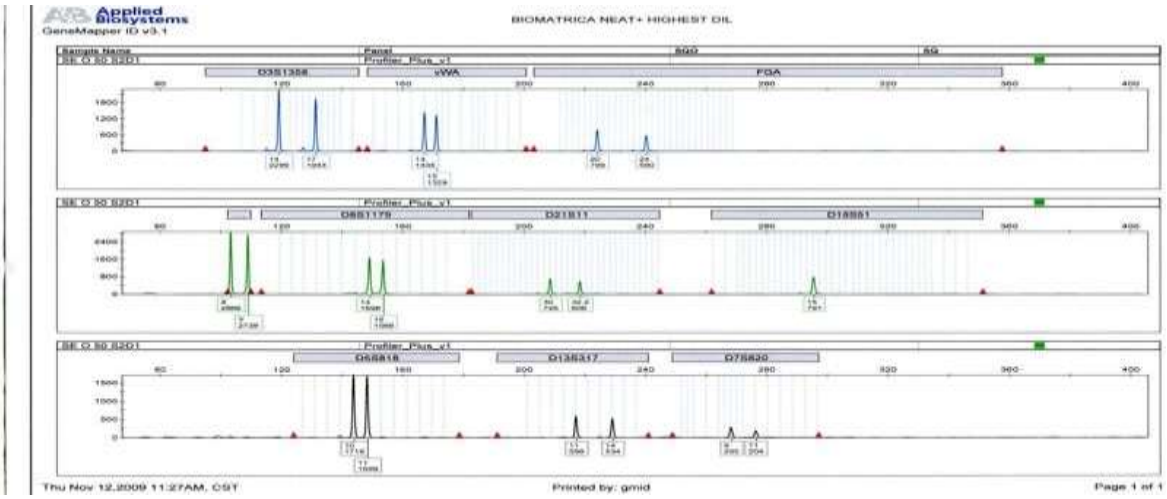

Fig. 195: 1:50 Semen SM2 Applied Dry 6 months (PCI)

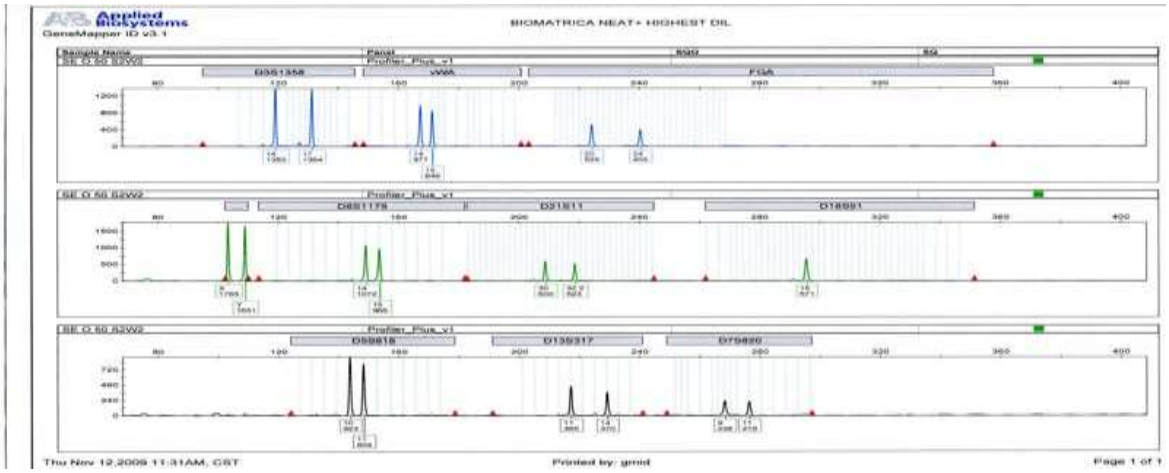

Fig. 196: 1:50 Semen SM2 Applied Wet 6 Month Storage (PCI)

CHAPTER 9: CONCLUSIONS

Conventional Serology Studies

Blood

Samples stored at -20°C largely displayed the most intense color changes when compared to samples stored under the remaining storage conditions. In the case of the phenolphthalein color test, SM1 appears to give relatively equivalent results to the frozen control. For the remaining two color tests (luminol and LMG), a difference was noted. The disparity between storage conditions was best observed at higher dilutions and at extended storage times. Samples stored frozen consistently displayed a positive reaction whereas samples stored at the remaining storage conditions failed to produce a reaction. At times, it appeared as if the samples coated with one of the SampleMatrix™ formulations performed marginally better than those stored unprotected at room temperature Studies performed by Biomatrica, Inc. that have demonstrated the ability of SampleMatrix™ to preserve the extracted DNA samples that were stored at room temperature. However, the SampleMatrix™ formulations were optimized to specifically protect the cellular components of blood. In the present study, it appears that hemoglobin is better preserved at freezing temperatures than at room temperature (with or without SampleMatrix™ protection). However, these results may instead be due to a greater availability of hemoglobin in the frozen samples to be utilized for a test since freezing can cause red blood cells to lyse. In comparing the performance of the two different SampleMatrix™ formulations, it appeared that SM1 performed slightly better than SM2 in the phenolphthalein and leucomalachite green tests. Additionally, it was discovered during the preliminary testing that the dyed incorporated into the SM2 formulation was incompatible with phenolphthalein and leucomalachite green since it produced a false positive. Given that the dye used was phenol red, it is recommended that this substance be added to the list of substances known to produce a false positive for presumptive blood tests. Finally, there is no indication that either SM1 or SM2 interferes with the three presumptive tests performed in this study.

Semen

Prostatic Acid Phosphatase Results

The study demonstrated that both SM1 and SM2 protected samples gave results for the AP test at higher semen dilutions and for shorter storage periods. This was evident in that the only positive reactions at the 1:1,000 dilutions were associated with the samples protected with SampleMatrix™ for the 1 day and two-week samples. In contrast, none of the room temperature or frozen samples gave a positive reaction at the equivalent dilution. Equivalent color intensity results were obtained for the one-month time period when controlling for dilution. That is, only the samples stored for two weeks or less gave more reactions that were positive at the higher dilutions as compared to the unprotected swabs. This inconsistency makes rendering a conclusion as to the protective properties of difficult SampleMatrix™. When considering the overall results, there is evidence to support that the samples protected with SampleMatrix™ gave superior results; the percentage of negative AP results is specified in parenthesis following the corresponding storage condition: unprotected at room temperature (27.2%), unprotected frozen (38.9%), SM1 at room temperature (16.7%), and SM2 at room temperature (6.9%).

Prostate-Specific Antigen Results

The results of the detection of PSA in semen were relatively equivalent among the four storage conditions tested. However, the SM1 formulation did appear to preserve the PSA more so than the frozen samples. For both the two week and one month time periods, the frozen samples resulted in a greater number of negative reactions relative to SM1. Further, the intensity of the assay result for the SampleMatrix™ protected samples was consistently higher than the frozen samples. For the majority of the dilutions, SM1 exhibited a test band that was of a higher intensity relative to the internal standard (T>q). In comparison, the test band obtained for the frozen samples was typically below or equivalent in intensity to the internal standard. The PSA proteins in the SampleMatrix™ samples appear to be somewhat protected from degradation effects. Consequently, the frozen samples are subject to not only degradation, but are also exposed to freezing and thawing conditions that may exacerbate degradation. This may explain why the protected samples are displaying higher intensity reactions relative to the unprotected control samples. It is important to note that, although the results for the SM2 protected samples were not as promising as those of the SM1 protected samples in terms of PSA detection, SM2 protected samples still compared favorably with respect to the frozen samples. However, difference in the results between SM2 protected samples and the frozen samples were marginal but consistent. As was the case the SM1 formulation, the majority of SM2 protected sample registered higher intensity reactions relative to the frozen samples.

Morphological Examination Results

The morphological evaluation results obtained for the protected and unprotected samples were essentially equivalent. An empirical observation was noted when viewing the protected samples microscopically. It was easier to locate and identify the spermatozoa and was particularly evident for the higher dilution samples. This was attributed to a clustering of spermatozoa that was evident in a number of samples that were protected with SampleMatrix™. It is unclear as to whether the hydrophobic protective shield that SampleMatrix™ forms around the sample may be contributing to this clustering of cells. Although these results are not necessarily definitive proof of the stabilizing effect of SampleMatrix™, they do appear to have a valuable and practical application. If SampleMatrix™ is causing these cells to cluster, this finding may prove useful in aiding the visualization during a microscopic examination. In regards to the morphological evaluations, it should be understood that the observation was subjectively based on researcher observations and warrants independent assessment.

Saliva

Radial Diffusion/α-amylase

The best results for the amylase radial diffusion tests were obtained for the samples that were protected with SM1 and stored at room temperature. No other storage methods compared as well to the consistently high amylase activity achieved with the SM1 samples. The samples protected with SM2 consistently showed activity across all dilutions and at all times. These samples did almost as well as the SM1 samples and actually demonstrated higher amylase activity for the corresponding lower diluted samples. The samples stored at room temperature with no protection gave the least promising results: no amylase activity was observed for the 1:50 dilution stored for three days; no activity for the 1:100 dilutions stored for one day, three days, two weeks and two months; and, no activity was observed for any of the 1:200 dilutions regardless of time period.

The frozen samples gave slightly better results compared to the room temperature samples with the exception of the 1:200 dilutions, which did not register any amylase activity at two weeks. This was surprising given that frozen storage was still expected to result in high amylase activity, just not to the extent observed for the samples protected by SampleMatrix™. Across all of the storage conditions, the results obtained for each time interval (excluding immediate) did not follow a particular pattern. For the frozen samples, patterns varied with the exception of the three day and one month samples. For SM1, three day and two months consistently gave the strongest diameter readings across all dilutions with the exception of the 1:50 dilution in both times. SM2 showed almost the same pattern as SM1 except for the samples stored for a two-month period. One potential explanation for the higher detection of amylase in the long-term samples may be because the Sample Matrix™ was given time to dry on the swab before being tested. The samples that were frozen for three days exhibited consistently higher amylase activity compared with samples that were frozen for extended times. As expected, the unprotected room temperature samples gave the highest amylase activity for the immediate samples and rapidly declined as the dilution factor and time period increased within this storage condition.

The results suggest that SampleMatrix™ may provide greater protection for samples subjected to long-term storage. The frozen samples gave the best results with the neat and 1:10 dilutions, but the results were inconsistent as the dilutions approached 1:200, suggesting that frozen storage might be the best option for samples stored for only short periods. The adverse effects of freezing and thawing may explain the decrease in amylase activity for the long-term samples stored under frozen conditions.

SALIgAE® Testing

The results obtained with the SALIgAE® method were dependent on the development of a color after ten minutes. Almost 50% of the room temperature control samples did not render a color change. The frozen stored samples gave comparable results to the room temperature control samples, with a slightly smaller percentage of the samples exhibiting no color change. The difference in the number of samples that gave a color change when comparing to the room temperature and frozen samples was marginal, although the frozen samples did perform slightly better. The reaction time was relatively consistent across all storage methods. However, given that the frozen samples showed no reaction for almost all the 1:100 and 1:200 samples, it was difficult to determine if the reaction time compared to samples applied Sample Matrix™.

Epithelial Morphology/Concentration

The evaluation of epithelial cell morphology showed that the frozen storage method was most favorable given that approximately 50% of the cells remained intact. In contrast, the SampleMatrix™ results fared the same as the room temperature samples with only 12% and 4.7% exhibiting intact cells in the SM2 and SM1 samples, respectively. The room temperature control samples gave slightly better results than the SM1 samples but were not quite as good as the SM2 samples. Given these results, it appears that SampleMatrix™ provides biostability to amylase but the formulation has not been optimized to maintain the integrity of the cellular components of the saliva. This explanation is consistent with the theory that nuclease activity is expected to increase at room temperature relative to frozen storage. In terms of the abundance of cells observed, none of the four storage conditions gave excellent results; however, the SM1 and

SM2 samples exhibited the greatest number of cells with 14% and 20% falling into the abundant category, respectively. In comparison, 11% of the frozen samples and 5% of the room temperature samples fell into the abundant category. Although these results would seem contradictory to the results obtained for the cell morphology, it should be noted that the results obtained for cell abundance only take into account the cells that are visualized and not their condition. The "plus" rating system was employed for determining epithelial cell concentration. This data does not take into consideration the actual epithelial cell counts per field of view. This rating system is intended to semi-quantify the data and was strictly used as a general guideline that is subject to variation.

Review of Hypothesis Statements

The data obtained for the presumptive testing of blood, semen and saliva samples do not support our hypothesis that samples stored at room temperature protected by SampleMatrix™ will give a greater number of positive test results, particularly at higher dilutions and at longer storage time periods, as compared with the control samples stored either at room temperature or frozen. Our data supports our hypothesis that SampleMatrix™ will neither interfere nor compromise the results of the presumptive tests.

Assessment of DNA Yields

The following conclusions are based on a comparison of the mean DNA yields of the various experimental variables. While clear differences in the mean DNA yield are evident when comparing the collection and storage conditions, in the majority of cases these differences were not statistically significant. For example, the results of the wetting studies indicate that SampleMatrix™ protected samples consistently gave greater mean DNA yields compared with unprotected control samples. However, the ANOVA and t test analyses determined that the differences observed are not statistically significant. This attributed to the large standard deviation associated with each mean. The factors contributing to the large standards deviation include the small number of replicate samples, the variation introduced during sample preparation and DNA extraction, and the use of multi-step analytical techniques that required extensive manual manipulations.

Wetting Agent Studies

The objectives of this study were to compare the ability of SampleMatrix™ to water, when used as a wetting agent, to assess the total amount of recovered DNA (dependent variable) from various substrates. This comparison incorporated different variables such as dilution factor, type of substrate, and compatibility to the downstream extraction method. Evidence suggesting improved efficiency in DNA recovery and preservation can result in an implementation of improved protocols for the collection and storage of DNA in forensic settings. Currently, the majority of biological stains are collected by swabbing prior to forensic testing. The swab is first moistened with sterile water to help solubilize the dried biological material then air dried, packaged, and frozen until the time of analysis. All of the samples in the study that were collected with SampleMatrix™ were stored at room temperature in paper envelopes. Statistical analyses (ANOVA and t test) support that there is no statistical difference in the mean recovery of DNA from each of the three wetting agents when collecting blood, semen, or saliva from

various substrates for the vast majority of samples. Two exceptions were identified 1) neat saliva recovered from carpet and extracted with the Qiagen chemistry indicated a significant difference at the 95% confidence level in favor of the unprotected samples 2) a 1:100 saliva dilution recovered from glass and extracted with PCI chemistry indicated a significant difference at the 95% confidence level in favor of the SampleMatrix™ protected samples.

Six-Month Stability of Blood

The results support that SampleMatrix™ demonstrates compatibility based on the higher mean yield a compared with the conventional method of using water as a wetting agent. The total mean DNA yield was higher using the Chelex extraction method with all three wetting agents when compared to Qiagen and Organic techniques. It has been reported in the literature that bloodstain samples sometimes result in PCR inhibition. Previous work alludes to prophyrin compounds from blood as the cause of the inhibition [71]. Therefore, a possible explanation for higher DNA recovery with the Chelex technique may be attributed to the lack of free-floating porhyrin compounds [71]. The second goal of the study was to determine if the protective properties of SampleMatrix™ provide advantages at higher dilutions. The SampleMatrix™ formulations demonstrate higher DNA recovery at all five different dilutions compared to water. SM2 ranked slightly higher than SM1 (> 1 ng) at three dilutions (1:100, 1:400, and 1:800). However, based on the total mean DNA recovered from all five dilutions SM1 gave highest mean recovery, followed by SM2, with H_2O as the wetting agent ranking third. SampleMatrix™ formulations SM1 and SM2 show greater DNA recovery with four of the five dilutions; the only exception was observed for the 1:800 dilution for Qiagen, where the recovery was considered equivalent (SM1= 0.45ng and H_2O = 0.46ng). At the 1:800 dilutions, SM2 ranked highest in DNA recovery with the Organic and Chelex extraction methods. The third objective compared the use of SM1, SM2 and water wetting agent in terms of recovering DNA from specific substrates. The results demonstrated that both SampleMatrix™ formulations performed effectively in DNA recovery at all five substrates, including cotton, cement, glass, carpet, and wood. Overall, both SampleMatrix™ formulations gave the highest DNA recovery for three out of the five substrates for all three of the extraction methods in comparison to water.

Six-Month Stability of Semen

SampleMatrix™ showed an improvement in overall DNA recovery over the traditional method of using water as a wetting agent when swabbing substrates for semen samples. The highest DNA recovery occurred with both the SM1 and the SM2 SampleMatrix™ formulations for the Qiagen and Chelex extractions. Whereas the Qiagen method recovered the highest amount of DNA overall, the Chelex extraction rendered the most consistent amount of recovery (lowest standard deviation values). The organic extraction recovered the least amount of sample. The type of substrate was also a factor when characterizing the properties of SampleMatrix™. Overall, SampleMatrix™ outperformed water across all dilutions. It was also noted that the DNA recovery increased in comparison to water at the higher dilutions for the Chelex and Qiagen extraction methods. In contrast, water outperformed SM1 at the higher dilutions when employing the organic extraction.

With the limited knowledge of the chemical components of SampleMatrix™, it was important to determine its compatibility with the three extraction methods. The preliminary

findings suggest that SampleMatrix™ does not inhibit or interfere with the downstream extraction process. In fact, SampleMatrix™ showed a marked improvement in overall DNA recovery over the traditional method of using water as a wetting agent when swabbing substrate for biological samples. The highest DNA recovery occurred with both the SM1 and the SM2 SampleMatrix™ formulations for the Qiagen and Chelex extractions. Whereas the Qiagen method recovered the highest amount of DNA overall, the Chelex extraction rendered the most consistent amount of recovery (lowest standard deviation values). The organic extraction recovered the least amount of sample, most likely due to the propensity for error in the multiple steps, as well as an inefficient manual extraction process. The opportunity to contaminate or reduce efficiency is inherent in longer-step protocols, and this could very well explain the lower results in the organic extraction. Regardless, the results of each extraction method demonstrated consistently better recovery rates for the SM1 and SM2 SampleMatrix™ formulations and therefore showed compatibility with each extraction method.

The type of substrate was also a factor when characterizing the properties of SampleMatrix™. Glass substrates allowed for the greatest recovery of DNA. This is most likely due to the smooth, non-porous surface, which minimized sample loss. This type of substrate allowed the swab close exposure to total surface area of the sample, and therefore allowed for extremely large recovery rates. On the contrary, swabbing cement for semen stains results in the smallest amount of recovered DNA, on average. Cement, though durable, is very porous and highly textured. This texture most likely allows biological samples such as semen to migrate into these recesses and thereby introducing recovery challenges, regardless of the wetting agent used. The wood substrate seemed to be the 'middle ground' substrate, in the sense that sufficient sample was absorbed to reduce recovery, but nonporous enough to increase sample recovery in comparison to carpet, cement, and cotton. The substrates cement, cotton, and carpet all recovered minimal amounts of DNA. Interestingly, water tended to recover more DNA than the various SampleMatrix™ formulations on wood. Unlike glass, where the collection allowed the entire sample to be swabbed, the cotton and carpet samples allowed the sample to absorb over a greater surface area due to each substrates porous and textured nature. In the glass substrate, the sample was not absorbed and remained centered on the substrate, where it was more easily recovered via swabbing. It was shown that substrate does play a role in the recovery of DNA; texture, absorption, and the porous nature of each substrate can play an important role in the recovery of biological stains. This study sought to determine the limits of detection for SampleMatrix™ at the various dilutions in comparison to the results obtained with water. Overall, SampleMatrix™ outperformed water across all dilutions; it was also noted that the DNA recovery increased in comparison to water at the higher dilutions for the Chelex and Qiagen extraction methods. In contrast, water outperformed SM1 at the higher dilutions when employing the organic extraction.

Six-Month Stability of Saliva

There is variation as to which storage condition is the more effective in stabilizing the DNA present in saliva. The results indicate that samples protected with SM1 or SM2 and stored at room temperature exceed the recovery obtained from the unprotected controls and tend to outperform frozen samples, except for the more concentrated dilutions extracted with the Qiagen method. The comparison is compounded by the lower recovery of DNA from saliva as compared to blood and semen. The lower amount of DNA in saliva is to be expected given the cellular

content of this body fluid combined with the reported higher nuclease activity. R&D scientists at Biomatrica are in the process of optimizing a formulation to stabilize saliva.

Coating Agent Studies

Six-Month Stability of Blood

A comparison of the recovery of DNA from samples that were stored without SampleMatrix™ at ambient temperature with samples stored at -20°C (frozen) led to some interesting preliminary findings. Forensic laboratories may store biological samples at frozen temperatures and therefore it was reasonable to expect that frozen temperatures would be more effective at preventing DNA degradation. The preliminary findings indicate that protected samples did result in a higher mean DNA recovery than unprotected samples stored at -20ºC; however, the mean recovery of DNA was lower for the SampleMatrix™ protected samples than the unprotected control samples that were also stored at ambient temperature. The differences in the mean recovery across these various storage conditions were not statistically significant. Considering only the Qiagen extraction method, SampleMatrix™ protected samples gave a higher DNA recovery relative to the other storage conditions. The data showed that, on average, DNA recovery was higher for the protected blood samples that were allowed to dry overnight prior to applying SampleMatrix™. An overall comparison showed that SM1 was marginally more effective in recovering DNA as compared to SM2. Further, these preliminary findings demonstrate that the three evaluated extraction methods were compatible with SampleMatrix™

The relative humidity within the laboratory used to store the samples was measured over the last month of the study and it was found to range between 61-75%. Biomatrica recommends including a desiccant when storing samples at room temperature, therefore the elevated humidity may have affected the results. The temperature was also recorded during this time and ranged between 19.8ºC and 27.6ºC. Despite the high humidity recorded during this time, unprotected samples remained the most effective storage condition. These results are again only preliminary and the effect of long-term storage on DNA degradation was not considered. In contrast, approximately 75% of blood samples stored at ambient temperatures protected by SampleMatrix™ resulted in higher DNA recovery than blood samples stored without sample matrix at -20°C.

The increased DNA recovery from blood samples that were stored without SampleMatrix™ at ambient temperature relative to freezer storage may be attributed to incomplete drying of the sample prior to storage. The sample may have dried more thoroughly during room temperature storage as compared to samples that were stored at -20ºC, thereby reducing the degrading effects of moisture at room temperature. The blood samples stored without SampleMatrix™ at -20°C may have a decrease in DNA recovery compared to those at ambient temperature again possibly due to water content. This may be an effect of the swab interior not completely drying. A longer drying period may be necessary prior to freezer storage. All of the samples were initially prepared in the same manner. The samples protected by SampleMatrix™ were prepared using two methods, dry or wet, and then allowed to dry overnight prior to final storage. It is possible that a longer drying period is necessary before the application of SampleMatrix™. It is also a possibility that SampleMatrix™ may not completely diffuse through the swab to penetrate the blood absorbed in the interior of the swab; therefore, the entire blood sample may not be benefiting from the protective properties of SampleMatrix™.

The method of applying SampleMatrix™ to the blood swab was investigated. In the dry method, blood was applied to the swab and allowed to dry overnight prior to the application of SampleMatrix™ whereas in the wet method SampleMatrix™ was immediately applied to the blood swab. The data showed that, on average, DNA recovery was higher for the blood samples that were allowed to dry overnight prior to applying SampleMatrix™. This may correlate to the need to remove as much moisture as possible in order to increase DNA recovery and prevent DNA degradation. Another factor that may contribute to the lower recovery from blood samples where SampleMatrix™ was applied wet is the degree of saturation of the cotton swab. Given that SampleMatrix™ was applied to the cotton swab immediately after the blood was applied, the absorption was much slower and required a longer application time. In some instances, the cotton swab appeared to be over-saturated and excess SampleMatrix™ was lost (ran down the cotton swab applicator). Blood may have been lost due to the over-saturation caused when SampleMatrix™ was applied to the sample. The preliminary findings suggest that the application ratio of blood to SampleMatrix™ may have not been optimal for the wet application method. The blood samples with SampleMatrix™ applied dry did not have these absorption issues.

An overall comparison showed that SampleMatrix™ SM1 was marginally more effective in recovering DNA. Further, these preliminary findings demonstrate that the three evaluated extraction methods were compatible with SampleMatrix™ and did not show a difference in DNA recovery relative to unprotected samples. The DNA recovery between the extraction methods was inconsistent as evident on the standard deviation values and therefore it is not possible to state whether one method is more effective than other in recovering DNA.

Six-Month Stability of Semen

When comparing the DNA recovery of semen samples that were untreated, either unprotected at room temperature or unprotected and stored frozen (-20 °C), there were some variation in the results. In this study, the semen samples that were stored frozen (-20 °C), on average, did result in higher DNA recoveries than those stored at room temperature. However, the average DNA recovery of the samples extracted using Qiagen was slightly higher when the samples were stored untreated at room temperature rather than frozen (-20 °C). Considering only the Qiagen extraction method, SampleMatrix™ protected semen samples gave a higher DNA recovery relative to the other storage conditions when considering the optimal coating approach of applying SM1 to a dried semen swab. An overall trend was seen throughout all the samples in which samples extracted by the Chelex method showed much lower DNA recovery values than those of Qiagen or Organic. Another trend observed across all samples was that extractions using the Organic method gave slightly lower recovery compared to Qiagen. Examining the preliminary results of samples treated with SampleMatrix™ in comparison to samples that were untreated and stored at -20 °C, only approximately 25% of samples treated with SampleMatrix™ had a higher recovery of DNA than samples that were stored frozen (-20°C). Similarly, when compared to samples stored at room temperature only approximately 25% of samples treated with SampleMatrix™ resulted in a higher recovery of DNA. All of these samples were extracted using the Chelex extraction method. The only SampleMatrix™ treated samples with consistently higher DNA recoveries compared to both room temperature and -20°C is the sample treated with SM1 applied wet and extracted using the Chelex method.

Examining the preliminary results of samples treated with SampleMatrix™ in comparison to samples that were untreated and stored at -20 °C, only approximately 25% of samples treated with SampleMatrix™ had a higher recovery of DNA than samples that were stored frozen (-20°C). Similarly, when compared to samples stored at room temperature only approximately 25% of samples treated with SampleMatrix™ resulted in a higher recovery of DNA. All of these samples were extracted using the Chelex extraction method. The only SampleMatrix™ treated sample with consistently higher DNA recoveries compared to both room temperature and -20°C are the samples treated with SM1 applied wet and extracted using the Chelex method. As stated earlier, samples extracted via the Chelex method gave consistently lower DNA recovery values than those extracted using the Organic or Qiagen method. For this reason (and considering standard deviation values), the higher DNA recovery values seen in the samples treated with SampleMatrix™ may not be not meaningful. During the final month of the study, the relative humidity within the laboratory used to store the samples was measured and it was found to range between 61 and 75 percent. Biomatrica recommends including a desiccant when storing samples at room temperature, therefore the elevated humidity may have affected the results. The temperature was also recorded during this time and ranged between 19.8 ºC and 27.6 ºC. These samples protected by SampleMatrix™ were prepared using two methods of application, dry or wet, and then allowed to dry overnight prior to storage. Other possible explanations for the lower DNA recoveries are that a longer drying period is necessary before the application of SampleMatrix™. Alternatively, SampleMatrix™ may not have completely diffused through the swab to penetrate the semen sample absorbed in the interior of the swab. This would prevent the entire semen sample from benefiting from the protective properties of SampleMatrix™.

Preliminary findings indicate that SampleMatrix™ does not cause interference with any of the extraction methods. As stated previously, there was a trend throughout the samples in DNA recovery between extraction methods. DNA recovery was highest when Qiagen extraction was utilized, followed by a slightly lower recovery using Organic and finally the lowest recovery was observed when Chelex extraction was used. However, because this trend was consistent when the samples were treated with SampleMatrix™ or left untreated, this was not attributed to an interference of the SampleMatrix™ polymer. These recovery differences are most probably a result of the limitations of extraction method in recovering high yields. Organic extractions, although much more involved, are not necessarily specific to the type of sample matrix (liquid, semen, swab) and may be less accurate than Qiagen, which does provide specific kits for different types of samples (semen, swab).

Another variable examined was the effect of the different SampleMatrix™ formulations (SM1 and SM2) on DNA recovery. Taking into account only the different SampleMatrix™ formulations, higher DNA recoveries were seen when SM2 was used at the storage method when extracted with either Qiagen or Organic. Once again, Chelex was inconsistent, but overall DNA recovery was much lower and standard deviation values make the Chelex results unreliable. The explanation for the difference in DNA recovery between the SampleMatrix™ formulations is difficult to assess due to the limited knowledge of composition of each formulation.

The final variable considered was the time delay in the addition of each SampleMatrix™ formulation. Focusing only on the wet or dry SampleMatrix™ application method, and excluding the inconsistent results obtained with the Chelex extractions (based on low recoveries and standard deviations) the dry application method of SampleMatrix™ produced a higher recovery of DNA from semen.

Six-Month Stability of Saliva

There is variation as to which storage condition is the more effective in stabilizing the DNA present in saliva. The results indicate that samples protected with SM1 or SM2 and stored at room temperature exceed the recovery obtained from the unprotected controls and tend to outperform frozen samples, except for the more concentrated dilutions extracted with the Qiagen method. As stated earlier, R&D scientists at Biomatrica have now optimized a formulation to stabilize saliva.

17-24 Month Stability of Blood

An unexpected preliminary finding was depicted from the comparison of DNA recovered from samples that were stored without SampleMatrix™ at room temperature to samples stored frozen at -20°C. Samples gave a greater amount of recovered DNA from storage conditions at room temperature as opposed to freezing temperatures. The Chelex extraction resulted in a DNA recovery following storage at ambient temperatures that was five times higher than the frozen control stored at -20°C. Considering only the Qiagen extraction method, SampleMatrix™ protected blood samples gave a higher DNA recovery relative to the other storage conditions when considering the optimal coating approach of applying SM1 to a dried blood swab. The temperature within the laboratory was measured during the sixth month of the study and it was found to be between 19.8°C and 27.6°C. The relative humidity in the laboratory used to store the samples was also measured over the sixth month of the study. This humidity level was determined to range from 61-75%. Biomatrica recommends that a desiccant should accompany samples stored at room temperature. Since water contributes to DNA degradation, the desiccant is necessary to minimize any moisture that might shorten the shelf life of samples. In addition, approximately 70% of blood samples protected by SampleMatrix™ and stored at room temperature resulted in a greater amount of recovered DNA than unprotected blood samples stored at -20°C. This decrease in DNA recovery at -20°C could result from possible water content in a swab that was not thoroughly dried. Prior to freezer storage, a drying period longer than 24 hours may be necessary. The samples protected by SampleMatrix™ were prepared using two methods, wet or dry, and then allowed to dry overnight prior to final storage. It is also possible that a longer drying period was needed prior to SampleMatrix™ application, as well.

Another factor that could explain why a lower DNA recovery was observed from protected samples stored at room temperature than from unprotected samples at ambient temperatures was that the amount of SampleMatrix™ applied was insufficient. An additional explanation could be that SampleMatrix™ may not have entirely diffused through the swab. This would result in an incomplete penetration of the blood that was absorbed in the interior of the swab. Therefore, the entire blood sample may not have benefited from the protective properties of either of the SampleMatrix™ formulations. In addition, the application method of SampleMatrix™ to the biological sample was examined. In the dry technique, the blood applied to the swab was dried overnight prior to SampleMatrix™ application. On the other hand, the wet technique involved the immediate application of SampleMatrix™ to the blood swab. The data indicated that DNA recovery was consistently greater for the dry application method as compared with the wet technique. This may also correlate to the importance of completely removing as much moisture as possible in order to prevent DNA degradation and increase DNA recovery. Another factor that may contribute to a lower DNA yield is an inconsistency in the degree of saturation of

SampleMatrix™ to the cotton swab. A cotton swab that was not sufficiently saturated by the wet technique could exhibit a longer application time and a slower absorption of SampleMatrix™ to the cotton swab. An over-saturated cotton swab could result in a loss of some of the SampleMatrix™ storage medium or even a loss in part of the blood sample. This provided further evidence of the fact that the wet technique displayed a greater inconsistency in DNA recovery, and was imprecise due to the large standard deviation values.

Based on the findings for the long-term storage of blood, it appears that the SM1 formulation resulted in a higher DNA recovery than the SM2 formulation for the Organic extraction method. Furthermore, it was difficult to predict the compatibility of the storage medium to the type of extraction method utilized because the composition of each formulation was unknown. The results did demonstrate that all three-extraction chemistries appeared to be compatible with SampleMatrix™. In addition, the overall amount of recovered DNA from unprotected samples was only marginally different from the samples protected with SampleMatrix™. It was also apparent that the extraction methods displayed inconsistency, as demonstrated by the widespread values. This was evident not only by large standard deviation values, but also by standard deviations that exceeded the average values.

17-24 Month Stability of Semen

Generally, it was observed that samples protected by SampleMatrix™ stored at either ambient temperature recovered lower amount of DNA than unprotected samples stored frozen or room temperature condition. However, there are inconsistent results for SampleMatrix™ protected samples extracted with the Chelex method, as these samples appear to show a higher average DNA recovery when compared to the unprotected control samples stored at room temperature. Semen stored at frozen temperature resulted in the highest average DNA recovery for the Chelex and Organic extraction method. Semen samples stored at frozen temperature had at least a ~30% greater recovery when compared to SampleMatrix™ protected samples or unprotected samples stored at room temperature. Considering only the Qiagen extraction method, SampleMatrix™ protected semen samples gave a comparable DNA recovery relative to the frozen samples but the recovery was still lower than the unprotected control samples when considering the optimal coating approach of applying SM1 to a dried blood swab.

Although all three extraction chemistries appeared to be compatible with SampleMatrix™, there was considerable variability within each extraction method. In this particular study, it was found that the Organic method gave the highest average amount of DNA recovered but also has the greatest standard deviation. The greater degree of manual manipulation with the Organic extraction method can partially explain the observed variable standard deviation. In this particular study, it was observed that the Chelex extraction method at the following dilutions (1:1,000, 1:2,000, and 1:4,000) exhibited the largest variability; some of the data at these higher dilutions yielded undetermined results. The samples recovered with the Chelex extraction method had a very high retentate volume (~500 to 900µL) while samples recovered with the Organic extraction were observed to have very low retentate volumes (~9 to 30µL). As a general trend, it was also observed that the Qiagen extraction method had the lowest amount of DNA and the lowest standard deviation. This most likely attributable to the fact that the Qiagen method is far more standardized than both the Organic and Chelex methods

Another objective was to determine whether SampleMatrix™ protected samples recover higher DNA yields as compared to the unprotected control samples. Generally, it was observed that samples protected by SampleMatrix™ stored at either ambient temperature recovered lower

amount of DNA than unprotected samples stored frozen or room temperature condition. However, there are inconsistent results for SampleMatrix™ protected samples extracted with the Chelex method, as these samples appear to show a higher average DNA recovery when compared to the unprotected control samples stored at room temperature. Semen stored at frozen temperature resulted in the highest average DNA recovery for the Chelex and Organic extraction method. Semen samples stored at frozen temperature had at least a ~30% greater recovery when compared to SampleMatrix™ protected samples or unprotected samples stored at room temperature. Considering only the Qiagen extraction method, SampleMatrix™ protected semen samples gave a comparable DNA recovery relative to the frozen samples but the recovery was still lower than the unprotected control samples when considering the optimal coating approach of applying SM1 to a dried blood swab.

17-24 Month Stability of Saliva

The findings suggest that all of the three extraction methods were compatible with SampleMatrix™ as there was no apparent difference in the DNA recovery relative to unprotected samples across all three chemistries. The DNA recovered within each extraction method demonstrated considerable variation as apparent from the standard deviation values. Of note, the Qiagen extraction method appeared to consistently recover less DNA than the Chelex or Organic methods, yet displayed the better standard deviation values. The Qiagen method produced a consistently low DNA recovery. The total DNA recovered from saliva samples stored without SampleMatrix™ at room temperature was compared with samples stored at -20°C (frozen). The preliminary findings suggest that samples stored at -20°C produced higher DNA recovery than samples stored at room temperature. Approximately 91% of saliva samples stored with SampleMatrix™ at room temperature resulted in lower DNA recovery than saliva samples stored at -20°C. Coincidently, the same holds true for saliva samples stored without SampleMatrix™ at room temperature. Biomatrica recommends including a desiccant when storing samples at room temperature, therefore any fluctuation in humidity may have affected the results. The method of applying SampleMatrix™ to the saliva swab was considered in this study. The data shows that DNA recovery was higher for saliva samples subjected to the dry application of SampleMatrix™.

Review of Hypothesis Statements

It was predicted that SampleMatrix™ would provide a greater mean DNA recovery than both the unprotected samples stored at room temperature and frozen samples. However, a statistical analysis of the data obtained for the wetting and coating agent studies do not support this hypothesis. The data supports our hypothesis that SampleMatrix™ does not interfere with the chemistry of the three extraction methods evaluated. Our data also support our hypotheses that SampleMatrix™ will not compromise the ability to quantitate DNA yields or interfere with genotyping techniques.

Environmental Insult Studies

Accelerated Aging @ 50°C Studies

A comparison of the recovery of DNA from samples stored under accelerated aging conditions, with and without SampleMatrix™, supports the conclusion that SampleMatrix™ technology does protect DNA in un-extracted biological samples. This hold true for all three biological fluids: blood, semen, and saliva. The amount of DNA recovered from a sample protected with SampleMatrix™ is consistently higher than the corresponding control samples without SampleMatrix™. An overall comparison of the data obtained from this study (holding all other variables constant) showed that SM1 recovered 20-60% more DNA than in samples protected by SM2. SM1 protected samples consistently gave a higher mean yield as compared with the unprotected control samples. However, the differences in the mean recovery were not statistically significant (ANOVA).

The major goal of this study was to examine the long-term performance of SampleMatrix™ with different biological fluids of varying dilutions. Knowledge of the product performance over extended times with different fluid samples would provide valuable information to forensic agencies who are considering storing case samples at room temperature using SampleMatrix™. Subjecting biological samples to an elevated temperature (50ºC) for varying times allows for the aging these samples in order to determine the long-term effectiveness of SampleMatrix™ for as much as 31 months.

The amount of DNA recovered after one week of incubation (equivalent to 70 days at room temperature) is relatively high for blood samples at all dilutions. The amount of DNA recovered dropped dramatically after two weeks of incubation (equivalent to 103 days at room temperature). A similar amount of DNA is recovered from samples incubated for between four to 19 weeks (equivalent to 215 to 930 days at room temperature). Generally, 1.5ng of DNA is needed to generate a full profile in STR analysis. DNA less than 250pg commonly results in a partial profile, which is less discriminating to the individuality of the sample. The amount of DNA recovered in blood samples of 1:10 dilution protected by SampleMatrix™ is higher than unprotected samples; however, the amount of DNA recovered in unprotected sample might also likely result in a full STR profile after 19 weeks of incubation under accelerated aging conditions. The amount of DNA recovered versus time fluctuates between 50-75% in semen samples of all dilutions. The amount of DNA recovered in equivalent samples protected by SampleMatrix™ increased. However, this may not impact the number of that meet the 1.5ng threshold for attaining a full STR profile given that all of the semen samples (both protected and unprotected) at 1:50 and 1:500 dilution recover more than 1.5ng of DNA during the 73-936 days stored at room temperature. However, the relative difference in DNA recovery for samples protected by SampleMatrix™ SM1 versus unprotected samples increases with dilution.

DNA was also recovered from semen samples protected by SM1 of 1:1,000 dilution subjected to one to 19 weeks of incubation. It is unknown whether the amount of DNA present would give a complete STR file; however, the presence of SampleMatrix™ would be critical if SampleMatrix™ does give protection to DNA under accelerated aging conditions. Semen samples diluted 1:2,000, both unprotected and protected by SM2, had almost no DNA recovery after storage at room temperature for 73 days. A higher amount of DNA was recovered from semen samples of the corresponding dilution protected by SM1; however, the amount of DNA recovered is still likely to be insufficient to generate a full STR profile. The amount of DNA

recovered from neat saliva samples, subjected to 62-936 days of storage at room temperature, and protected by SampleMatrix™ (both SM1 and SM2) is sufficient to generate a full STR profile; however, the amount of DNA recovered from unprotected neat saliva samples drops below 1.5ng after 619 days of storage at room temperature. The use of SampleMatrix™ in this case is significant if neat saliva samples were to be stored beyond 619 days at room temperature. For lower concentration samples, the accelerated aging profile showed fluctuation between 75-100%. One potential explanation to account for such fluctuation is that DNA at lower concentrations (1:50 and 1:100) in saliva degrades rapidly to result in fragments shorter than 62bp (PCR amplification fails). The amount of DNA recovered in all saliva samples (both protected and unprotected) of 1:50 and 1:100 dilutions may fall below the threshold of STR typing after 1week of incubation under accelerated aging conditions (equivalent to 62 days at room temperature). Saliva samples at the lowest concentration (1:200) show no DNA recovery after 12 weeks of incubation (equivalent to 619 days at room temperature).

Elevated Temperature (55°C) and Humidity (60%) Studies

The goal of this study was to determine if SampleMatrix™ protects blood samples when exposed to both elevated temperature (55°C) and humidity (60%). The amount of DNA recovered under these conditions was compared to unprotected samples that were stored at room temperature or frozen. According to Biomatrica, Inc., the SampleMatrix™ formulations will remain stable at room temperature, and should withstand up to 40% humidity and elevated temperature, allowing for increased protection of biological samples under such conditions. This study further hypothesized that SampleMatrix™ would withstand elevated temperatures and that blood samples protected with the coating agent would allow for greater recovery of DNA than samples not coated with one of the three SampleMatrix™ formulations investigated in this study. Additionally, it was hypothesized that SampleMatrix™ would assist in the protection of DNA at a moderate level of 60% humidity, allowing for a greater yield of recoverable DNA.

Prior to analysis in forensic laboratories, biological samples are typically stored frozen, without protection by SampleMatrix™. This practice allows for the reasonable expectation that unprotected frozen samples allow for greater recovery of DNA than unprotected samples stored at room temperature. This expectation was supported by the findings of this study. However, samples coated with SampleMatrix™ and stored at room temperature exhibited increased recovery of DNA as compared to frozen samples coated with SampleMatrix™. Given that the samples were prepared in an equivalent manner prior to storage, this finding may be explained by the fact that the SampleMatrix™ formulations contain water and may not have been dried thoroughly prior to storage in the freezer whereas storage at room temperature allowed for increased drying of the samples. It is known that the presence of water contributes to DNA degradation by interfering with hydrogen bonding, providing a potential explanation for the observed results. Further, SampleMatrix™ has been designed to protect DNA at room temperature rather than under frozen conditions, and the findings of this study support this premise. Additionally, the data illustrate that samples protected with SampleMatrix™ and stored at an elevated temperature and humidity allowed for a greater yield of recoverable DNA when compared to unprotected samples exposed to the same elevated conditions. These two findings support that the SampleMatrix™ formulations are able to withstand room temperature and elevated temperature and humidity conditions, resulting in improved stability of the biological sample and increased DNA yield.

The higher amount of DNA recovered in the protected samples at room temperature as compared to elevated temperature and humidity is most likely due to the detrimental effects of elevated temperature. The relative humidity in the laboratory was monitored over the four-week incubation period at room temperature and was found to range from 3% to 58%, but did attain a high of 78% for one particular day. Given that the previous findings support that SampleMatrix™ can withstand moderate levels of humidity, the relative humidity range measured in the laboratory (3-58% as compared to 60% in the humidity chamber) may be considered negligent, leading to the assumption that increased temperature was primarily responsible for the decrease in amount of recoverable DNA. As discussed in the introduction and literature review chapters of this thesis, when the temperature is elevated even slightly above ambient temperature, hydrogen bonds are broken and the DNA double helix is denatured into its primary form. As the temperature increases even further above the melting temperature of DNA, the primary structure is degraded, resulting in irreversible damage. Furthermore, the results indicate that the SampleMatrix™ formulations may be able to withstand elevated temperature and humidity conditions up to a certain point, after which the amount of recoverable DNA decreases.

The data indicate that the SM1 and SM2 formulations afforded the same protection at elevated temperature and humidity, but coating the blood samples with the SM2 formulation resulted in an increased yield of DNA at room temperature. As expected, protection with the lysis formulation resulted in an increase in DNA yield as compared with either the SM1 or SM2 formulations. This is most likely attributed to the fact that the formulation lyses the cell upon contact, allowing for direct stabilization of human DNA contained within the cell. Based on the data, it appears that the SampleMatrix™ lysis buffer affords increased protection to more concentrated samples. The general trend indicates that the lysis buffer is less effective at higher dilutions, particularly for extended storage periods.

Review of Hypothesis Statements

All of the samples protected by SampleMatrix™ were predicted to yield a higher DNA recovery in comparison with unprotected samples when subjected to environmental insult conditions. However, a statistical analysis of the data does not support this hypothesis.

Cell Morphology Studies

Properties of SM3-treated Whole Blood and Application of Handling Results

While the basis of the study was the effectiveness of the SM3 formulation in preserving whole blood cells at room temperature, it became necessary to first develop basic parameters for handling the formulation in order to obtain the most consistent results possible. Not all of these parameters were anticipated during the initial design of the study. SM3 initially has a consistency similar to water, making it easy to handle during pipetting, other, and other manipulations. Once dried, SM3 forms to a viscous consistency and drying appears to occur from the outside inward. In some cases, a solidified SM3 pellet has a consistency that permits intact removal from a micro-titer well plate; however, samples that have not thoroughly dehydrated should not be removed from their wells, as they may break apart.

SM3 displays complete functional compatibility with major whole blood components. When SM3 is mixed with plasma is appears to be fully homogeneous, and the environment maintains

RBC and WBC morphology. While it is unclear whether SM3 is preserving/providing a substitute for blood plasma or directly sustaining the individual cells themselves, it was observed that blood cells in SM3-treated samples display the same initial morphology as those in untreated samples. For the purposes of forensic whole blood specimen collection, the use of SM3 can be considered equivalent to the use of physiological saline solution (0.85% NaCl).

The most significant obstacle in the handling of SM3 is its resistance to rehydration, which is discussed further below. Additionally, SM3 has a tendency to froth and form bubbles of high-viscosity fluid. As blood tends to immediately emulsify in SM3, practical experience in handling SM3 led to the conclusion that it was best to mix the emulsification via agitation with a pipette. If the proper technique (as outlined in the results section) is not used, there is a significant tendency for the SM3 to foam, and the resultant air pockets do not readily dissipate. This can increase the likelihood of contamination from two major sources: overflow from a frothing well to an adjacent well and transfer on to the well plate lid when closed, leading to capillary flow across the underside of the lid and subsequent cross-contamination of adjacent wells, in both rows and columns. Due to frothing and overflow issues, it is recommended that samples be prepared such that the combined sum of the SM3, blood, and rehydration solution volume should not exceed 75% of the well volume.

Storage Ratio and Rehydration Interval Assessments

This experiment involved varying the ratio of initial SM3: blood. The baseline consisted of a mixture of 50µL SM3 to 25µL neat blood. As pure dried SM3 proved significantly more difficult to rehydrate than pure dried blood, it was hypothesized that reducing the volume of SM3 would facilitate more thorough rehydration. However, results are inconclusive, as samples initially mixed at 1:1 both underperformed and outperformed samples initially mixed at 2:1, depending upon the sample set. In addition, the rehydration time periods were varied. While previous specimens had been allowed to rehydrate a maximum of 15 minutes, some specimens were subsequently allowed to rehydrate for periods of 1 – 3 hours. For samples aged one and six weeks, mixtures that had rehydrated for 3 hours showed increased downstream DNA recovery over mixtures rehydrated for 1 hour. However, the results are inconsistent: shorter rehydration periods produced higher yields in some instances. It appears that the inconsistencies observed with initial volume ratio and rehydration period experiments, as well as inconsistencies observed between samples with varied rehydrated periods, are based in the difficulty of obtaining thorough rehydration of SM3 / blood mixtures, leading to differential purification and recovery.

Preservation Properties of SM3

In the course of initial experiments with SM3 and mixed blood, it was observed that whole blood consistently forms an emulsification when deposited into a larger volume of SM3; of note is the observation that even samples that are initially mixed will re-form a blood droplet emulsification within a few hours. Once dried, the emulsification retains its form and is located in a concentrated, discrete area at the bottom center of the well. This led to the question of whether SM3 is penetrating cells and operating at the cytoplasmic level, whether it is acting as a replacement or preservative for blood plasma, or whether is simply forms a protective hermetic seal and provides an environment conducive to plasma stability. During the qualitative portion of the study, a sample of the treated and dried emulsification was removed from its well and sectioned and the core of the dried blood portion allowed to age while exposed to the environment. The exposed blood portion demonstrated intact blood cells at a level comparable to

that of emulsified samples that had not been sectioned and exposed to air. This appears to indicate that SM3 provides more than a mere hermetic seal and is at least penetrating the plasma portion of the mixture.

Homogeneity of SM3 / Blood Mixtures

Of the twelve pairs of mixed *v*. unmixed samples of blood and SM3 that were compared over the course of 104 days, there was no apparent difference in the degradation rate of RBC or WBC between mixed (manually agitated) and unmixed samples. If anything, the morphology of RBC and WBC in unmixed samples may sustain their morphology slightly better over time than for mixed samples; this may be due to mechanical damage from repeated pipetting in mixed samples, or may merely be an artifact of the subjective rating system used. However, it can be asserted that while whole blood deposited directly into a pool of SM3 will form an emulsification, the SM3 does appear to permeate the emulsification, rendering mechanical mixing unnecessary.

Staining of SM3-Treated Samples

H and E staining is often used to enhance the visualization of RBC and WBC. Eosin functions as a basophilic dye, staining basic structures with a blue tint. Multiple dilution ratios were tested, and while a ratio of 10:1 proved ideal for viewing of untreated stained samples, dilutions of even 100:1 presented with fields obscured by blue tint, indicating the possible adherence of the basophilic hematoxylin dye to some portion of the SM3 matrix. The suggestion is that SM3 should be modified to remove the component that has an affinity to eosin affinity in order to be compatible with the H and E staining process.

Effectiveness of SM3 in Preserving Whole Blood Component Morphology

Overall, SM3 was more effective in preserving blood cell morphology in whole blood samples as compared with the untreated control samples. Whereas the concentration of intact RBC and WBC in untreated samples declined with a half-life of approximately four days, treated samples continued to demonstrate identifiable cells for periods in excess of 220 days. It is noteworthy that SM3 appears to be more effective in preserving RBC than WBC, particularly given that RBC's are more susceptible to lysing than WBC. Also noteworthy is the apparent preservation of free nuclei. While SM3 is effective in preserving blood cells over the length of the study, there is an obvious declining trend. Degradation of WBC nuclei was not observed over the length of the study. This suggests that SM3 may be more effective in preservation nuclei than intact cells.

Review of Hypothesis Statements

It was hypothesized that SM3 would preserve the integrity of whole blood (RBC and WBC) stored at room temperature to the extent that the morphology would be comparable with that of freshly drawn whole blood. The data obtained in the present study supports this hypothesis.

DNA Genotyping Analysis

Coating Agent Study (6 Months)

In reference to the coating study, it was hypothesized that the extraction method would not affect the quality of DNA recovered. This hypothesis proved correct as in all instances full or partial DNA profiles were recovered from blood, semen, and saliva with the three extraction methods. A greater number of full profiles were obtained when blood coated with both SampleMatrix™ formulations applied wet and dry was extracted with Qiagen and Chelex. In the case of semen samples, the Qiagen extraction method resulted in a greater number of full profiles regardless of the sampleMatrix™ formulation and application used. Semen samples coated with either formulation applied wet resulted in higher number of full profiles when extracted with Chelex. Saliva samples coated with SM1 applied dry provided the highest number of full profiles when Organic extraction was performed. Those samples coated with SM1 applied wet, as well as both applications of SM2 provided the highest number of full profiles using Qiagen and Chelex.

In regards to storage conditions, it was hypothesized that samples stored at room temperature protected with SampleMatrix™ would yield better quality DNA profiles. The samples treated with SM1 resulted in a greater number of full DNA profiles compared to SM2 with each of body fluid studied. However, untreated saliva samples frozen at -20˚C and extracted with Qiagen showed higher quality DNA profiles as compared to unprotected samples at room temperature.

Wetting Agent Study (6 Months)

Regarding the wetting study it was hypothesized that the extraction method would not affect the quality of DNA recovered. This hypothesis proved correct as in all instances full or partial DNA profiles were recovered from blood, semen, and saliva. However, no obvious pattern was noted when comparing the quality of DNA obtained using different extraction methods for each body fluid.

In regards to storage conditions, it was hypothesized that samples stored at room temperature protected with SampleMatrix™ would yield better quality DNA profiles compared to untreated frozen at -20˚C. The hypothesis was supported in case of blood samples, where SampleMatrix™ formulation SM1 provided the highest quality DNA profiles followed by SM2. The frozen blood samples yielded the lowest quality DNA profiles. The results obtained from both semen and saliva samples showed the highest quality DNA profiles when SampleMatrix™ formulation SM1 was used. Samples treated with SM2 showed the lowest quality DNA profiles. There was a clear advantage of samples treated with SampleMatrix™ stored at room temperature over frozen samples.

It was hypothesized that more diluted samples would result in decreased DNA quality, which was supported by the results. As a general trend, the higher dilutions of body fluids resulted in a lower quality of DNA profiles. For neat blood samples, Qiagen provided the highest quality of DNA profiles; however, at the higher dilutions Organic extraction appeared to provide better results. Chelex appeared to provide the highest quality DNA profiles at 1:50 dilution of semen samples. The quality of DNA profiles recovered from semen samples using each extraction method was directly proportional to the dilution factor. Higher quality DNA profiles were recovered from neat saliva samples using Chelex, followed by Qiagen and organic extractions. At the higher dilutions, lower quality of DNA profiles were obtained using each extraction method, although Organic extraction appeared to provide slightly better results.

However, no general conclusion regarding all body fluids can be drawn, as results were not obtained at all dilutions.

Coating Agent Study (17-24 Months)

The objectives of this study were to evaluate the quality of DNA recovered from blood, semen and saliva samples deposited on a cotton swab following storage under different conditions for 17-24 months and extracted by two different extraction chemistries. In reference to the storage conditions evaluated, it was hypothesized that samples stored at room temperature, protected by SampleMatrix™ would result in the recovery of higher quality DNA and therefore would produce more complete STR profiles as compared to samples stored without SampleMatrix™, either stored at room temperature or frozen. Samples protected with SampleMatrix™ either appeared to provide similar results when comparing the quality of the profiles to unprotected samples stored at room temperature or frozen with respect to the percentage of alleles detected. "Good profiles" were considered those that resulted in either a full profile or a profile with at least 75% of the total alleles present. A total of 51% of the samples protected with SampleMatrix™ resulted in "good profiles" in comparison with samples stored frozen or unprotected at room temperature that resulted in "good profiles" for 57% and 51% of the samples, respectively.

An additional approach to the analysis of STR profiles was to address the CODIS eligibility of the profiles obtained using SampleMatrix™ as a protecting agent in comparison to samples stored either frozen or unprotected at room temperature. Samples protected with Biomatrica's SampleMatrix™ appeared to provide higher quality DNA for STR typing than samples stored unprotected at room temperature, when addressing STR profiles for CODIS eligibility. 38% of the samples stored unprotected at room temperature did not qualify for either CODIS database, meaning that those profiles had representation at less than seven loci. In comparison, 97% of the frozen samples and 87% of the samples protected with SampleMatrix™ at room temperature were eligible for both CODIS databases.

The second issue addressed in this study is whether the SampleMatrix™ formulation affects the quality of DNA recovered for STR genotyping. There was no expectation that one formulation would be more effective in protecting DNA. SampleMatrix™ formula SM1 appeared to provide higher quality DNA for STR typing when evaluating the percentage of alleles present in the profile. However, when approaching the results from a CODIS eligibility standpoint, SM1 and SM2 appeared to be comparable in terms of the quality of the DNA profiles obtained.

Based on this study, the application method (wet vs. dry) did not affect the quality of recovered DNA in terms of the STR genotyping results. The general trend in terms of the percentage of full profiles, partial profiles (greater than 75% of the full profile), partial profiles (less than 75% of the full profile), and no profiles was consistent between the two formulas. This general trend continued when CODIS eligibility was also evaluated. Approximately 60% of both application methods were eligible for NDIS, 22% (wet) and 29% (dry) were eligible for SDIS, and 16% (wet) and 10% (dry) were not eligible for either CODIS database. The implications of these results suggest that it is not necessary that the sample be dried on the swab prior to the addition of SampleMatrix™.

Limitations

This study explored a novel application of SampleMatrix™, that is, the stabilization of whole body fluid. The technology was developed for the stabilization of DNA. However, the authors recognized the potential of this technology as an evidence collection tool. That being said, the formulations used in this study may not be optimized as a wetting or coating agent. Further, given the different composition of each body fluid, it may be necessary to design a specific formulation that is optimized for each body fluid. We will be providing our preliminary findings to the Biomatrica R and D scientists for this purpose.

The nature of the samples evaluated in the studies presented here stand in sharp contrast to the purified DNA extracts examined in previous studies that have investigated the biostability properties of SampleMatrix™ formulations. Our research was designed to be broad in scope in order to simulate the collection of body fluids at a crime scene and to replicate the post-collection analytical procedures used in forensic laboratories. The analysis of crime scene samples requires many procedures, each of which is comprised of multiple steps. These procedures are performed manually and are limited by systematic and random errors that contribute to variations in the results. These errors are propagated across the various manipulations to affect the quantitative data. The combination of performing the analyses in duplicate and the large standard deviations observed do complicate the interpretation. Clearly, a greater number of replicates are required in order to account for the variance. Further, the statistical analysis was limited to a comparison of mean values for a small number of replicate samples.

Future Research

Despite the limitations specified above, there are some clear trends in the final analyses. The data indicates that SampleMatrix™, specifically the SM1 formulation, presents advantages when used as a wetting agent for the recovery blood and semen stains deposited on several substrates. This is in comparison with the standard practice of forensic laboratories to collect blood and semen by wetting the swab with water and storing the swab in a frozen condition. The results obtained with saliva stains are more ambiguous; however, the data suggest that the performance of SampleMatrix™ is comparable to the standard method. The optimization of a formulation that improves the stabilization of saliva should be considered for future research. In phase II of our original proposal (not funded), we considered the development of a crime scene collection kit based on the SampleMatrix™ technology. Based on the preliminary findings presented in this report, SampleMatrix™ may be a viable alternative or superior approach to the standard method of crime scene sample collection. Our research indicates that none of the SampleMatrix™ formulations interferes with conventional serological testing. In fact, enzymatic-based testing showed improved results with SampleMatrix™-treated samples.

Research on the optimization of SampleMatrix™ as a coating agent should be pursued given that evidentiary samples may be submitted which were collected by the standard method (an example includes the collection of sexual assault kits). The findings of our accelerated aging study support the advantage of SampleMatrix™ as a coating agent relative to unprotected samples. One approach to the treatment of swabs would be to consider the optimization of the coating formulation. Again, our data supports an advantage of the formulation in protecting blood samples, particularly when the SM1 formulation is added to a dried. However, the current

formulation appears to favor more concentrated blood samples; the formulation may need to be modified to extend the benefits to more dilute samples.

Our findings show a clear advantage in the ability of the SM3 formulation in maintaining the morphology of WBC and fragile RBC. Few forensic methods are available for the positive identification of blood. Future research should explore the potential of SM3 as a wetting agent to recover bloodstain for the dual purpose of identifying the stain as blood and determining the genotype of the stain.

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Dissemination of Research Findings

Roberts KA and Johnson DJ. Investigations On The Use Of SampleMatrix™ To Capture and Stabilize Crime Scene Biological Samples For Optimized Analysis and Room Temperature Storage. 61st Annual American Academy of Forensic Sciences Meeting, Denver, CO Feb. 2009.

Roberts KA and Johnson DJ Investigations On The Use Of SampleMatrix™ To Capture and Stabilize Crime Scene Biological Samples For Optimized Analysis and Room Temperature Storage. California Association of Criminalist Spring Seminar, Lake Arrowhead, CA May 2009.

Roberts KA (Invited Speaker). California Association of Crime Laboratory Directors. Hosted by Long Beach Police Department. April 2010.

Roberts KA (Invited Speaker) Forensic Science Education Programs An Update of the CSULA Criminalistics Program Research Activities. The California Association of Crime Laboratory Directors Fall Meeting, Santa Clarita, CA, Nov. 2008.

Muller R, Muller-Cohn J, de Rozieries S, Guroff S, Hadinoto G, Johnson DJ, Roberts KA (presenting author). Evaluation of Dissolvable SampleMatrix® for Long-Term Room Temperature Storage of Forensic DNA. Presented at the 18th International Symposium on Human Identification. Hosted by Promega: Hollywood, CA. Oct 1-4, 2007.

Roberts KA and Johnson DJ. Investigations On The Use Of SampleMatrix™ To Capture and Stabilize Crime Scene Biological Samples For Optimized Analysis and Room Temperature

Storage. Presented at the 18th International Symposium on Human Identification. Hosted by Promega: Hollywood, CA. Oct. 2008.

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