Effective Long-term Preservation of Biological Evidence

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Abstract

The preservation of biological evidence is necessary in order to maintain the quality and quantity of valuable DNA collected for forensic casework analysis. Once evidentiary material (blood, semen, vaginal fluid, etc.) is collected on a substrate, it is subject to degradation by nucleases from environmental microbes as well as oxidation from environmental forces. This presents a problem as some evidence may be stored for months or years before a crime lab receives it for analysis. Many current forensic evidence collection substrates (swabs, cloth, etc.) do not include methods for DNA preservation. The goal of this project was to identify the optimum method to preserve DNA associated with forensic evidence using commercial off the shelf (COTS) chemical preservatives that have been used for decades in the food and cosmetics industries. These COTS preservatives are inexpensive and generally recognized as safe, and they could be easily applied to cotton swabs by the forensic investigator at a crime scene. Four main categories of chemical preservatives were tested: nuclease inhibitors, antimicrobial agents, chelators/fixatives, and antioxidants. It was hypothesized that the use of COTS preservatives on cotton swabs following DNA collection would reduce the risk of DNA degradation and would result in improved profile quality or increased peak height values of analyzable alleles.

The study was conducted over three phases. Phase I consisted of real-time aging and accelerated aging studies that tested twelve chemical preservatives individually with forensically relevant fluids. In Phase II, the preservatives demonstrating the most promising results were combined to examine whether this would enhance the efficacy of the preservation. Phase III examined Zinc and Zinc-EDTA in conjunction with collection substrates that lent themselves to direct amplification.

The results of this study demonstrated that COTS preservatives can be used to protect DNA from degradation. In particular, Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate generated peak height values that demonstrated statistically significant increases when compared to the untreated control samples. Additionally, statistically significant differences were observed from most of the preservative combination treated samples when compared to the untreated control samples. In Phase III, successful direct amplification of treated blood samples was achieved with Promega's PowerPlex® Fusion kit.

This study described novel mechanisms for the preservation of biological evidence collected on a swab. No expensive instruments or specialized skills are required, and these techniques can easily be adopted by any state crime laboratory regardless of funding level. The application of preservatives to biological evidence now could aid in the processing of cold cases in the future by preventing the degradation of DNA evidence kept in long-term storage.

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Executive Summary

The preservation of DNA found in biological fluids is of general interest to the forensic community. While this project aimed to identify an effective method of preservation specifically for forensically relevant biological fluids, the results of this study can be applied across many different disciplines, such as pharmaceutical science, biorepository management, and homeland security. In each of these fields, the preservation of biological evidence is necessary to maintain the quality and quantity of valuable DNA for a wide range of downstream processing. As soon as evidentiary material, such as blood or saliva, is collected at a crime scene or police booking station, the DNA present in the sample may then undergo degradation by nucleases from environmental microbes or by oxidation from environmental forces. Poor storage conditions such as moist or warm environments may also exacerbate these degradative effects. Unfortunately, it could be months or even years before the evidence is received by a forensic laboratory and is able to be processed to a state where current preservation methods are useful. Therefore, in order to preserve the integrity of the DNA for future analyses, it would be beneficial to preserve the sample from environmental damage as soon as it is collected. The ability to apply a DNA preservative directly to a swab after collection could potentially eliminate the risk of DNA degradation and could result in the generation of quality DNA profiles over long periods of time. The need exists for an inexpensive and user-friendly product that can be applied to the swab at the time of sample collection.

The goal of this project was to identify the optimum method for the long-term preservation of DNA associated with forensic evidence using commercially available off-the-shelf (COTS) chemical preservatives that were directly applied to evidence collection substrates. Chemical preservatives that have been used for decades in the food and cosmetic industries to protect the quality and increase the longevity of their products may have direct applications in forensics for the preservation of biological evidence. These preservatives are inexpensive, safe, and could easily be applied to cotton swabs by the forensic investigator at the crime scene or in the lab. Based on published literature, four different types of COTS chemical preservatives were selected for use on forensically relevant biological fluids. The four categories are nuclease inhibitors, antimicrobial agents, chelators/fixatives, and antioxidants.

The proposed study was conducted in three phases:

1. Phase I encompassed two separate timed studies that evaluated the long-term functionality of various chemical preservatives applied to cotton swabs containing biological material.

2. Phase II was comprised of a single timed study that examined whether the long-term stability of biological evidence on a swab was enhanced by combining the preservatives that demonstrated the most promising results in the Phase I study. This study also examined preservative combinations that have been shown to have synergistic effects in existing literature.

3. Phase III explored the use of two preservative solutions in conjunction with direct amplification techniques.

In Phase I, twelve different individual COTS preservatives were tested on forensically relevant biological materials commonly found at crime scenes. The following preservatives were applied to blood, saliva, semen, or vaginal fluid on cotton swabs: Aurintricarboxylic acid (ATA), Actin, Sodium Azide, Nisin, Bronopol, Chitosan, Parabens, Lysozyme, Ethylenediaminetetraacetic Acid (EDTA), Zinc, Propyl Gallate and Ascorbic Acid. Known amounts of biological fluids were applied to the cotton swabs, followed by the addition of the preservatives of interest. Appropriate reagent concentrations were selected per recommendations in existing literature. Extensive controls (untreated swabs) were employed to ensure the accuracy and validity of all reported results. Across Phase I, a total of 1,584 samples were tested including controls. Real-time room temperature aging and accelerated aging studies were conducted simultaneously. Accelerated aging is a technique used to simulate aging of medical devices when real-time aging is not feasible, and it has previously been used to simulate the aging of DNA extracts [1].

Phase II sought to determine if enhanced preservative effects could be achieved through preservative combination. Preservative combinations were chosen based on the results obtained in Phase I and combinations recommended in peer reviewed literature. During this phase, nine preservative combinations were tested on blood, saliva, semen and vaginal fluid and were subjected to real-time room temperature aging and accelerated aging at 50°C to determine if greater preservation functionality was achieved. The preservative combinations examined in Phase II were Zinc-EDTA, Sodium Azide-EDTA, Parabens-EDTA, Propyl Gallate-EDTA, Nisin-EDTA, Lysozyme-EDTA, Nisin-Lysozyme, Zinc-EDTA-Sodium Azide, and Nisin-Lysozyme-EDTA.

During Phases I and II, the samples were extracted on the BioSprint 96 workstation with the BioSprint 96 DNA Blood Kit (QIAGEN, Valencia, CA) and quantified using the Quantifiler™ Duo DNA Quantification Kit (Life Technologies, Foster City, CA) on the Applied Biosystems® 7500 Real-Time PCR System. A DNA concentration of 1.5 ng was targeted for amplification. Samples displaying quantification values less than 0.15 ng/µl were concentrated with Vivacon 500-30K columns (Vivaproducts, Littleton, MA). Depending on the experiment, samples were amplified with the PowerPlex® 16 or the PowerPlex Fusion System (Promega, Madison, WI). Amplification products were subjected to capillary electrophoresis on the 3130xl Genetic Analyzer (Life Technologies, Foster City, CA), and data was analyzed with GeneMapper ID® v3.2.1 with an analytical threshold of 75 relative fluorescence units (RFU) and a stochastic threshold of 200 RFU.

In Phase III, Zinc and Zinc-EDTA were tested in conjunction with alternative collection substrates that lent themselves to faster processing with the use of direct amplification. The following sample and substrate combinations were investigated: saliva on indicating FTA paper, saliva on Buccal DNA Collectors, and blood on FTA paper. Samples underwent real time aging at room temperature and accelerated aging at 50°C, followed by direct amplification with the PowerPlex® Fusion Kit. All data obtained was compared to the control samples and to the data obtained from the other time points.

For each phase, the effect of the preservatives on the DNA was evaluated by examining the quantification values, percent profiles, peak height values, and overall profile balance of the profiles generated. Results for treated samples were compared against the controls, as well as the results from the previous time points. Statistical analyses were completed to determine if the treated samples differed significantly from the untreated control samples. In Phase I, the Forensic

Index (FI), a numerical index used to assess the quality of DNA profiles, was also used for data analysis [2]. The FI assigns a quality score to profiles by taking into consideration the overall peak height of a profile, the profile balance within each locus, and the balance between all loci in a profile.

In Phase I, after examining twelve COTS preservatives that covered a range of preservative types, the most effective preservatives were found to be Sodium Azide (antimicrobial), Parabens (antimicrobial), EDTA (chelator), Zinc (fixative), and Propyl Gallate (antioxidant). Across varying time points and specific biological fluids (saliva and vaginal fluid), the peak height values and percent profiles generated by these preservatives demonstrated statistically significant increases when compared to the untreated samples. Upon review of the results, all of the best performing preservatives were found to have antioxidant activity. The worst performing preservative was Bronopol, which consistently generated peak height values and percent profiles that were significantly lower than those generated by the untreated control samples. In this study, the Forensic Index was found to be a useful method for the assessment of profile quality. When comparing the FI values from the treated samples to those from the untreated control samples, statistically significant increases in FI were observed from the Sodium Azide treated, Parabens treated, EDTA treated, Zinc treated, and Propyl Gallate treated samples. It was also observed that the Bronopol treated samples generated the lowest quality profiles across all time points and biological fluids. In general, the results from the Forensic Index rankings and the previous data analyses were in concordance.

In the first part of Phase II, preservative combinations were examined, and the samples were processed with PowerPlex 16. All of the preservative combinations, except for Nisin-Lysozyme, demonstrated statistically significant increases in profile quality (peak height and percent profile). Zinc-EDTA, Sodium Azide-EDTA, Parabens-EDTA, Propyl Gallate-EDTA, Nisin-EDTA, Lysozyme-EDTA, Zinc-EDTA-Sodium Azide, and Nisin-Lysozyme-EDTA were shown to generate statistically significant differences in percent profiles and peak height values when compared to the control blood, saliva, and vaginal fluid samples. This demonstrated that combining preservatives can be effective; however, further studies will have to be conducted to determine if the combined preservatives were more effective that the individual preservatives. Phase II also included a comparison of the PowerPlex 16 and PowerPlex Fusion amplification kits. An additional 5 year 50°C accelerated aging time point was processed with PowerPlex Fusion and was compared to the 5 year 50°C PowerPlex 16 data. Overall, the data gathered from this study indicated that the PowerPlex Fusion kit is a robust and sensitive system. With the advent of new amplification kits such as PowerPlex Fusion, it will be possible to obtain more information from lower quality DNA samples, consequently diminishing the need for preservatives such as those examined in this study.

Phase III examined direct amplification of preservative treated blood and saliva samples on FTA cards and Buccal DNA Collectors. Direct amplification with Promega's PowerPlex Fusion kit was successful with the Zinc treated and Zinc-EDTA treated blood on FTA samples. While no statistically significant differences were observed between the peak height values and profiles balance ratios from the untreated control samples and the Zinc treated blood samples, statistically significant decreases in peak heights values and statistically significant increases in profile balance ratios were observed when the Zinc-EDTA treated blood on FTA samples were compared to both the untreated control samples and the Zinc treated samples. Direct amplification was not successful with saliva samples on FTA and Buccal DNA Collectors that were treated Zinc and Zinc-EDTA.

While the untreated control saliva samples produced high partial to full profiles, the treated saliva samples generally failed to generate profiles. Because the untreated control saliva samples produced profiles, it is most likely that the direct amplification of the treated samples failed due amplification inhibition.

This study outlined a method by which biological evidence collected on swabs may be effectively preserved for extensive periods of time. Evidence at crime scenes is frequently collected on sterile cotton tipped swabs and stored for future analysis. Often, evidence is stored for months to years before it is submitted to a crime laboratory or while crime laboratories await state or federal funding. During that time, precious sample may be lost to degradative insults from microbes, nucleases, or poor environmental conditions. Commercial off the shelf (COTS) preservatives used for decades in the food and cosmetics industries may have direct applications for forensic practices to preserve biological evidence. These COTS preservatives are inexpensive and generally recognized as safe, and they can easily be applied to cotton swabs by the forensic investigator at a crime scene. If samples are collected in this manner, the forensic investigator could be confident that the samples being collected will generate favorable results, regardless of when the evidence is processed. This method represents a novel mechanism for preservation of sample as currently there are no measures being taken to prevent degradation of unextracted evidence. This method does not require expensive instruments or specialized skills and can easily be adopted by any state crime lab regardless of funding level. With continued research, the application of preservatives now could aid in the processing of cold cases in the future by preventing the degradation of DNA evidence kept in long-term storage.

Future studies could examine additional environmental conditions that would be conducive to bacterial growth. To encourage bacterial growth, 37°C temperature conditions and varying humidity levels could be studied. It would also be beneficial to test the efficacy of the preservatives by examining them in conjunction with plated colonies of known microbes frequently associated with forensic samples. This study did not attempt to determine if preferential amplification of the smaller loci was a result of sample degradation or amplification inhibition by the preservatives. In future studies, gel electrophoresis could be used to assess the quality of the DNA prior to amplification. In Phase III of this study, profiles were not successfully produced following direct amplification of saliva samples on FTA paper and Buccal DNA Collectors. It was strongly believed that this was due to inhibition by the preservatives. To address inhibition issues, further research could be conducted to examine various preservative concentrations and determine the ideal concentration that maintains the preservative effect without inhibiting the samples. Furthermore, the data generated in this study can be used to augment and refine the preservation methods used in forensic science. Additional COTS preservatives that function via the same mechanisms of action as the effective preservatives should be identified and examined for their efficacy when used to preserve biological evidence. Finally, a true long-term room temperature storage study can be performed, and the results from this study can be compared to the accelerated aging results generated in Phase I. By pursuing these avenues for further research, it will be possible to strengthen and expand upon the data already generated.

Introduction

Statement of the Problem

Establishing a preservation technique for biological evidence is necessary to protect the integrity of DNA as it awaits analysis. After it is collected at the crime scene, DNA evidence is typically stored in a law enforcement evidence room before it is submitted to a crime laboratory for processing; however, the evidence may not be immediately submitted to the crime lab. In a survey of 2,250 law enforcement agencies, it was found that 14% of the unsolved homicides that occurred over a five year time period (2003-2008) contained forensic evidence that had not been submitted to a crime laboratory, and 18% of the unsolved rape cases that occurred during the same five year time period contained forensic evidence that had not been submitted to a crime laboratory [3]. Of these cases, 40% (12,548 cases) were estimated to contain DNA evidence. For unsolved property cases during that time period, 23% contained forensic evidence. Although there were a variety of reasons why forensic evidence from unsolved crimes was not submitted to a crime laboratory for testing, almost half of the law enforcement agencies reported that evidence may not have been submitted if a suspect was not identified. This indicated that "no suspect" cases may be assigned a lower priority, resulting in delayed submission or lack of submission to the crime laboratory. Other reasons that evidence was not submitted for further analysis are as follows: the subject adjudicated without forensic evidence testing, the case was dismissed, the law enforcement officers were uncertain of the usefulness of the forensic evidence, analysis of the evidence was not requested by the prosecutors, a suspect was identified but not formally charged, and laboratory resource or timeliness issues [3]. Further processing delays may also occur at the crime laboratory. Even in instances when biological evidence is submitted to a crime laboratory in a timely manner, the average turnaround time for DNA testing of forensic evidence is 123 days, based on a survey given to a small sampling of state and local crime laboratories [4]. Laboratory delays often result from staffing and funding shortages. Between law enforcement and laboratory delays, it may be months or years before biological evidence is processed.

The ability to preserve biological evidence would also benefit evidence subjected to long-term storage after processing has occurred. Stored biological evidence has increasingly been re-examined in efforts to exonerate wrongfully convicted individuals. Since 1989, there have been 312 post-conviction DNA exonerations in the United States [5]. Faced with the possibility that biological evidence may be used to exonerate the innocent, 34 states have passed laws defining the criteria for evidence retention [6]. Depending on the state, these statutes cover a variety of offenses from all criminal cases to only felony sex offenses or homicide. The period of time the law enforcement agency is required to retain the evidence also varies by state. In Arizona, for instance, the evidence associated with a cold case must be retained for 55 years or until a person is convicted of the crime and remains incarcerated or under supervised release for that offense. Other states may only require evidence from cold cases to be retained for the length of the statute of limitations [7**,** 8]. For crimes in which a conviction occurred, many states require the evidence to be stored for the duration of the sentence. In general, states that have evidence retention laws require the evidence to be stored for years after it was collected, and any biological material present on the evidence remains unprotected from degradation.

Swabs are the collector of choice at crime scenes and in sexual assault collection kits, and, unlike FTA cards, swabs are not treated with chemical preservatives. This leaves collected samples vulnerable to bacterial and/or fungal growth and probable DNA degradation by nucleases from environmental microbes or by oxidation from environmental forces especially if stored under

moist or warm conditions. There are several commercially available products that are used for the preservation and storage of extracted DNA; however, it could be months or years before the evidence is received by a forensic laboratory and processed to a state where these liquid preservation methods are useful. In order to preserve the integrity of the DNA and reduce the risk of DNA degradation, the evidence should be preserved from environmental damage as soon as it is collected. In turn, greater profile quality, increased RFU values, and improved profile balance could result. Commercial off the shelf (COTS) preservatives that have been used for decades in the food and cosmetic industries to protect the quality and increase the longevity of their products may have direct applications in forensics for the preservation of biological evidence. These COTS preservatives are inexpensive and safe, and they could easily be applied to cotton swabs by the forensic investigator at a crime scene or in the lab.

Literature Citations and Review

In forensic science, proper evidence collection and storage techniques are important to prevent or inhibit the growth of bacteria and fungi on evidence. Bacterial and fungal growth can degrade and damage DNA present in biological substances [9]; however, it may not always be possible to achieve ideal storage conditions for evidence, especially when stored for extensive periods of time. This presents a challenge in the forensic community when cases may be stored for years before they can be processed and cold case evidence may be archived for future analysis. Successful DNA extraction after long-term storage can be compromised by nuclease and microbial activities and fluctuating environmental conditions. Many current forensic evidence collection substrates (swabs, cloth, etc.) do not include methods for DNA preservation; therefore, the DNA is vulnerable to degradation by biological substances and environmental conditions that may degrade DNA.

Commercially available chemically treated collection cards, such as the Whatman FTA® Cards (Whatman, Clifton, NJ), are used to collect and store reference samples from known contributors. FTA paper is a solid medium on which DNA can be collected, stored, and preserved. It consists of an absorbent cellulose-based paper and four chemical substances that protect the DNA molecules from degradation and preserve the paper from bacterial growth [10]. While this type of collector has obvious advantages, there are drawbacks. Because of the chemical components, the treated paper may not be placed directly in a subject's mouth. Samples, such as blood or saliva, must be applied directly to the paper. Alternatively, a foam applicator may be used to rub the inside of the subject's mouth. Each side of the applicator is then pressed onto the indicating circle on the FTA card. Although these methods are sufficient for the collection of reference samples, they are not applicable to evidence collected at a crime scene, where low copy DNA may be present and the evidentiary stains are applied directly to the collection device. Despite the chemical treatments present on these cards, they have still been shown to exhibit statistically significant DNA degradation after seven years under various storage conditions [11].

In addition to FTA cards, other products available to the forensic community enable long-term stabilization of DNA. Products such as DNA Stable (Biomatrica, San Diego, CA) and GenTegra (Integnex, Pleasanton, CA) enable the long term preservation and stability of DNA at room temperature after the DNA has been extracted from the sample. These liquid preservation

products offer forensic laboratories many benefits, but they are only useful after the DNA has been extracted. It may be months or years before the evidence is received by a forensic laboratory and is processed to a state where these liquid preservation methods are useful. Biomatrica also has a line of products that enable the collection, preservation, and room temperature storage of liquid blood (DNA stable Blood) and saliva (DNAguard Saliva) for a defined period of time. These products are similar to FTA cards in that they are only useful to the forensic community when used to collect reference samples from individuals rather than unknown evidence or biological stains from a crime scene. Because of this, cotton tipped swabs are still the collector of choice at crime scenes. Unlike FTA cards, cotton tipped swabs are not treated with preservative, and they are left vulnerable to bacterial and fungal growth and DNA degradation, especially if the swabs are stored when still moist or under warm conditions [12, 13]. The ability to apply a DNA preservative directly to the swab would eliminate the risk of DNA degradation and could allow for the generation of better quality profiles or improved fluorescence values of analyzed alleles.

For years, the cosmetics and food industries have been using commercially available preservatives to protect the quality and increase the longevity of their products. These additives have been used based on their inherent properties to prolong the shelf life of products and have been shown to be safe at specific concentrations. These products can be categorized into four main sub-types: nuclease inhibitors, antibacterial/antifungal agents, chelators/fixatives, and antioxidants.

The first sub-type of potential preservatives, nuclease inhibitors, act on naturally occurring nucleases shown to degrade DNA. The enzyme that mostly affects nucleic acid degradation is DNase I. Aurintricarboxylic acid (ATA) is a general inhibitor of nucleases and has also been shown to inhibit DNase I, RNase A, SI nuclease, exonucelase III, and a variety of restriction endonucleases [14]. ATA has also been shown to prevent DNA strand breaks, fragmentation, and cell death in renal tubular epithelial cells by inhibiting endonuclease activation [15]. In gene transfer technology, ATA has been used to enhance gene transfer efficiency by interfering with the endo- or exo-nucleolytic cleavage of “free” polynucleotides [16]. Because most nucleic acid binding proteins are sensitive to ATA, it might be a suitable chemical for the prevention of DNA degradation by nuclease activity. Furthermore, Actin, a nuclease inhibitor found in muscle cells, inhibits the enzymatic activity of DNase I [17]. Actin-bound DNase I is enzymatically inactive and is unable to degrade DNA inside cells. In forensics, the use of actin as a preservative may prevent DNA degradation on substrates, thus enabling long-term storage of evidentiary items.

The second sub-type of preservatives consists of additives that are considered to be bacteriostatics (inhibiting bacterial growth), bacteriocidals (killing bacteria), and antifungals [18]. For example, sodium azide, a bacteriostatic, has been shown to inhibit Gram-negative bacterial growth in concentrations as low as 0.01% [19]. Sodium azide can be used to preserve raw milk samples without compromising its quality [20]. It has also been used to preserve urine samples for DNA typing. A study has shown that in the presence of sodium azide, DNA can be obtained from urine samples stored at 4ºC for up to 20 days [21].

Nisin is a broad-spectrum bacteriocin that is effective against many Gram-positive bacteria and pathogens. Bacteriocins, a sub-type of bacteriostatics, are antibacterial proteins produced by bacteria that kill or inhibit growth of other bacteria by forming pores in their target membranes. Nisin is a natural, toxicologically safe antimicrobial food preservative that has been used in cheese, meats, and beverages to extend shelf-life for over 50 years [22]. Although the effectiveness of

nisin against Gram-negative bacteria is low, if coupled with a chelator, such as EDTA, nisin has been shown to inhibit Gram-negative bacteria as well.

Bronopol is another commercially available antimicrobial agent that may prevent DNA degradation. It has a high activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa* which is often found in water [23]. Bronopol can be combined with other antimicrobial agents, such as parabens, to increase its antimicrobial activity [24]. It has been used since 1970 in a variety of cosmetics and topical medications, and it has been found to be non-toxic and safe when used in low concentrations (0.01-1.0%) [25].

Methyl, ethyl, butyl, and propyl parabens are alkyl esters of *p*-hydroxybenzoic acid. This group of preservatives displays greater effectiveness against fungi than bacteria, while its antibacterial activity is stronger against Gram-positive bacteria. Many bacteria that are commonly present in the environment are Gram-positive. Studies have shown that Gram-positive *Staphylococcus* and *Micrococcus* bacteria comprised 11% and 41% of bacterial samples collected from urban air, respectively [26]. For greater coverage against both Gram-positive and Gram-negative bacteria, parabens may be combined with imidazolidinyl urea or diazolidinyl urea [25]. Parabens are non-poisonous and non-irritating, are stable over a wide pH and temperature range, and are soluble in water. Methyl and propyl parabens are the most commonly used parabens as antimicrobial agents in cosmetics. Propyl paraben has been used as a preservative in foods, such as fruit juices and baked goods, for over 50 years [27].

Lysozyme is an antimicrobial that has the ability to lyse the cell walls of certain bacteria. More specifically, lysozyme acts by hydrolyzing the peptidoglycan walls of gram-positive bacteria [28]. Gram-negative bacteria have less peptidoglycan in their cell walls and are therefore less susceptible to cellular lysis with lysozyme; however, it has been demonstrated that the addition of EDTA to lysozyme increases the susceptibility of gram negative bacteria to cellular lysis [29]. Chung et al. have also demonstrated that a combination of nisin and lysozyme demonstrates synergistic effects against many gram positive bacteria that cause food spoilage [30].

The third sub-type of preservatives that may be used to preserve biological materials is fixatives and chelators. Zinc based fixatives have been used primarily in pathology laboratories for the preservation of nucleic acids in tissues. These fixatives are non-toxic, non-carcinogenic, inexpensive, and not temperature sensitive. A solution consisting of 0.5% zinc chloride, 0.5% zinc trifluoroacetate, 0.05% calcium acetate in 0.1M Tris-HCl, pH 6.4-6.7 has been shown to be an effective fixative. Samples fixed in this solution and archived for 14 months produced DNA with similar quality to freshly fixed and processed samples [31]. Chelating agents bind divalent metal ions, such as magnesium and calcium, which promote DNA degradation by acting as cofactors for nucleases [10]. EDTA chelates free magnesium, preventing nucleases from destroying DNA. It has been shown to preserve parasite DNA in blood for up to three months [32]. In forensics, EDTA is included in elution buffers, such as TE^{-4} Buffer and Qiagen's Buffer AE, as a preservative for extracted DNA.

One agent that may be of particular interest for DNA preservation is chitosan because it has multiple modes of action. Chitin is a component found in the exoskeletans of crustaceans and arthropods and in the cell walls of some fungi. Chitosan is a deacetylated derivative of chitin that is produced from chitin by alkali treatment. Multiple studies have been conducted investigating the antimicrobial and chelating properties of chitosan. Studies show that decay caused by *B. cinerea* and *R. stolonifer* was reduced in fungi inoculated fresh strawberries dipped in chitosan solutions; however, chitosan will not affect the growth of fungi which contains chitosan as a major

cell wall component [33]. Both chitosan glutamate and chitosan lactate have demonstrated antibacterial properties against Gram-positive and Gram-negative bacteria. Chitosan also selectively chelates iron, copper, cadmium, and magnesium ions [34]. Because of its multi-functional mode of action, chitosan is a compound of interest.

The final preservative sub-type is antioxidants. Under physiological conditions, endogenous oxidants are produced at a high rate, resulting in extensive oxidative damage to proteins, lipids, and DNA. In living cells, DNA damage is expected to be repaired, but damaged residues that remain may be converted to mutations during replication [35]. Therefore, antioxidants were examined as possible preservatives for biological fluids. It has been demonstrated that ascorbic acid, a dietary antioxidant, plays a critical role in protecting germ cells against oxidative damage. Fraga et al. demonstrated that subnormal levels of naturally occurring ascorbic acid in seminal plasma resulted in increased DNA damage [36]. This study also demonstrated that when semen was incubated with 60-1,400 µM ascorbic acid, DNA damage did not increase as would have been expected if transition metals were available to catalyze the oxidation reaction. Ascorbic acid also inhibits light induced DNA damage while many other antioxidants do not [37].

Propyl Gallate is a synthetic antioxidant that exhibits antimicrobial activity and has been used in food and cosmetic products since 1948 [38, 39]. It is typically used to prevent rancidity in meat products, such as rendered fats and pork sausage, in quantities up to 0.02% of the fat or oil content of the food product [40]. Propyl gallate has been shown to inhibit the growth of the following common food-borne bacteria: *Alcaligenes faecalis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes* Scott A, *Listeria monocytogenes* IAI, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Shigella flexneri*, and *Yersinia enterocolitica* [41].

In summary, the preservation of DNA in biological fluids over time is essential for the forensic DNA community. Having a wide array of tools for the preservation of a sample collected from an individual or a crime scene is necessary especially if laboratories have a large backlog of cases or do not have the staff to process cases in a rapid manner. Testing a wide variety of chemical preservatives that act on nucleases, bacteria, or fungi via different mechanisms may reveal trends regarding what type of preservatives are most effective for maintaining the integrity of biological evidence. Identification of the most effective DNA preservation mechanisms will open the door for further research and allow for the development of improved preservation techniques in the future.

Statement of Hypothesis

The goal of this project was to identify the optimal method for preserving DNA associated with forensic evidence using COTS chemical preservatives that can be directly applied to evidence collection substrates. Nuclease inhibitors, antimicrobial agents, chelators/fixatives, and antioxidants were investigated.

Methods

Experimental Design

The goal of this research was to improve the long-term stability of DNA evidence collected on a commonly used cotton swab. It was hypothesized that the application of preservatives to the evidence immediately after collection would allow for a full DNA profile to be obtained after long-term storage of the substrate. This goal was accomplished by meeting the following research objectives in three separate phases:

1. Phase I encompassed two separate timed studies that evaluated the preservation functionality of various chemical preservatives on biological fluids collected on cotton swabs.

2. Phase II was comprised of a time study that examined whether long-term stability of biological evidence on a swab was enhanced by combining preservative solutions. The preservatives that demonstrated the most promising results during Phase I were combined to see if their preservative effects were enhanced. Established synergistic preservative combinations were also examined.

3. In Phase III, two of the best performing preservatives from Phases I and II were tested in conjunction with alternative collection substrates (FTA paper and Buccal DNA Collectors) that lent themselves to faster processing with the use of direct amplification.

The best performing preservatives were chosen based on the following properties: DNA quantification values, percent profiles, peak height values, and overall profile balance of the profile generated.

Solution Preparation

The appropriate concentrations for the preservative solutions were selected per recommendations in existing literature. Table 1 contains the protocols used to make each preservative solution. Unless otherwise specified, DNA grade water (Fisher Scientific, Waltham, MA) was utilized to make all solutions. All solutions were stored in amber colored glass bottles as noted below.

Table 1: Preservative Solutions

Type	Preservative	Protocol	Reference
Nuclease Inhibitor	Aurintricarboxylic Acid (ATA)	Reconstitute ATA (ACS reagent, Sigma-Aldrich, St. Louis, MO) in sterile water to yield a 0.1M stock solution. From the stock solution, create a 1mM working solution with sterile water. Store solutions at RT.	[**14**, **42**]
	Actin	Reconstitute purified rabbit-derived actin (≥85%, Sigma-Aldrich, St. Louis, MO) in sterile water to yield a 100µg/mL stock solution. From the stock solution, create a 10µg/mL working solution with sterile water. Store solutions at -20 °C.	[**17**]
Antimicrobial Agent	Sodium Azide*	Reconstitute sodium azide (ReagentPlus®, Sigma-Aldrich, St. Louis, MO) in sterile water to yield a 0.25% w/v working solution. Store working solution at RT for up to three months.	[**21**]
	Nisin	Nisin (Sigma-Aldrich, St. Louis, MO) is most stable in low pH environments. The activity should be >900 IU/mg. To a 0.02M HCl solution, add NaCl to create a 0.75% w/v solution. Add Nisin (0.025% w/v) and mix until dissolved. Store working solution at 4°C.	[**43**, **44**]
	Bronopol	Bronopol is most stable at acidic pH levels (pH 3-8) and should be reconstituted in a low pH buffer. Dissolve Bronopol (2-Bromo-2-nitro-1,3-propanediol, 98%, Sigma-Aldrich, St. Louis, MO) in McIlvaine Buffer, pH 3.2, to a concentration of 0.1% w/v. Stir until all solid is dissolved, with the addition of heat if necessary. Store solution at RT.	[**23**]
	Chitosan	Dissolve Chitosan (practical grade, Sigma-Aldrich, St. Louis, MO) in a 1% HCl solution to create a 1% w/v solution of chitosan in 1% HCl. Stir with heat (60 °C) to dissolve the chitosan. Once dissolved, adjust the pH of the solution to 5.6 using 1M NaOH. Store solution at RT.	[**45**]
	Parabens	Methyl paraben (≥99%, Sigma-Aldrich, St. Louis, MO) and propyl paraben (Sigma-Aldrich, St. Louis, MO) were combined together in one solution for this study. Dissolve methyl and propyl paraben to a concentration of 1% methyl/0.5% propyl paraben (w/w) in 200-proof RT ethanol. Store solution at RT.	[**27**, **46**]
	Lysozyme	Combine 10 mg/ml lysozyme (Sigma-Aldrich, St. Louis, MO) with 25 mM sodium acetate with 50% glycerol. Dilute lysozyme to a working concentration of 0.5mg/ml with sterile water. Store solution at -20 °C.	[**30**, **44**, **28**]
Chelator	EDTA	Dilute the 0.5 M EDTA solution (molecular biology grade, Sigma-Aldrich, St. Louis, MO) to a working concentration of 0.2M with sterile water. Store solutions at RT	[**32**]
Fixative	Zinc	Add 0.5% w/v zinc chloride (molecular biology grade, Sigma-Aldrich, St. Louis, MO), 0.5% w/v zinc triflouroacetate (Sigma-Aldrich, St. Louis, MO), and 0.05% w/v calcium acetate (ReagentPlus®, Sigma-Aldrich, St. Louis, MO) to 0.1M Tris-HCl pH 7.4. Stir until all solids are dissolved. The final pH should be 6.5 – 7.0. Store the solution at RT.	[**31**]
Antioxidants	Propyl Gallate	Prepare a 0.1M stock solution of propyl gallate (98%, Acros Organics, Geel, Belgium) in a 9:1 glycerol:10X PBS mixture. From the stock solution, create a 0.25µM working solution with 200-proof ethanol. Store solution at RT.	[**47**, **48**]
	Ascorbic Acid	Prepare a 0.1M stock solution of ascorbic acid (99%, Acros Organics, Geel, Belgium) in sterile water. From the stock solution, create a 100µM working solution with sterile water. Store solution at RT.	[**37**]

*Hazardous: when working with the solid, wear a filter mask to block particles, do not inhale over bottle, and work under a chemical hood until the solid has dissolved.

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Phase I: Testing of Individual Chemical Preservatives

During Phase I, twelve different preservatives were tested on various forensically relevant biological fluids (blood, saliva, semen and vaginal fluid) that were deposited on standard cotton swabs (Puritan®, Guilford, Maine) (Table 2).

Table 2: List of preservatives that were evaluated during Phase I

Type	Preservative	Mode of Action
Nuclease Inhibitor	Aurintricarboxylic Acid (ATA)	Inhibition of nucleases
	Actin	Inhibition of DNase I
Antimicrobial Agent	Sodium Azide	Bacteriostatic (Gram–negative)
	Nisin	Broad-spectrum bacteriocin (Gram-positive)
	Bronopol	Inhibits bacteria (Gram-negative)
	Chitosan	Antibacterial, Antifungal, Chelating properties
	Parabens	Antibacterial, Antifungal
	Lysozyme	Damages bacterial cell walls
Chelator	EDTA	Binds free metal ions
Fixative	Zinc	Preservation of DNA
Antioxidant	Propyl Gallate	Prevents oxidation
	Ascorbic Acid	Prevents oxidation

Blood, saliva, and semen from three separate male donors were purchased from Biological Specialty Corporation (Colmar, PA). The blood samples purchased for this study contained potassium-EDTA preservatives to prevent coagulation. Although the saliva and semen were purchased commercially, neither biological fluid contained additional preservatives. Upon receipt, the samples were thawed and cell counts were performed using a disposable hemocytometer (Incyto, Korea) and a light microscope. The cell counts were verified by quantifying the amount of DNA present within 10 µl of each sample. In triplicate, 10 µl of each biological fluid was subjected to DNA extraction utilizing the EZ1 DNA Investigator Kit (QIAGEN, Valencia, CA) 200 µl lysis protocol on the EZ1 Advanced Instrument (QIAGEN, Valencia, CA). After extraction, samples were then quantified using the Quantifiler® Duo DNA Quantification Kit on the Applied Biosystems 7500 Real-Time Quantification System.

Vaginal fluid was collected from two female donors using sterile cotton tipped swabs following Bode Technology's Internal Review Board (IRB) guidelines. The vaginal cells were eluted from the swabs into 500 µl of 1X PBS. In order to retain the integrity and environment of the vaginal fluid, the cells were not purified by washing in 1X PBS. Cell counts for all vaginal fluid samples were conducted as described above. Prior to sample preparation for Phase I, each fluid was DNA typed with Promega's PowerPlex® 16 System in order to obtain each donor's DNA profile.

Equivalent amounts of cells, by fluid, were applied to each swab to ensure that full STR profiles were achieved for each of the four biological fluids tested. Per donor, 10 µl of blood and 15 µl of

semen were applied to the swabs. Varying amounts of saliva were applied to the swabs in order to deposit approximately 150 cells/µl: 10 µl of Donor A, 15 µl of Donor B, and 10 µl of Donor C. As for the vaginal fluid, 13 µl of the eluate from Donor A was applied to the appropriate swabs and 5 µl of the eluate from Donor B was applied to the appropriate swabs. A vast difference was observed between the cell counts for vaginal fluid Donors A and B. Due to this, 5 ng of DNA was targeted from Donor A, while 10 ng was targeted from Donor B.

Following application of the biological fluids, all swabs were allowed to dry for approximately one hour. The chemical preservative was then applied to the tip of each swab using a dropper bottle. Two drops of preservative were applied to each swab. After treatment with the chemical preservative, each swab was dried at room temperature (RT) and was then placed in a cardboard swab box. Samples were incubated at RT or transferred to an incubator at either 50°C or 60°C. The real-time room temperature aging and accelerated aging studies were conducted simultaneously. A total of 1,584 samples were tested during Phase I (Table 3). Twelve preservative reagents were tested for each sample type at the following time points: 0, 2, 6, 8 and 10 month (real-time/RT) and 1 (50°C and 60°C), 2.5 (50°C), 5 (50°C and 60°C), and 10 year (60°C) accelerated aging time points.

Table 3: Phase I – Sample Numbers

Sample Type	Storage Condition	Control Samples	Containing Preservative
Blood	RT	15	185
Saliva		15	175
Semen		15	185
Vaginal Fluid		15	175
Blood	50°C	9	111
Saliva		9	105
Semen		9	111
Vaginal Fluid		9	105
Blood	60°C	9	111
Saliva		9	105
Semen		9	111
Vaginal Fluid		9	105

Accelerated Aging

Accelerated aging is a technique used to simulate aging of medical devices when real-time aging is not feasible, and it has previously been used to simulate the aging of DNA extracts [1]. The Simplified Protocol for Accelerated Aging, or the 10-degree rule, was utilized for this study [49, 50]. This protocol states that a temperature increase of 10°C corresponds to a twofold increase in shelf life ($Q_{10} = 2$). The formula employed is as follows:

$$\mathbf{TIME_{T1} = TIME_{RT}/Q_{10}^{(T1-TRT)/10}}$$

where T_1 = oven aging temperature, T_{RT} = room temperature (22°C), and Q_{10} = reaction-rate coefficient [49].

For Phase I, it was proposed that samples be tested at 50°C for the equivalent of 1 year (52 days), 2.5 years (131 days) and 5 years (262 days). In addition, samples were tested at 60°C for the equivalent of 1 year (26 days), 5 years (131 days) and 10 years (262 days). The calculations for Phase I are listed below.

Storage at 50°C:

1 Year: $TIME_{T1} = 365 \text{ days}/2^{(50-22)/10}$

$365/2^{2.8} = 365/6.96 = 52$ days

2.5 Years: $TIME_{T1} = 913 \text{ days}/2^{(50-22)/10}$

$913/2^{2.8} = 913/6.96 = 131$ days

5 Years: $TIME_{T1} = 1825 \text{ days}/2^{(50-22)/10}$

$1825/2^{2.8} = 1825/6.96 = 262$ days

Storage at 60°C:

1 Year: $TIME_{T1} = 365 \text{ days}/2^{(60-22)/10}$

$365/2^{3.8} = 365/13.92 = 26$ days

5 Years: $TIME_{T1} = 1825 \text{ days}/2^{(60-22)/10}$

$1825/2^{3.8} = 1825/13.92 = 131$ days

10 Years: $TIME_{T1} = 3650 \text{ days}/2^{(60-22)/10}$

$3650/2^{3.8} = 3650/13.92 = 262$ days

Sample Processing

To ensure that equivalent numbers of treated cells were processed, the entire swab head was cut using sterile techniques and was placed into a SlicPrep™ 96 Device (Promega, Madison, WI). In preparation for DNA extraction on the BioSprint 96 workstation with the BioSprint 96 DNA Blood Kit (QIAGEN, Valencia, CA), a cocktail of Buffer ATL (480 µl) and Proteinase K (20 µl) was added to each sample well of the device. When processing the samples containing

semen, 20 µl of 1.0 M DTT was also added to the digestion solution. Samples were incubated at 56°C with shaking at 900 rpm for 1 hour. The SlicPrep device was briefly centrifuged and the collar was inserted. The SlicPrep device was centrifuged again at 1,500 x g (~3,000 rpm) for 10 minutes. After centrifugation, the collar and the 96-well spin basket (containing the swab heads) were removed and the sample lysate (~500 ml) was then split into two S-blocks (~250 µl each). Each S-block was then processed independently on the BioSprint workstation following the manufacturer's recommended protocol for the purification of DNA from buccal swabs [51]. The eluates for each sample across the two trays were combined for a total of 250 µl.

After DNA extraction, all samples were quantified using the Quantifiler™ Duo DNA Quantification Kit (Life Technologies, Foster City, CA) using 12.5 µl reaction volumes on the Applied Biosystems® 7500 Real-Time PCR System. A DNA amount of 1.5 ng was targeted for amplification. Samples displaying quantification values less than 0.15 ng/µl underwent concentration with Vivacon 500-30K columns (Vivaproducts, Littleton, MA). Samples were amplified with the Powerplex® 16 System (Promega, Madison, WI) using 12.5 µl reaction volumes and a 30 cycle amplification. Amplification products were subjected to capillary electrophoresis on the 3130xl Genetic Analyzer (Life Technologies, Foster City, CA), and data was analyzed with GeneMapper ID® v3.2.1 with an analytical threshold of 75 RFU and a stochastic threshold of 200 RFU.

Phase II: Testing of Chemical Preservative Combinations

In Phase II, the preservatives that generated the most promising results in Phase I were combined to determine if this would enhance their preservative effects. Preservative combinations that were shown to be effective in the published literature were also included. The nine preservative combinations of interest are displayed in Table 4.

Table 4: Phase II preservative combinations

Preservative Combination	Type of Preservative
Zinc/EDTA	Fixative - Chelator
Sodium Azide/EDTA	Antimicrobial - Chelator
Parabens/EDTA	Antimicrobial - Chelator
Propyl Gallate/EDTA	Antioxidant - Chelator
Nisin/EDTA	Antimicrobial - Chelator
Lysozyme/EDTA	Antimicrobial - Chelator
Nisin/Lysozyme	Antimicrobial - Antimicrobial
Zinc/EDTA/Sodium Azide	Chelator - Fixative - Antimicrobial
Nisin/Lysozyme/EDTA	Antimicrobial - Antimicrobial - Chelator

Samples were prepared as described in Phase I, treated with one of the preservative combinations listed above, dried at RT, and placed in a cardboard swab box. For each set of control and preservative combinations samples, biological fluids were obtained from two donors and were examined in triplicate. A total of 720 samples were processed during this part of Phase II (Table 5). Accelerated aging was performed by incubating the samples and controls at 50°C for a specific

number of days in order to achieve the RT storage time equivalent (1 year, 2.5 years and 5 years). Samples were processed as described in Phase I.

Table 5: Sample types tested during Phase II

Sample Type	Storage Condition	Amplification Kit	Control Samples	Containing Preservative
Blood				
Saliva			6	54
Semen			6	54
Vaginal Fluid			6	54
Blood	50°C	PowerPlex 16	12	108
Saliva			12	108
Semen			12	108
Vaginal Fluid			12	108
Blood	50°C	PowerPlex Fusion	6	54
Saliva			6	54
Semen			6	54
Vaginal Fluid			6	54

An additional set of samples that was prepared at the same time as the other Phase II samples and were stored alongside at 50°C for 262 days (equivalent to five years at RT) was tested with the PowerPlex Fusion System. This set of experimental samples was processed alongside the original Phase II 5 year accelerated aging time point so that a direct comparison of the data generated from the PowerPlex 16 and Fusion Systems could be performed. A total of 240 samples including controls were amplified with PowerPlex Fusion.

Samples were subjected to DNA extraction and quantification as described in Phase I. Based on internal sensitivity studies, 1.0 ng of DNA was targeted for amplification with the PowerPlex® Fusion System (Promega, Madison, WI). A 12.5 µl reaction volume with a 29 cycle amplification was performed. Amplification products were subjected to capillary electrophoresis on the 3130xl Genetic Analyzer, and data was analyzed with GeneMapper ID® v3.2.1 with an analytical threshold of 75 RFU and a stochastic threshold of 200 RFU.

Phase III: Direct Amplification

During Phase III, Zinc and Zinc-EDTA were tested using biological fluids that had been applied to alternative collection substrates (Table 6). The blood samples utilized in this experiment were purchased from Biological Specialty Corporation (Colmar, PA), and the saliva samples were collected from individuals following Bode Technology's Internal Review Board (IRB) guidelines. Samples were prepared as follows: 25 µl of blood were applied to FTA mini cards (GE Healthcare, Pittsburgh, PA) and 25 µl of saliva were spotted three times (the sample was allowed to dry in between applications) to the center of the Indicating FTA mini cards (GE Healthcare, Pittsburgh, PA) and to the center tip of the Buccal DNA Collectors (Bode Technology, Lorton, VA). All

samples were allowed to dry overnight at RT prior to application of the preservative solutions. Zinc and Zinc-EDTA were applied to each substrate using a dropper bottle (~ 2 drops). After treatment with the chemical preservative, the samples were dried at RT. The samples that were to be stored at an accelerated aging temperature of 50°C were transferred into the incubator. The real-time room temperature aging and accelerated aging studies were conducted simultaneously. Samples were tested at the following time points: 0 months (RT), 3 months (RT), 6 months (RT), 1 year (accelerated aging for 52 days at 50°C), and 2.5 year (accelerated aging for 131 days at 50°C). A total of 324 samples including controls were tested during Phase III.

Table 6: List of sample types tested during Phase III

Sample Type	Substrate	Storage Condition	Control Samples	Containing Preservative
Saliva	FTA Indicating	RT	18	36
Saliva	Buccal Collector		18	36
Blood	FTA		18	36
Saliva	FTA Indicating	50°C	18	36
Saliva	Buccal Collector		18	36
Blood	FTA		18	36

Sample Processing

At each time point, two 1.2 mm punches were taken from each treated and untreated Buccal DNA Collector sample and placed into a 96-well plate. Ten microliters of Promega's Punch Solution was then added to each Buccal DNA Collector sample and incubated in a heat block for 30 minutes at 70°C. One 1.2 mm punch was taken from each treated and untreated (control) blood sample on FTA paper. Two 1.2 mm punches were taken from each treated and untreated saliva sample on indicating FTA paper**.** Samples were directly amplified with the Powerplex® Fusion System using 12.5 µl reaction volumes and a 26 cycle amplification following the manufacturer's thermal cycling parameters. Amplification products were subjected to capillary electrophoresis on the 3130xl Genetic Analyzer, and data was analyzed with GeneMapper ID® v3.2.1.

Data Analysis

The effects of the preservatives on the DNA were evaluated by examining quantification values, profile accuracy, percent profile, the peak height values, and the overall profile balance of the profiles generated. Results for treated samples were compared against the controls, as well as the results from the previous time points. A stochastic threshold of 200 RFU was utilized for data analysis.

The percent profile for each sample was calculated by dividing the number of observed alleles by the number of expected alleles followed by multiplying the calculated value by 100 (e.g. 22/32 = 0.6876 x 100% = 68.76%).

The average peak height across a sample was determined by first calculating the average peak height at each locus, followed by calculating the average of all peak heights across the profile.

The DNA profiles' overall profile balances were calculated as an indicator of the presence of an amplification inhibitor or the occurrence of DNA degradation within a sample. Samples that produced DNA profiles that were imbalanced and displayed preferential amplification of the smaller alleles over the larger alleles, appearing as a "ski-slope," may have contained inhibitors or degraded DNA. In order to determine a profile's balance, the average peak height per locus for all loci was first calculated. Next, the value obtained for the locus containing the maximum average peak height was divided by the value obtained from the locus containing the minimum average peak height. This value (ratio) represented a profile's overall profile balance. DNA profiles that displayed consistent peak heights per locus across an entire profile had low profile balance ratios (e.g. Maximum Peak Height/Minimum Peak Height = 2,000/1,000 = 2), whereas DNA profiles that appeared as a "ski-slope" had higher profile balance ratios (e.g. Maximum Peak Height/Minimum Peak Height = 5,000/500 = 10)

A statistical analysis system program, JMP® Software: Classic Design of Experiments (DOE), was used for the Phase I experimental design and for creating the graphs presented in the results section. Microsoft Excel was utilized for the single factor Analysis of Variance (ANOVA) computations. An F-Test for Variance and a Student's T-test were employed to determine if the null hypotheses were supported. In addition, the JMP Software, StatistiXL 1.8, and Microsoft Excel were used to perform all statistical analysis associated with the forensic DNA profile index (FI) [**2**, **52**].

Forensic Index (FI) Analysis

In addition to analyzing the data using percent profile, average peak height, and overall profile balance, the data was also analyzed utilizing a recently developed ranking system, the forensic DNA profile index (*FI*) developed by Hedman et al [2, 52]. *FI* is a numerical index intended to be used as a means to provide unbiased and quantitative quality assessment of a DNA profile. This index assesses the quality of a DNA profile by providing a single quantitative value that takes three factors into consideration: overall peak height (Total Peak Height - *TPH*), peak height balance within each locus in a profile (mean Local Balance - *MLB*), and the profile balance across all loci of a profile (Shannon Entropy - *SH*).

The following equations are used to calculate *TPH*, *MLB* and *SH*:

TPH (Total Peak Height): $\sum_{i=1}^{M} PH_i$

- M is the number of STR loci analyzed

-PH_i is the sum of the two peaks heights or single peak height of a locus i

MLB (Mean Local Balance): $M^{-1} \sum_{i=1}^{M} LB_i$

$$-LB_i = \begin{cases} \frac{\text{Height of the lower peak}}{\text{Height of the higher peak}} & \text{for a heterozygous locus} \\ 1 & \text{for a (true) homozygous locus} \end{cases}$$

SH (Shannon Entropy): $-\sum_{i=1}^{M} p_i * \ln p_i$

$-p_i$ is the contribution from marker i to the total sum of peak heights

$$p_i = \frac{TPH_i}{\sum_{i=1}^{M} TPH_i}$$

Principal component analysis (PCA) is used to combine these three factors into one single and easily interpretable numerical index. PCA calculations were performed using StatistiXL 1.8 software.

Principal component analysis was performed on a calibration set consisting of 0 month samples, 10 months samples, and 1 year 50°C RT equivalent samples. Multiple time points were included in the calibration set so that both low and high quality profiles were taken into account. Each of the three factors (*TPH*, *MLB*, and *SH*) was standardized using sample means and sample standard deviations before applying PCA.

$$pc_i = a_1 * tph_i + a_2 * mlb_i + a_3 * sh_i \, , \; i = 1, \; \ldots n$$

Factor loadings (a_1, a_2, a_3) obtained from PCA are adjusted through validation against a manual profile grading scale (prg). The relationship between prg and pc is demonstrated by fitting both variables to a linear model and validated utilizing leave-one-out cross validation to "shrink" the parameters of the model. New factor loadings (c_1, c_2, c_3) are estimated from this linear prediction model. The adjusted principal component (apc) is then used to form the final model for calculation of the FI.

$$FI = apc + c_1 * a_1 * tph + c_2 * a_2 * mlb + c_3 * a_3 * sh$$

The approach for calculating the *FI* that was used in this study differed from that of Hedman. To obtain the *FI* for the Phase I sample data, the *pc* values for the Phase I samples were plotted against the equation of the line obtained from the calibration set, as described in the results section. The *FI* values obtained equate to profile quality such that a higher value is indicative of a better quality DNA profile.

Results

Phase I

Due to the large number of preservatives investigated, the preservatives were divided into two groups: (1) those that demonstrated a statistically significant increase in peak height from any

untreated control samples (blood, saliva, semen, or vaginal fluid samples) and (2) those that demonstrated no statistically significant difference or a statistically significant decrease in peak height from the untreated control samples. The first group will review the results from the samples that were treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The second group will review the results from the samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme.

Mean Quantification Values

Blood

The untreated control blood samples generated average quantification values ranging from 0.493 ng/µl – 0.960 ng/µl until the 10 year RT equivalent time point. At this time point, the average quantification values for the untreated blood samples decreased to 0.234 ng/µl.

The mean quantification values for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 1. Reproducible quantification values greater than 0.5 ng/µl were generated for the Sodium Azide treated blood samples for the 0 month - 1 year RT equivalent at 50°C time points. Beginning at the 1 year RT equivalent at 60°C time point, the Sodium Azide treated blood samples generated variable quantification results that ranged from 0.1 ng/µl – 0.7 ng/µl. The Parabens treated, EDTA treated, and Zinc treated blood samples produced average quantification values greater than 0.6 ng/µl across all time points until the 10 year RT equivalent time point. At this time point, the average quantification values decreased to approximately 0.2 ng/µl. Reproducible quantification values that ranged from 0.5 ng/µl – 1.0 ng/µl were generated for all blood samples treated with Propyl Gallate up until the 5 year RT equivalent at 60°C time point when the quantification values decreased to 0.1 ng/µl – 0.2 ng/µl.

Figure 2 displays the mean quantification results generated for the blood samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Reproducible quantification values that ranged from 0.6 ng/µl – 1.0 ng/µl were generated for the ATA treated and Actin treated blood samples for the 0 - 10 month time points. Beginning at the 1 year RT equivalent at 50°C time point, a decrease in quantification value (0.03 ng/µl – 0.35 ng/µl) was observed over time. Reproducible quantification values greater than 0.6 ng/µl were generated for the Nisin treated and Bronopol treated blood samples at the 0 month - 1 year 50°C RT equivalent time points. Beginning at the 1 year RT equivalent at 60°C time point, variable quantification results that ranged from 0.05 ng/µl – 0.60 ng/µl were generated for the Nisin treated blood samples, whereas low quantification results that ranged from 0.04 ng/µl – 0.09 ng/µl were obtained from the samples treated with Bronopol. Until the 5 year RT equivalent at 60°C time point, the Chitosan treated samples generated quantification values of approximately 0.25 ng/µl. Quantification values less than 0.10 ng/µl were generated for the remaining two time points. Blood samples that were treated with Ascorbic Acid produced quantification results that ranged from 0.4 ng/µl - 1.0 ng/µl up until the 2.5 year RT equivalent time point. At each time point thereafter, quantification values less than 0.10 ng/µl were generated. On average, quantification values greater than 0.5 ng/µl were generated for the blood samples treated with Lysozyme until the 10 year RT equivalent time point where a decrease in quantification values was observed.

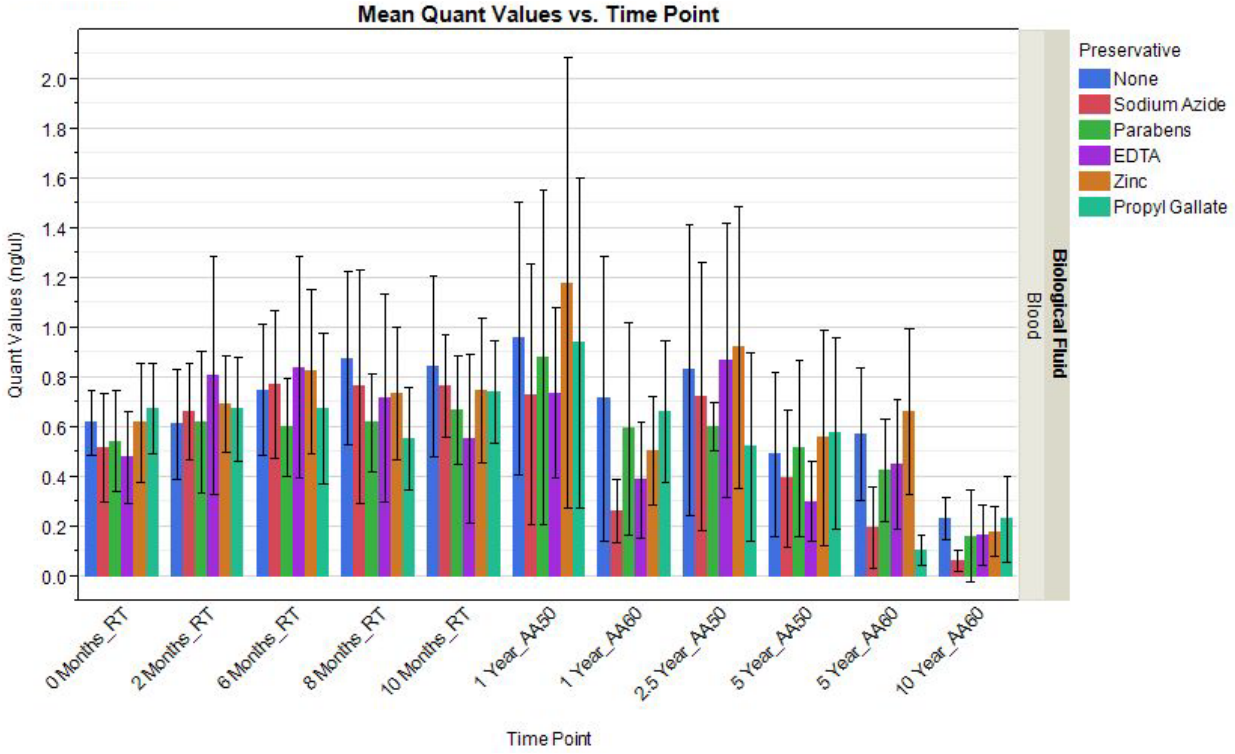

Figure 1: Mean Quantifiler Duo Human results (ng/µl) for the blood samples that were treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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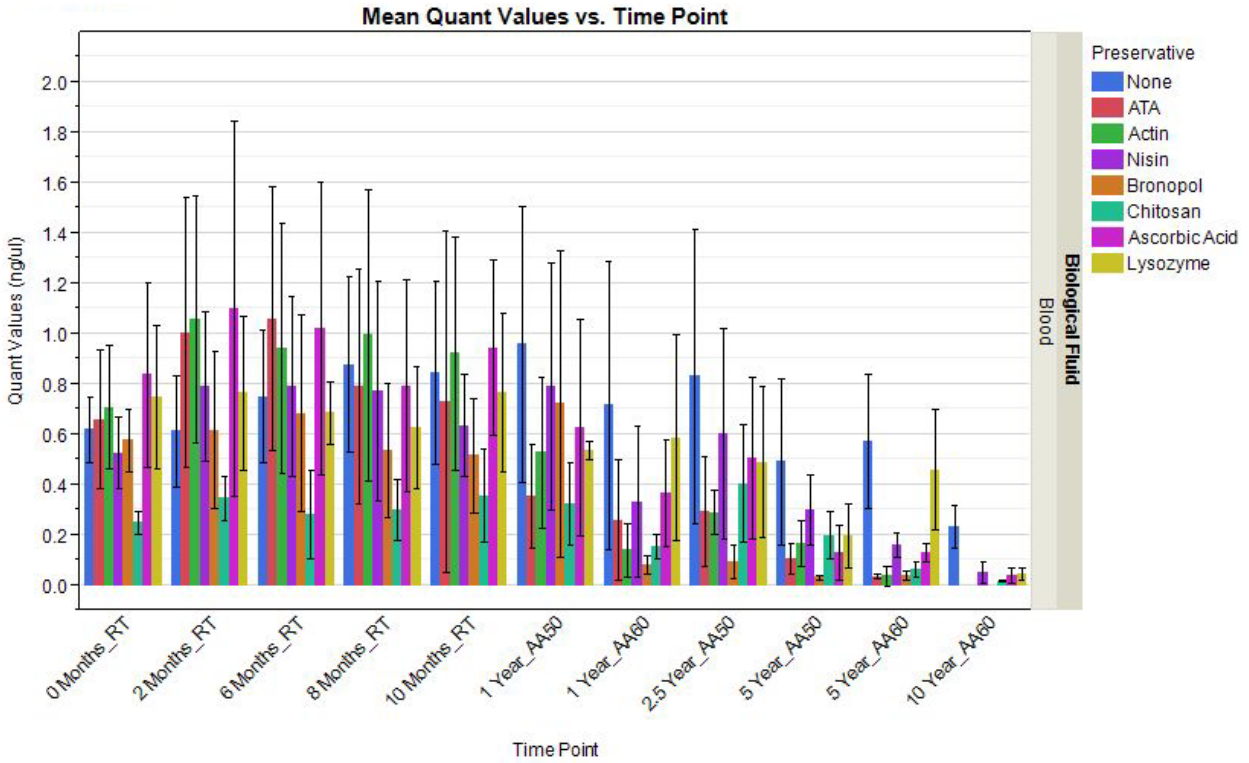

Figure 2: Mean Quantifiler Duo Human results (ng/µl) for the blood samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Saliva

In general, the mean quantification results were lower and more variable for the saliva samples than for the blood samples. The mean average quantification value for the untreated control saliva samples at the 0 month time point was 0.510 ng/µl. At the following time points, the control saliva samples produced lower mean quantification values that ranged from 0.007 ng/µl – 0.345 ng/µl.

The mean quantification values for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 3. At the 0-10 month time points, the Sodium Azide treated saliva samples produced a mean average quantification value of 0.150 ng/µl. At the following time points, the mean quantification value for the Sodium Azide treated samples decreased to 0.099 ng/µl. For the Parabens treated, Zinc treated, and Propyl Gallate treated saliva samples, mean quantification values ranging from 0.320 ng/µl – 0.360 ng/µl were observed at the 0-10 month time points. Beginning at the 1 year RT equivalent at 50°C time point, decreasing quantification values were observed at different rates, with the lowest values observed at the 10 year RT equivalent time point. Across the 0-10 month and 1 year RT equivalent at 50°C/60°C time points, variable quantification results that ranged from 0.080 ng/µl - 0.328 ng/µl were obtained for the saliva samples treated with EDTA. At the following time points, the EDTA treated saliva samples generated quantification values that ranged from 0.143 ng/µl – 0.321 ng/µl.

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Figure 4 displays the mean quantification results generated for the saliva samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. At the 0 month time point, the ATA and Actin treated saliva samples generated average quantification values of 0.289 ng/µl and 0.373 ng/µl, respectively. Decreased quantification values were observed at each time point thereafter. Across the 0, 2, 6, 8, and 10 month time points, variable quantification results ranging from 0.108 ng/µl – 0.457 ng/µl and 0.014 ng/µl - 0.341 ng/µl were obtained from the Nisin treated and Bronopol treated saliva samples, respectively. For the remaining time points, the mean quantification values for the Nisin treated samples decreased to 0.061 ng/µl, whereas the mean quantification values for the Bronopol treated samples decreased to 0.006 ng/µl. The mean quantification values at the 0 month time point for the Chitosan treated and Lysozyme treated saliva samples averaged 0.279 ng/µl and 0.448 ng/µl, respectively. Over time, the quantification values decreased until they reached 0.013 ng/µl and 0.019 ng/µl at the 5 year RT equivalent at 60°C time point. For the Ascorbic Acid treated saliva samples, average quantification values of 0.365 ng/µl were obtained at the 0 month time point. Decreasing quantification values were observed at each time point thereafter.

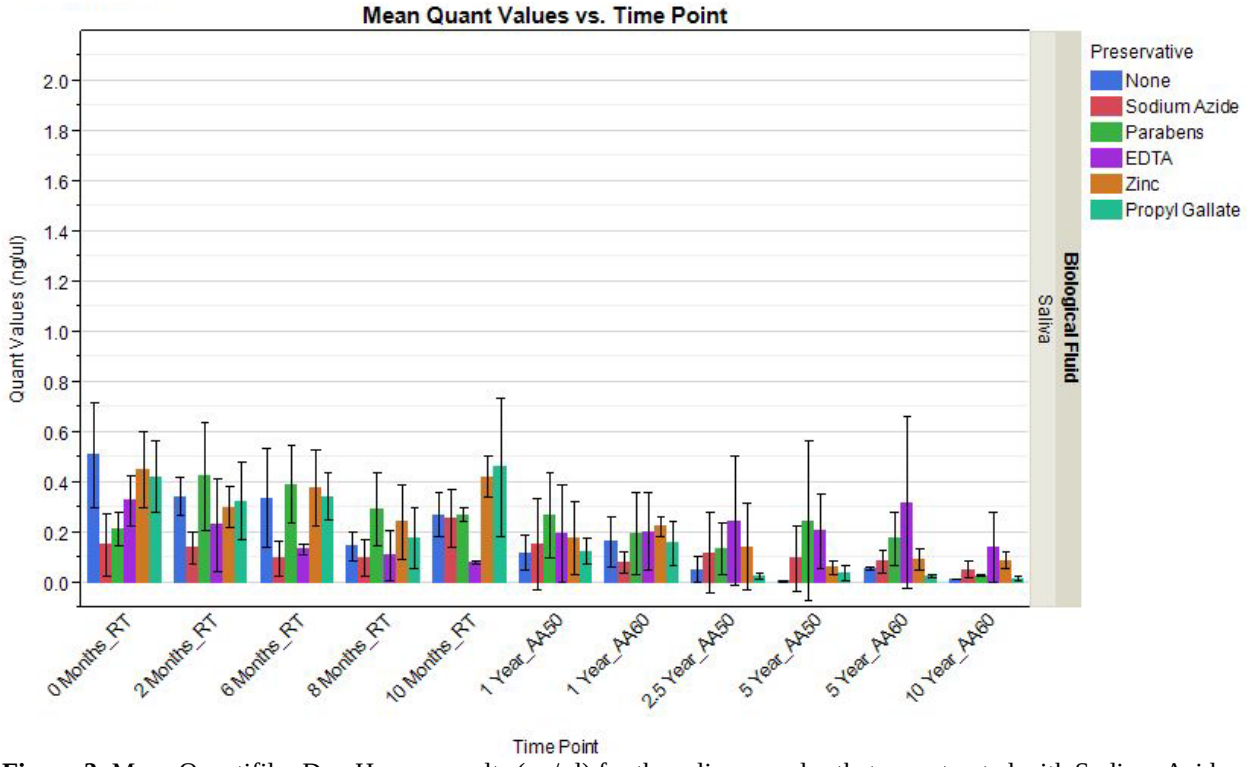

Figure 3: Mean Quantifiler Duo Human results (ng/µl) for the saliva samples that were treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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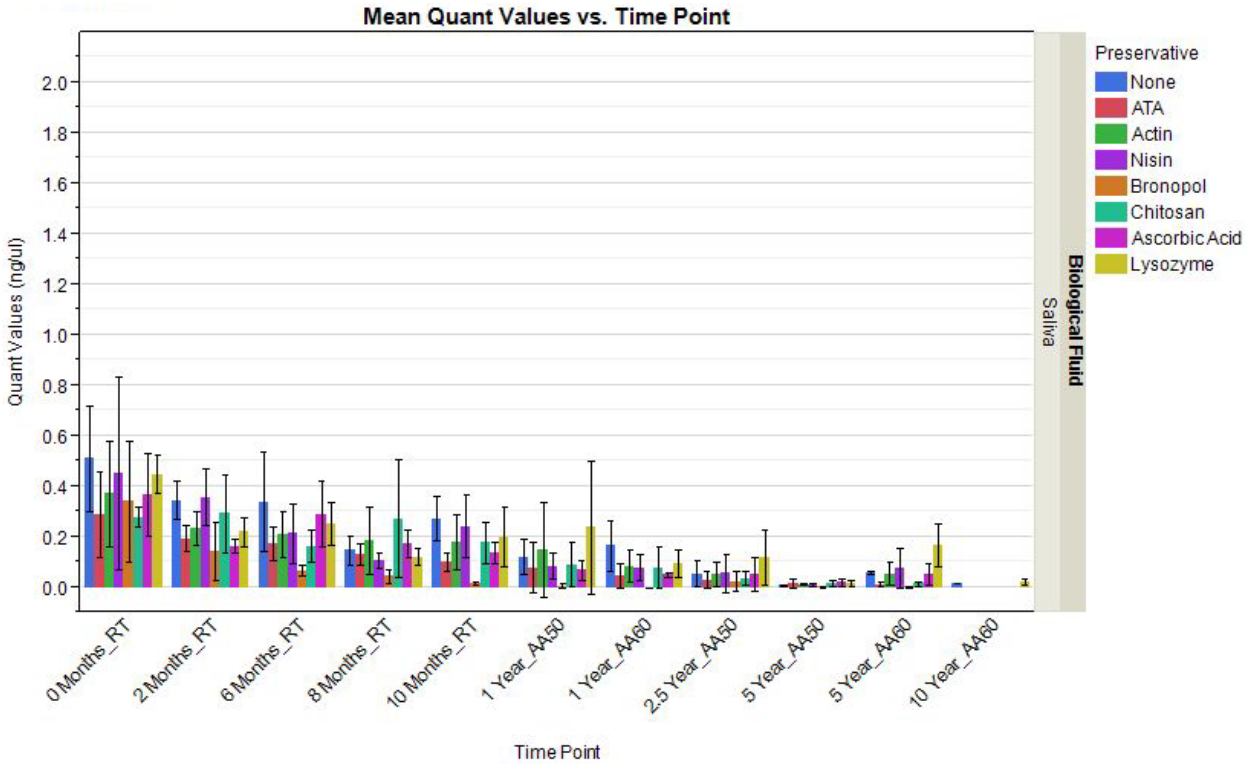

Figure 4: Mean Quantifiler Duo Human results (ng/µl) for the saliva samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Semen

Large standard deviations were observed within each treated and untreated set of semen samples because Donor 2 consistently generated quantification values less than 0.10 ng/µl. Mean average quantification values greater than 2.0 ng/µl were generated from all untreated control semen samples until the 10 year RT equivalent time point. At this time point, a mean quantification value of 0.855 ng/µl was observed.

The mean quantification values for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 5. Despite the low quantification values generated by Donor 2, mean quantification values of approximately 4.5 ng/µl were generated for the Sodium Azide treated semen samples until the 10 year RT equivalent time point where the average quantification value decreased to 0.76 ng/µl. Across all time points until the 10 year RT equivalent time point, the Parabens treated, EDTA treated, Zinc treated, and Propyl Gallate treated semen samples produced quantification values averaging approximately 5.0 ng/µl. At the 10 year RT equivalent time point, the quantification values for those samples decreased to 1.5 ng/µl – 2.1 ng/µl.

Figure 6 displays the mean quantification results generated for the semen samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Mean quantification values greater than 2.0 ng/µl were generated from all ATA treated, Actin treated, and Nisin treated

semen samples from all time points except for the 10 year RT equivalent. At this time point, mean quantification values of approximately 0.5 ng/µl – 0.7 ng/µl were generated. For the 0 month - 1 year RT equivalent at 50°C time points, the Bronopol treated samples generated quantification values that ranged from 2.5 ng/µl – 5.6 ng/µl. Beginning at the 1 year RT equivalent time point at 60°C, variable quantification values that ranged from 0.03 ng/µl – 1.5 ng/µl were generated. Mean average quantification values greater than 4.0 ng/µl were generated from most Ascorbic Acid treated semen samples from all time points until the 5 year RT equivalent at 50°C time point. At the 5 and 10 year RT equivalent time points, mean quantification values of 0.8 ng/µl – 3.1 ng/µl were generated. Mean average quantification values of 5 ng/µl were generated from the Lysozyme treated semen samples across all time points except for the 10 year RT equivalent. At this time point, the mean quantification value decreased to 0.292 ng/µl.

Over time, the quantification values presented in Figures 5 and 6 displayed a Gaussian distribution rather than exponential decay. It is unknown why a Gaussian distribution was generated from the semen samples but not the other biological fluid samples. The quantification assays performed within the validated parameters. For each time point, the R^2 value of the standard curve was greater than the validated minimum value of 0.98 and the slope of the standard curve was within the validated range of $^{-}3.0$ - $^{-}3.6$. The y-intercepts were also within the recommended range of 28 +/- 3.0. The cycle threshold (Ct) values of the standards and the internal positive controls (IPCs) were within the validated range and were similar across all assays. Additionally, this trend was not observed for the vaginal fluid samples that were quantified on the same trays. Based on this analysis, the lower quantification values observed at the 0 month and 2 month time points did not result from a quantification issue. It is possible is that the cellular structure of the sperm heads weakens over time, which results in an increase in free floating DNA and increased extraction efficiency.

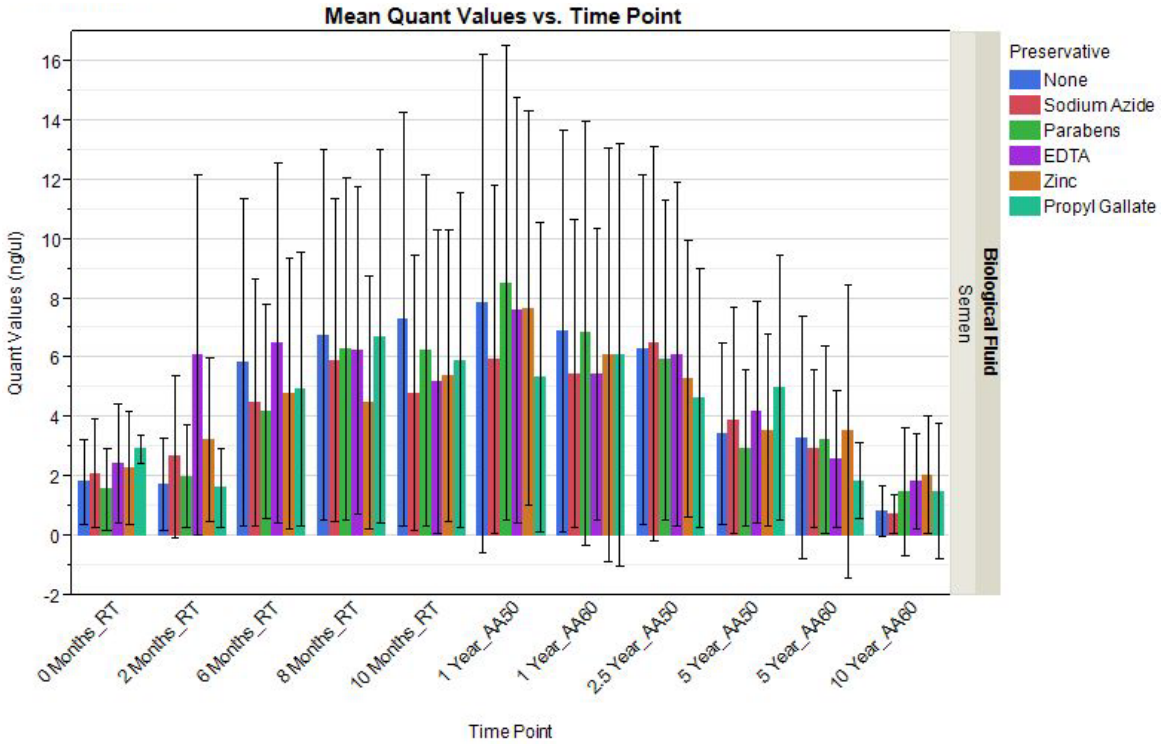

Figure 5: Mean Quantifiler Duo Human results (ng/µl) for the semen samples that were treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

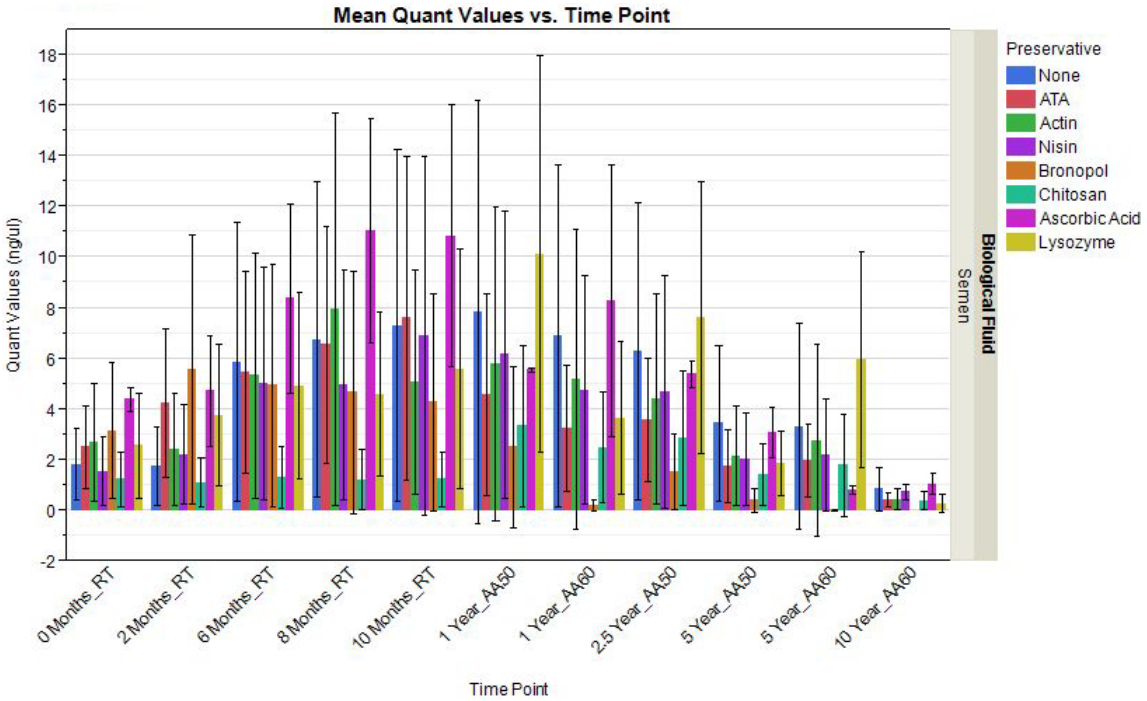

Figure 6: Mean Quantifiler Duo Human results (ng/µl) for the semen samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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Vaginal Fluid

Similar to the saliva samples, variable quantification results were obtained across all time points for the treated and untreated vaginal fluid samples. At the 0 month time point, an average quantification value of 0.281 ng/µl was generated for the untreated control vaginal fluid samples. For the remaining time points, the average quantification values ranged from 0.001 ng/µl – 0.171 ng/µl.

The mean quantification values for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 7. An average quantification value of 0.136 ng/µl was obtained from the Sodium Azide treated vaginal fluid samples at the 0 month time point. Decreased quantification values were obtained at each time point thereafter. Across the 0 - 10 month time points, mean average quantification values of 0.122 ng/µl, 0.075 ng/µl, 0.194 ng/µl, and 0.386 ng/µl were obtained from the Parabens treated, EDTA treated, Zinc treated, and Propyl Gallate treated vaginal fluid samples, respectively. Across the accelerated aging time points, mean average quantification values that ranged from 0.03 ng/µl – 0.05 ng/µl were generated.

Figure 8 displays the mean quantification results generated for the vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme.
Mean quantification values of 0.07 - 0.16 ng/µl were generated for the ATA treated and Actin treated vaginal fluid samples at the 0 - 10 month time points. Quantification values less than 0.025 ng/µl were generated for the remaining time points. At the 0 month time point, average quantification values of 0.106 ng/µl and 0.215 ng/µl were obtained for the Nisin treated and Bronopol treated vaginal fluid samples, respectively. Decreased quantification values were generated at each time point thereafter. Mean average quantification values for the Chitosan treated, Ascorbic Acid treated, and Lysozyme treated samples for the 0, 2, 6, 8 and 10 month time points were greater than 0.10 ng/µl. Mean quantification values less than 0.05 ng/µl were generated for all remaining time points.

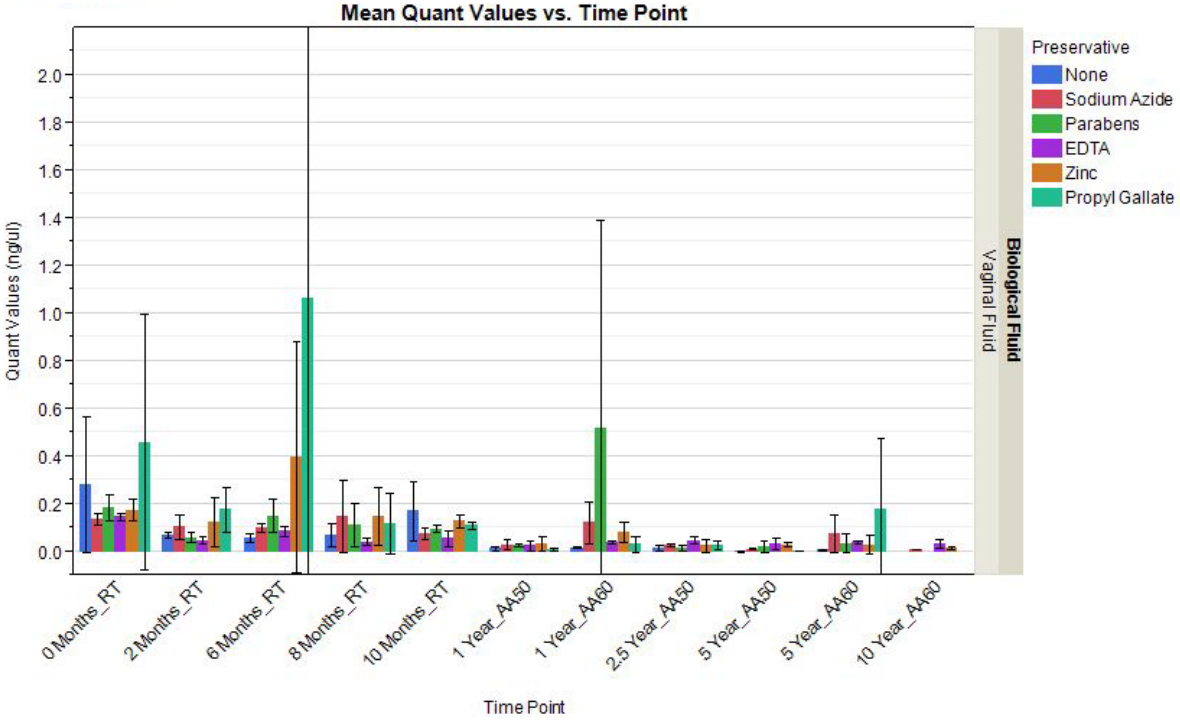

Figure 7: Mean Quantifiler Duo Human results (ng/µl) for the vaginal fluid samples that were treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

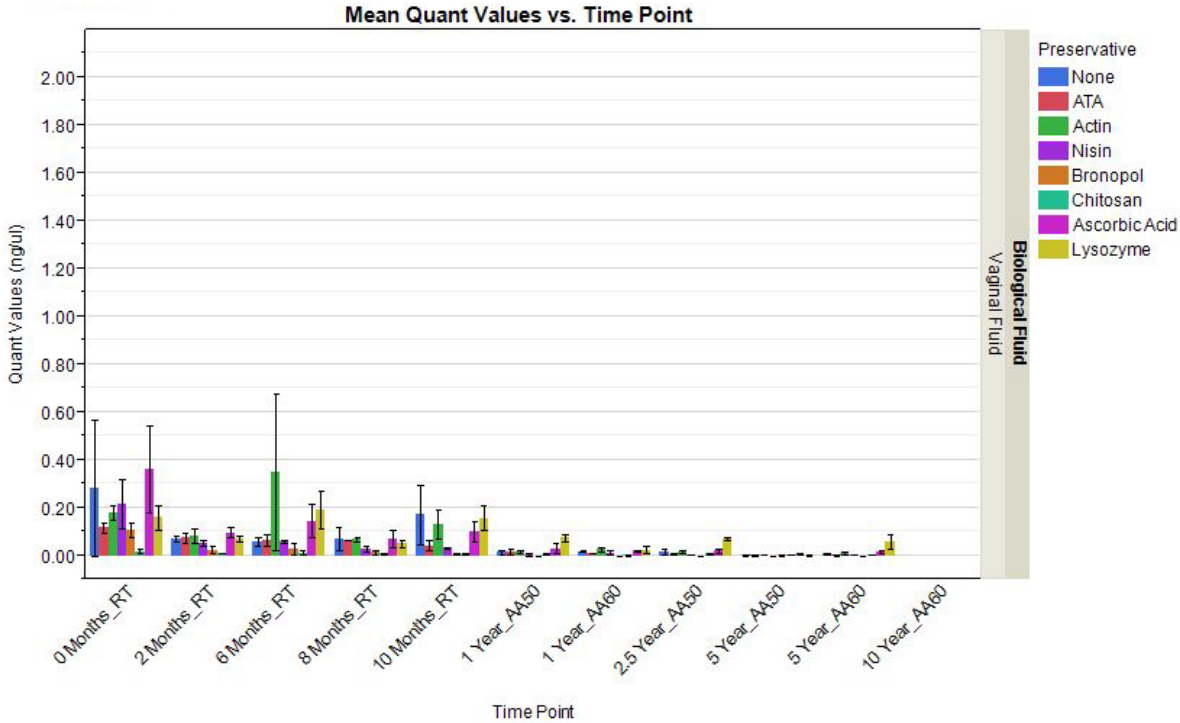

Figure 8: Mean Quantifiler Duo Human results (ng/µl) for the vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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Mean Percent Profile

No statistically significant differences in percent profile were observed between the preservative treated blood and semen samples and their respective controls (Table 7). When compared to the control, statistically significant differences in percent profile were observed in saliva samples treated with EDTA, Zinc, Propyl Gallate, Actin, and Chitosan and vaginal fluid samples treated with Sodium Azide, EDTA, and Zinc.

Table 7: Summary of the statistically significant percent profile data for each preservative and fluid from Phase I. Statistically significant percent profile when compared to control is represented as "+", no effect or decrease in percent profile when compared to the control is represented as "-".

Preservative	Blood	Saliva	Semen	Vaginal Fluid
Sodium Azide				
Parabens	-	-	-	-
EDTA	-	+	-	+
Zinc	-	+	-	+
Propyl Gallate	-	+	-	-
ATA	-	-	-	-
Actin	-	+	-	-
Nisin	-	-	-	-
Bronopol	-	-	-	-
Chitosan	-	+	-	-
Ascorbic Acid	-	-	-	-
Lysozyme	-	-	-	-

Blood

The 10 year RT equivalent time points contain incomplete sample data due to an amplification issue. Full DNA profiles were generated for the untreated control blood samples across all time points.

The mean percent profile values for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 9. Across all time points, full to high partial profiles were generated for all blood samples treated with Sodium Azide. Full DNA profiles were generated for the Parabens treated blood samples until the 2.5 year RT equivalent time point when a slight decrease in mean percent profile was observed. Regardless of the length of storage time, all EDTA treated, Zinc treated, and Propyl Gallate treated blood samples produced full DNA profiles.

Figure 10 displays the mean percent profile results generated for the blood samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Full to high partial profiles were generated for all blood samples that were treated with ATA and Actin at all time points except for the 5 year RT equivalent at 60°C. At this time point, the treated samples demonstrated percent profiles of approximately 50%. Full to high partial profiles were also generated for all blood samples treated with Nisin across all time points. Until the 5 year RT equivalent at 50°C time point, the blood samples treated with Bronopol generated full to high

partial profiles. At this time point and beyond, a decrease in percent profile was observed. The Chitosan treated blood samples generated full profiles at every time point until the 5 year RT equivalent at 60°C time point. At this time point and beyond, a decrease in percent profile was observed. Similarly, the Lysozyme treated blood samples generated full profiles until the 5 year RT equivalent at 50°C time point. Regardless of length of time, all Ascorbic Acid treated blood samples produced full to high partial DNA profiles.

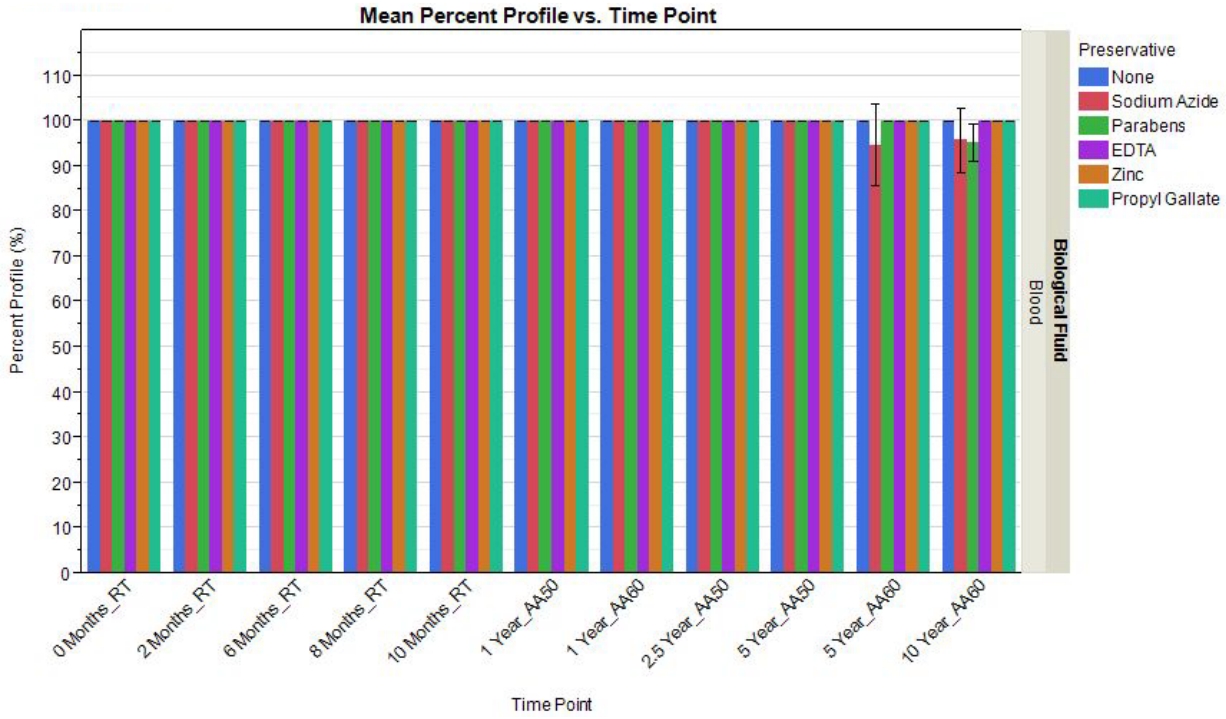

Figure 9: Mean percent profile (%) generated for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

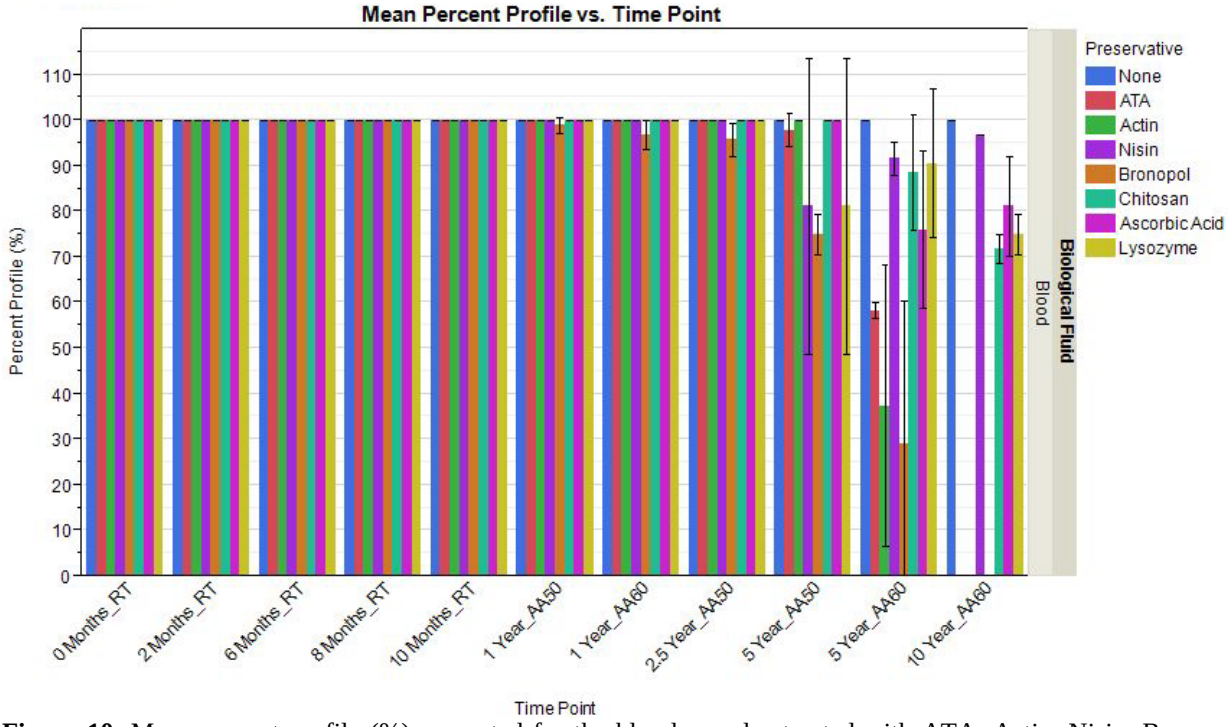

Figure 10: Mean percent profile (%) generated for the blood samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Saliva

The 10 year RT equivalent time point contains incomplete sample data due to an amplification issue. Up until the 2.5 year RT equivalent time point, the untreated control saliva samples produced full profiles. Decreasing percent profiles were generated at each time point thereafter.

The mean percent profile values for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 11. Full to high partial profiles were generated for all Sodium Azide treated, EDTA treated, and Zinc treated samples across all time points. For the Parabens treated saliva samples, full to high partial profiles were observed through the 5 year RT equivalent at 60°C time point, whereas decreasing percent profiles were generated at the 10 year RT equivalent time point. Until the 5 year RT equivalent at 50°C time point, the Propyl Gallate treated saliva samples generated full profiles. At the remaining time points, high partial profiles (greater than 60%) were generated. The following treated saliva samples demonstrated statistically significant increases in percent profile when compared to the untreated control samples: EDTA treated saliva samples at the 5 year RT equivalent at 50°C time point ($p = 0.0161$), Zinc treated saliva samples at the 5 year RT equivalent time points at 50°C and 60°C ($p = 0.0161$, $p = 0.0422$), and Propyl Gallate treated saliva samples at the 5 year RT equivalent at 50°C time point ($p = 0.0238$).

Figure 12 displays the mean percent profile results generated for the saliva samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The ATA treated and Actin treated saliva samples produced full and high partial profiles at the following

time points: 0 month, 2 month, 6 month, 10 month, 1 year RT equivalents at 50°C/ 60°C, and 2.5 year RT equivalent at 50°C time points. A reduction in average percent profile was observed at the 5 year RT equivalents at 50°C/ 60°C and 10 year RT equivalent time points. Up until the 2.5 year RT equivalent time point, the Nisin treated samples produced full profiles. Decreasing percent profiles were generated at each time point thereafter. Beginning at the 6 month time point, decreasing percent profiles were generated for the samples treated with Bronopol. For the Chitosan treated, Ascorbic Acid treated, and Lysozyme treated saliva samples, full profiles were observed through the 1 year RT equivalent at 60°C time point after which decreasing percent profiles were generated. When compared to the untreated control samples, the Actin treated and Chitosan treated saliva samples demonstrated statistically significant increases in percent profile at the 5 year RT equivalent at 50°C time point ($p = 0.0494$, $p = 0.0494$).

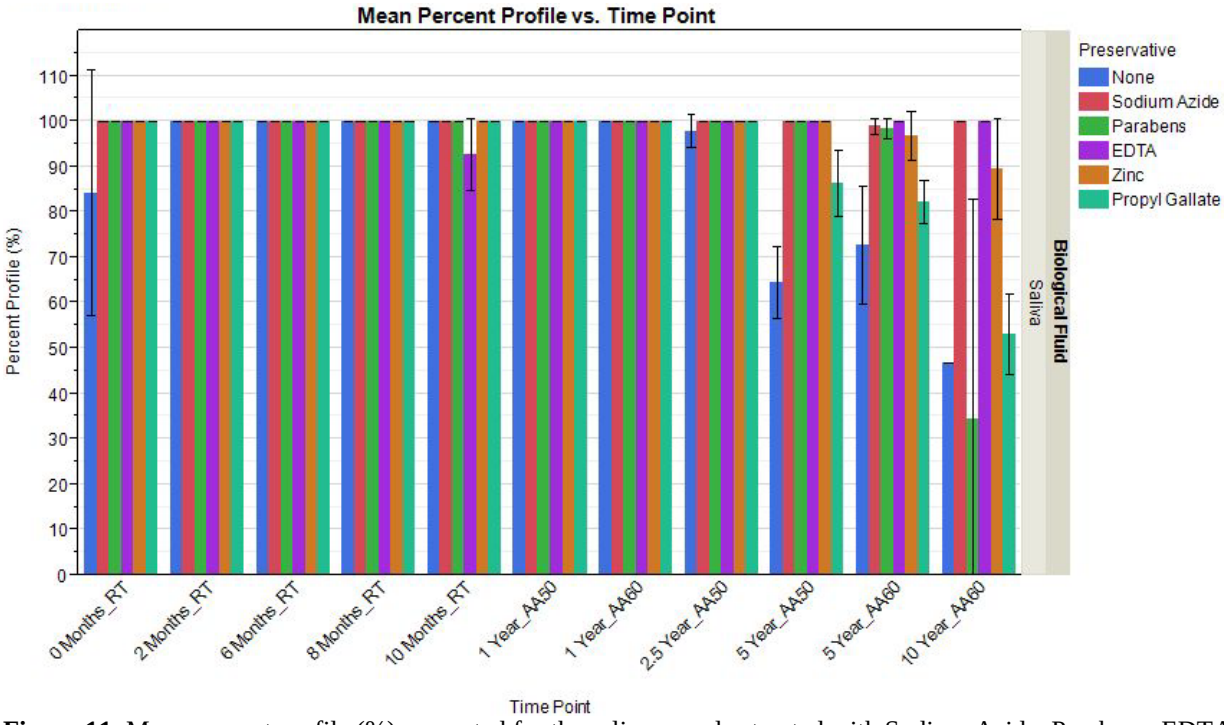

Figure 11: Mean percent profile (%) generated for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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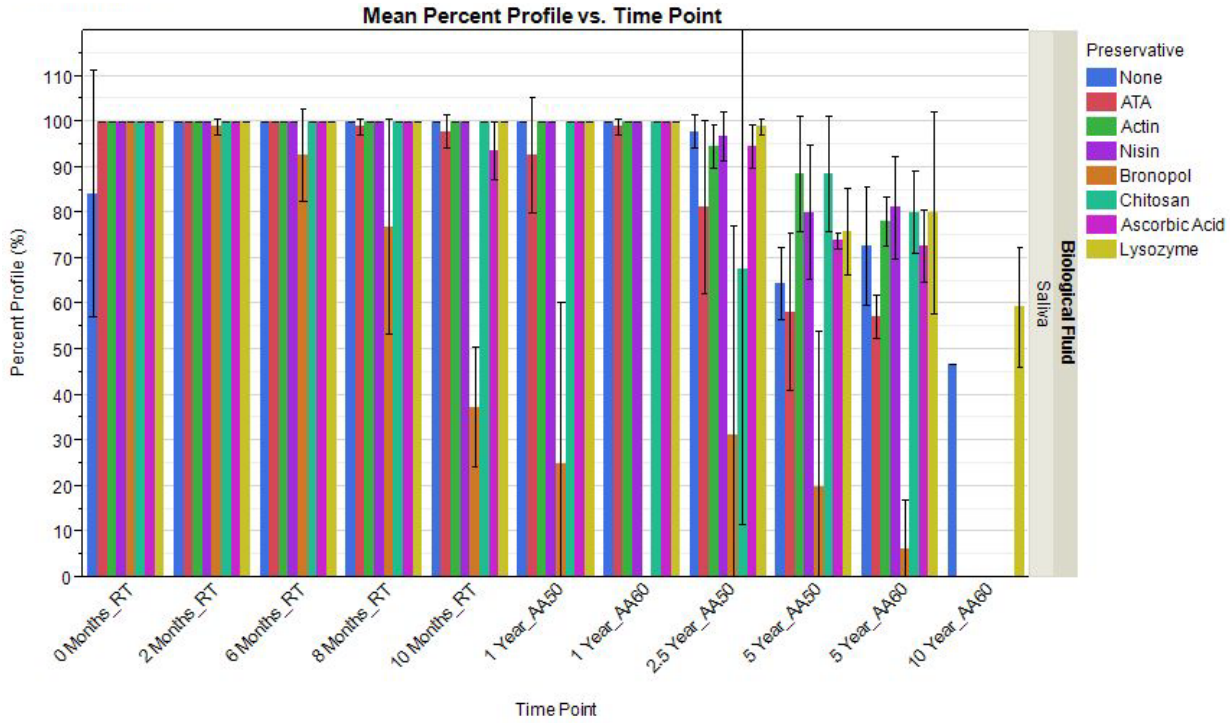

Figure 12: Mean percent profile (%) generated for the saliva samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Semen

Full profiles were observed for all untreated control semen samples across all time points.

The mean percent profile values for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 13. Across all time points, full to high partial profiles were achieved for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, or Propyl Gallate.

Figure 14 displays the mean percent profile results generated for the semen samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Across all time points, the ATA treated, Actin treated, Nisin treated, Chitosan treated, and Ascorbic Acid treated samples generated full to high partial profiles. Partial profiles at the 1 year (60°C) and 5 year RT equivalent time points were generated for the semen samples that were treated with Bronopol. Decreasing percent profiles were observed for the Lysozyme treated semen samples beginning at the 5 year RT equivalent at 60°C time point.

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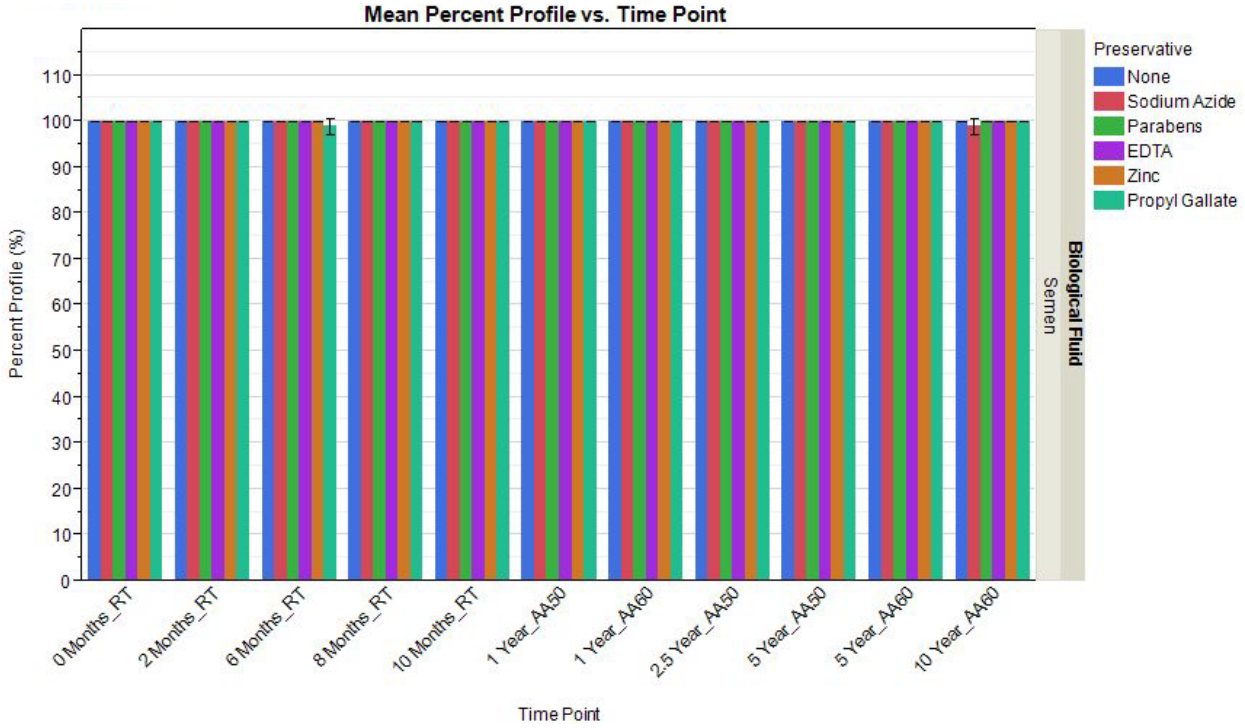

Figure 13: Mean percent profile (%) generated for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

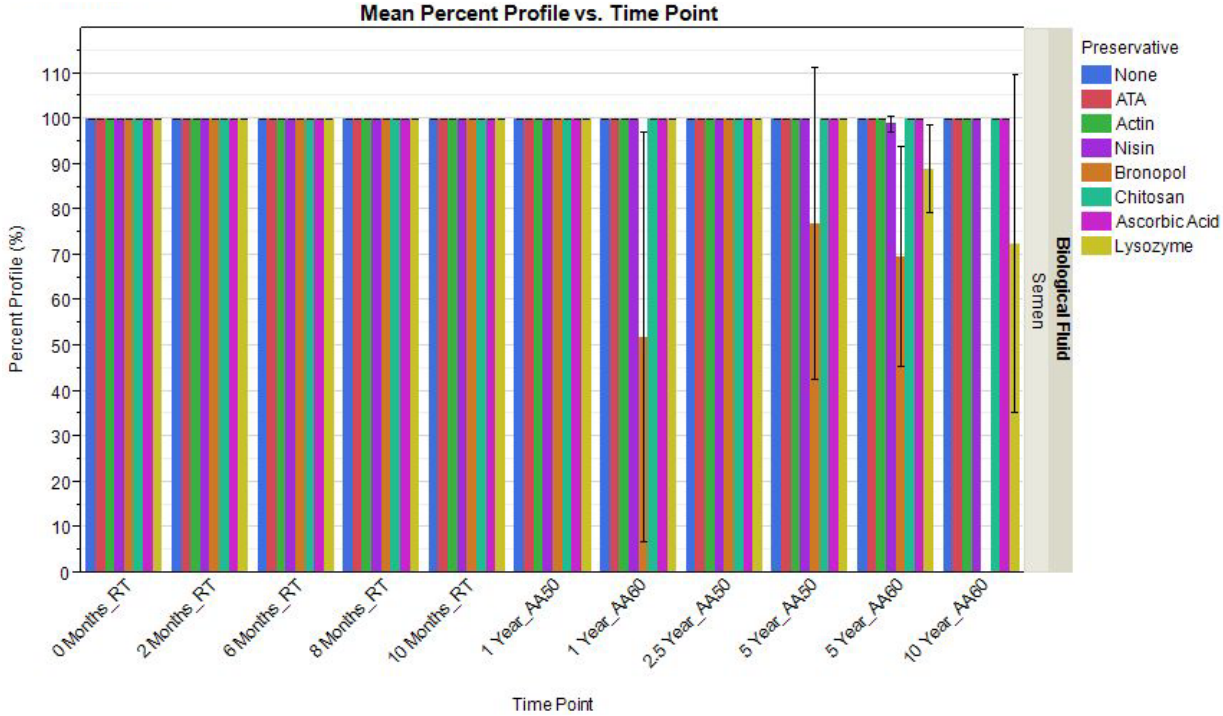

Figure 14: Mean percent profile (%) generated for the semen samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Vaginal Fluid

For the 0 month time point through the 2.5 year RT equivalent time point, the untreated control vaginal fluid samples produced full and high partial profiles. A reduction in percent profile was observed at the subsequent 5 and 10 year RT equivalent time points.

The mean percent profile values for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 15. Full to high partial profiles were observed across all time points for the Sodium Azide treated, EDTA treated, and Zinc treated vaginal fluid samples. For the Parabens treated samples, full profiles were generated through the 2.5 year RT equivalent time point. The Propyl Gallate treated vaginal fluid samples produced full and high partial profiles at all time points except for the 5 year RT equivalent at 50°C. The following treated vaginal fluid samples demonstrated an increase in percent profile when compared to the untreated control samples: Sodium Azide treated vaginal fluid samples at the 5 year RT equivalent at 50°C time point ($p = 0.0002$), EDTA treated vaginal fluid samples at the 5 year RT equivalent at 50°C time point ($p = 0.0032$), and Zinc treated vaginal fluid samples at the 5 year RT equivalent at 50°C time points ($p = 0.0032$).

Figure 16 displays the mean percent profile results generated for the vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The ATA treated and Actin treated vaginal fluid samples produced full and high partial profiles at all RT time points, the 1 year RT equivalent at 50°C/60°C, and the 2.5 Year RT equivalent time points with one exception. Percent profiles less than 50% were generated for two out of three ATA treated samples at the 1 year RT equivalent at 60°C time point. A reduction in percent profile was observed at the 5 year RT equivalent at 50°C and 60°C time points. Full DNA profiles were generated for the Nisin treated samples up until the 10 month time point. For all time points after 10 months, a wide range of partial profiles were generated. Decreasing percent profiles were observed for the Bronopol treated vaginal fluid samples beginning at the 6 month time point. Full profiles were generated for all Chitosan treated vaginal fluid samples until the 8 month time point. A decrease in overall percent profile was observed at each time point thereafter. Full profiles were generated for all Ascorbic Acid treated and Lysozyme treated vaginal fluid samples until the 10 month time point. A decrease in overall percent profile was generated at each time point thereafter.

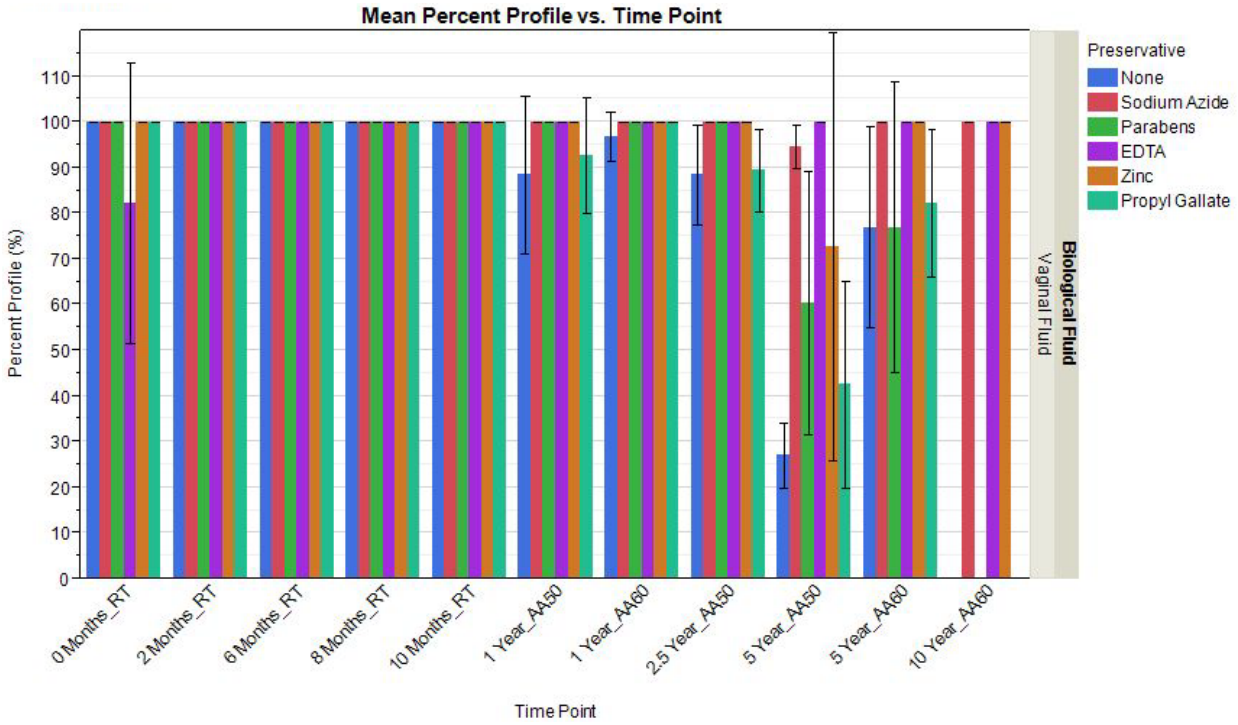

Figure 15: Mean percent profile (%) generated for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

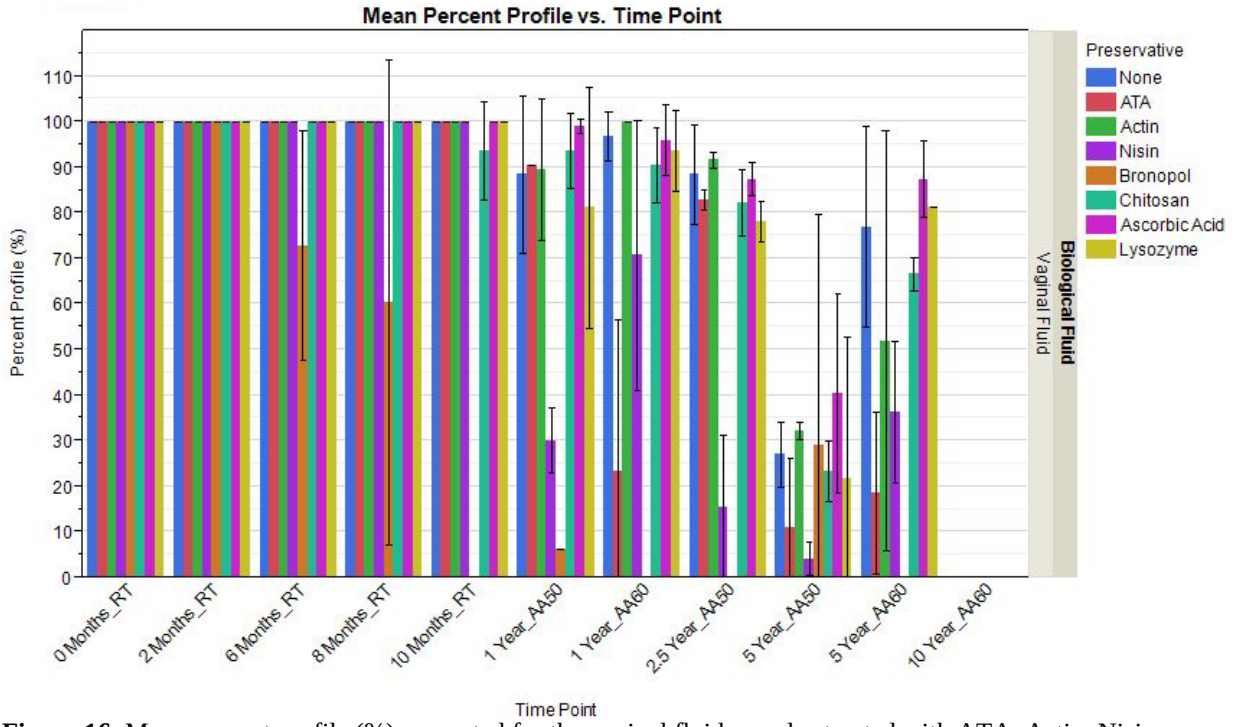

Figure 16: Mean percent profile (%) generated for the vaginal fluid samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Mean Peak Height Values

When compared to the control, statistically significant differences in peak height values were generated by the following samples: blood samples treated with Parabens and Propyl Gallate; saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate; and vaginal fluid samples treated with Sodium Azide, EDTA, Zinc, and Propyl Gallate (Table 8). No statistically significant differences in percent profile were observed between any of the preservative treated semen samples and the control.

Table 8: Summary of the statistically significant peak height values for each preservative and fluid from Phase I. Statistically significant peak height when compared to control is represented as "+", no effect or decrease in peak height when compared to the control is represented as "-".

Preservative	Blood	Saliva	Semen	Vaginal Fluid
Sodium Azide		+		+
Parabens	+	+	-	-
EDTA	-	+	-	+
Zinc	-	+	-	+
Propyl Gallate	+	+	-	+
ATA	-	-	-	-
Actin	-	-	-	-
Nisin	-	-	-	-
Bronopol	-	-	-	-
Chitosan	-	-	-	-
Ascorbic Acid	-	-	-	-
Lysozyme	-	-	-	-

Blood

Across the 0 month, 2 month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, the untreated control blood samples generated a mean average peak height (PH) value of approximately 3200 RFU. At the subsequent times points, the mean average PH value decreased to approximately 1600 RFU.

The mean peak height values for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 17. Across the 0 month - 2.5 year RT equivalent time points, mean average PH values of 3000 RFU, 3500 RFU, 2800 RFU, 3300 RFU, and 4000 RFU were observed for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate, respectively. The mean average PH values for all subsequent time points ranged from 1500 – 1700 RFU. The blood samples treated with Sodium Azide, EDTA, and Zinc did not demonstrate any statistically significant increases in mean average peak height when compared to the untreated control samples. When compared to the untreated control samples, the Parabens treated samples and the Propyl Gallate treated samples generated statistically significant increases in average peak heights at the 2.5 year RT equivalent time points ($p = 0.0087$, $p = 0.0315$).

Figure 18 displays the mean PH results generated for the blood samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Across the 0 month, 2

month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, the blood samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme generated mean average PH values that ranged from 1000 - 3000 RFU. For all subsequent time points, the ATA treated, Actin treated, Nisin treated, Chitosan treated, Ascorbic Acid treated, and Lysozyme treated blood samples generated mean average PH values that ranged from 900 – 1100 RFU, whereas the Bronopol treated blood samples generated mean average PH values of 350 RFU. The blood samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, and Lysozyme did not demonstrate any statistically significant improvements in mean average peak height when compared to the untreated control samples; however, a statistically significant decrease in average peak heights was observed between the untreated control samples and the Ascorbic Acid treated samples at the 2.5 year RT equivalent time point ($p = 0.0086$). Statistically significant decreases were also observed across most of the time points for the Bronopol treated samples.

The blood samples at the 1 year RT equivalent time point at 60°C and the 2.5 year RT equivalent time point at 50°C generated higher average peak height values than those generated by the other time points. The cause for this is unknown.

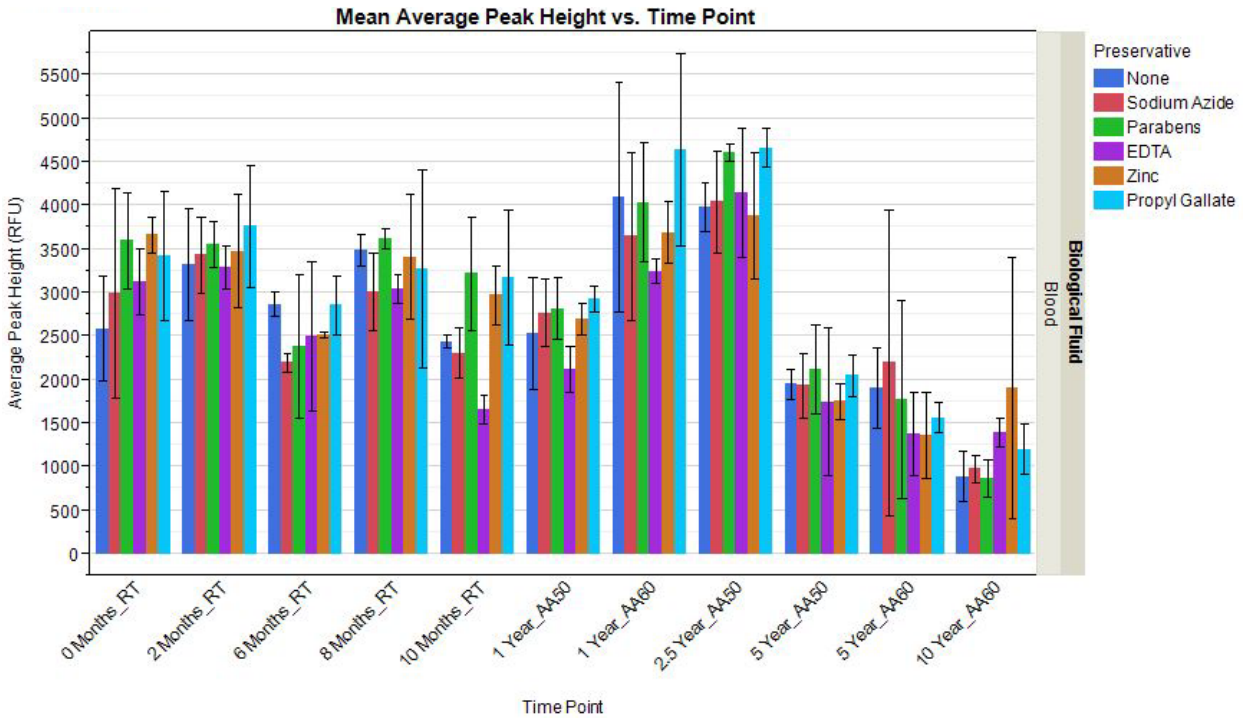

Figure 17: Mean average peak heights generated for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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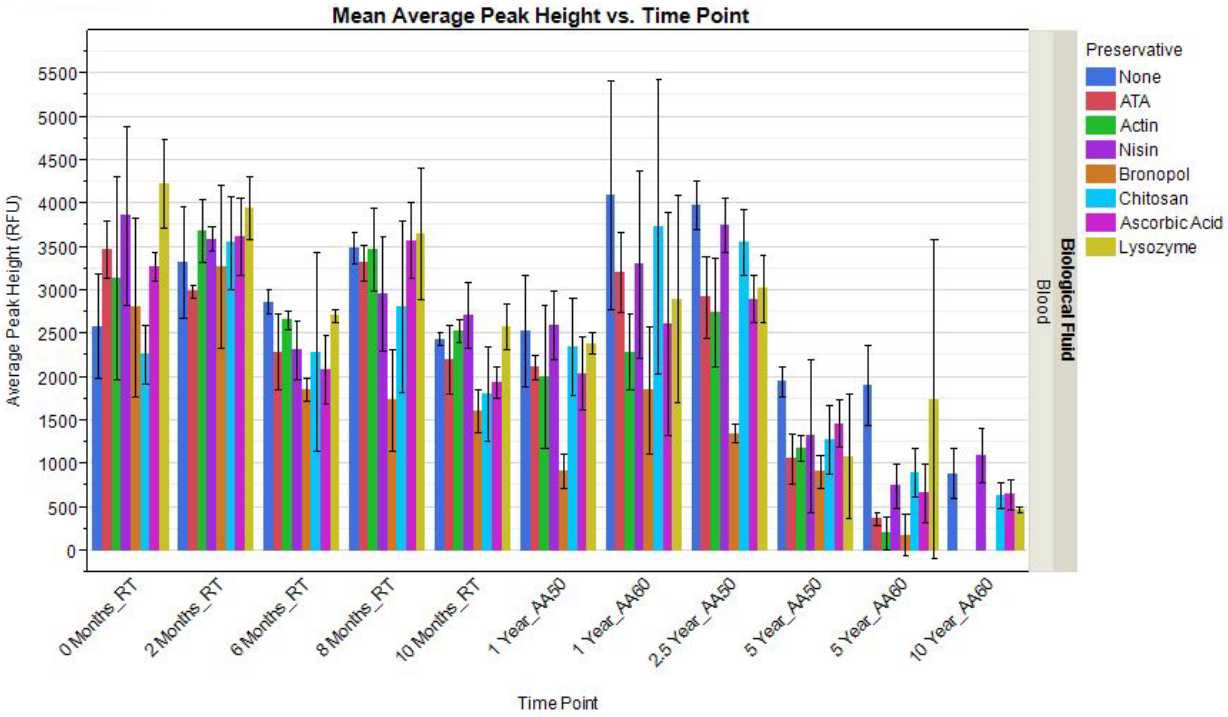

Figure 18: Mean average peak heights generated for the blood samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Saliva

Across the 0 month, 2 month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, the untreated control saliva samples generated a mean average peak height (PH) value of approximately 1500 RFU. At the subsequent times points, the mean average PH value decreased to approximately 300 RFU.

The mean peak height values for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 19. The mean average peak heights for the saliva samples treated with Sodium Azide varied across time points. Peak height values that ranged from 650 RFU – 3000 RFU were observed. The mean average peak heights for the saliva samples treated with Parabens varied across time points; however, mean average peak heights of 1800 RFU were generated prior to the 5 year RT equivalent time points. For all subsequent time points, the mean average PH for the Parabens treated saliva samples averaged 700 RFU. The mean average peak heights for the EDTA treated saliva samples ranged from 900 – 2800 RFU across all time points except for the 10 month time point. The mean average peak heights for the saliva samples treated with Zinc ranged from 900 – 2800 RFU across all time points until the 5 year RT equivalent at 60°C time point. At the 5 year RT equivalent at 60°C time point and the 10 year RT equivalent time point, mean average peak heights of 600 RFU were observed. From the 0 month time point to the 2.5 year RT equivalent time point, the Propyl Gallate treated saliva samples generated mean PH values of 1500 RFU. For all subsequent time points, the mean PH value was 500 RFU. Statistically significant increases in average peak heights were observed between the untreated controls and the following treated saliva samples: Sodium Azide treated saliva samples at the 5

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year RT equivalent time point at 50°C and 60°C ($p = 0.0018$, $p = 0.0288$), Parabens treated saliva samples at the 5 year RT equivalent at 50°C time point ($p = 0.0018$), EDTA treated saliva samples at the 5 year RT equivalent at 50°C time point ($p = 0.0008$), Zinc treated saliva samples at the 5 year RT equivalent at 50°C time point ($p = 0.03211$), and Propyl Gallate treated saliva samples at the 5 year RT equivalent at 50°C time point ($p = 0.0128$).

Figure 20 displays the mean PH results generated for the saliva samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The mean average peak heights for the ATA treated and Actin treated saliva samples at each time point leading up to the 2.5 year RT equivalent ranged from 500 – 2600 RFU. At the subsequent time points, the mean average PH values decreased to 300 – 500 RFU. The mean average peak heights for the Nisin treated saliva samples ranged from 900 – 2400 RFU across all time points until the 5 year RT equivalent at 60°C time point. Mean average peak heights of 550 RFU were generated for the remaining time points. For the Bronopol treated saliva samples, the mean average PH values decreased from 1800 RFU at the 0 month time point to 6 RFU at the 5 year RT equivalent at 60°C time point. Mean average peak heights of 1200 RFU, 1500 RFU, and 1450 RFU were generated for the Chitosan, Ascorbic Acid, and Lysozyme treated samples prior to the 5 year RT equivalent time points. The mean average PH for all subsequent time points averaged 700 RFU for the Chitosan treated samples, 460 RFU for the Ascorbic Acid treated samples, and 600 RFU for the Lysozyme treated samples. No statistically significant improvements in mean average peak heights were observed between the untreated controls samples and the saliva samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme; however, statistically significant deceases were observed across most time points for the Bronopol treated samples.

The saliva samples at the 1 year RT equivalent time point at 60°C and the 2.5 year RT equivalent time point at 50°C generated higher average peak height values than those generated by the other time points. The cause for this is unknown.

Figure 21 demonstrates the preservative effects of the Zinc fixative on a saliva sample that was stored at 50°C for 225 days (equivalent to 5 years of storage at room temperature). The Zinc treated saliva sample at the 5 year RT equivalent at 50°C time point generated peak height values that were comparable to those of an untreated control saliva sample at the 0 month time point.

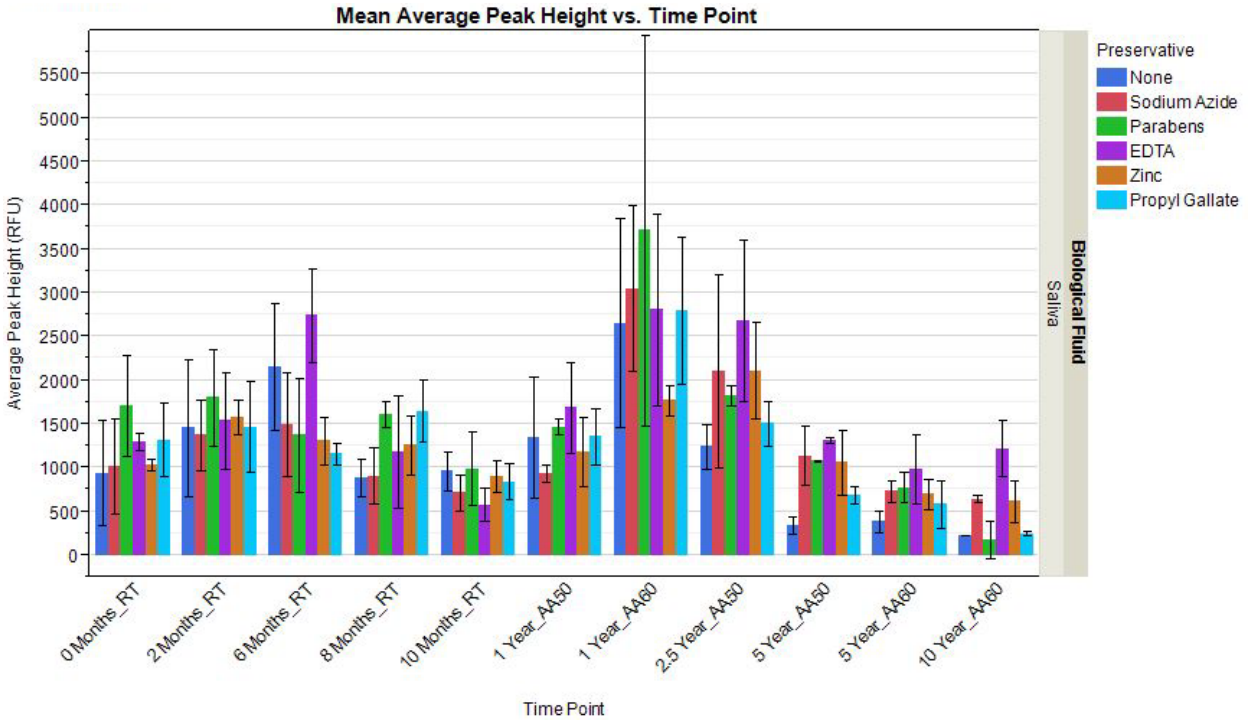

Figure 19: Mean average peak heights generated for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

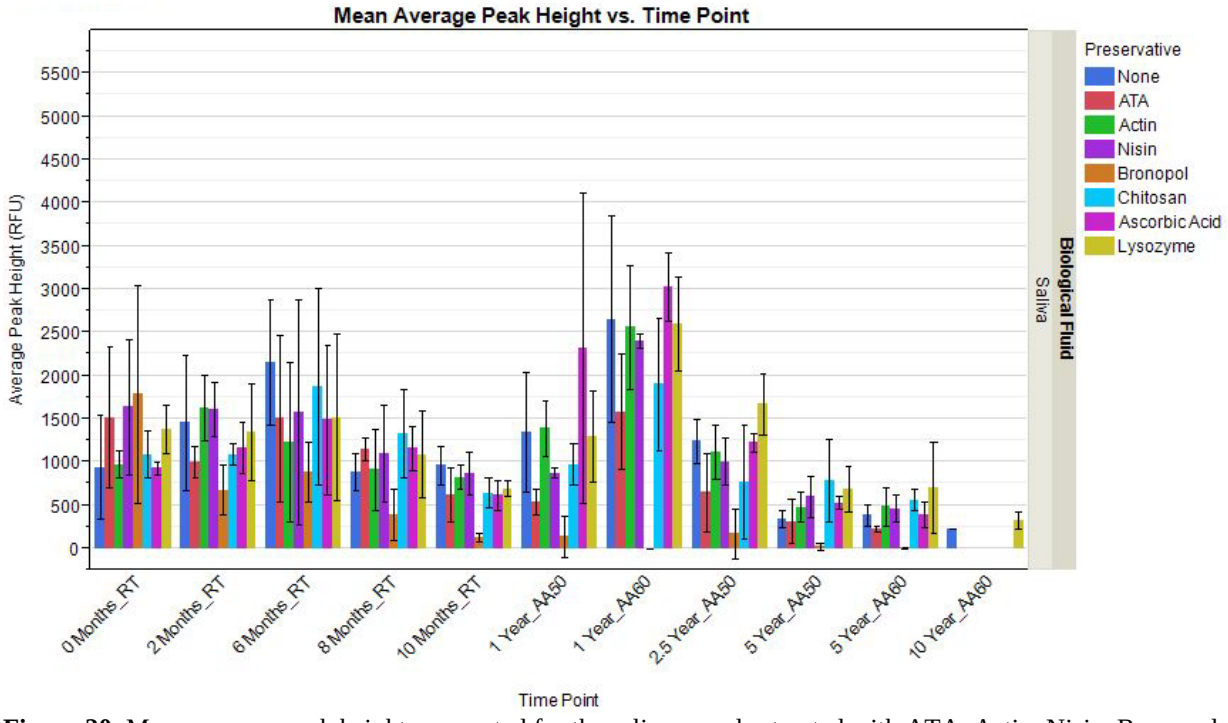

Figure 20: Mean average peak heights generated for the saliva samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

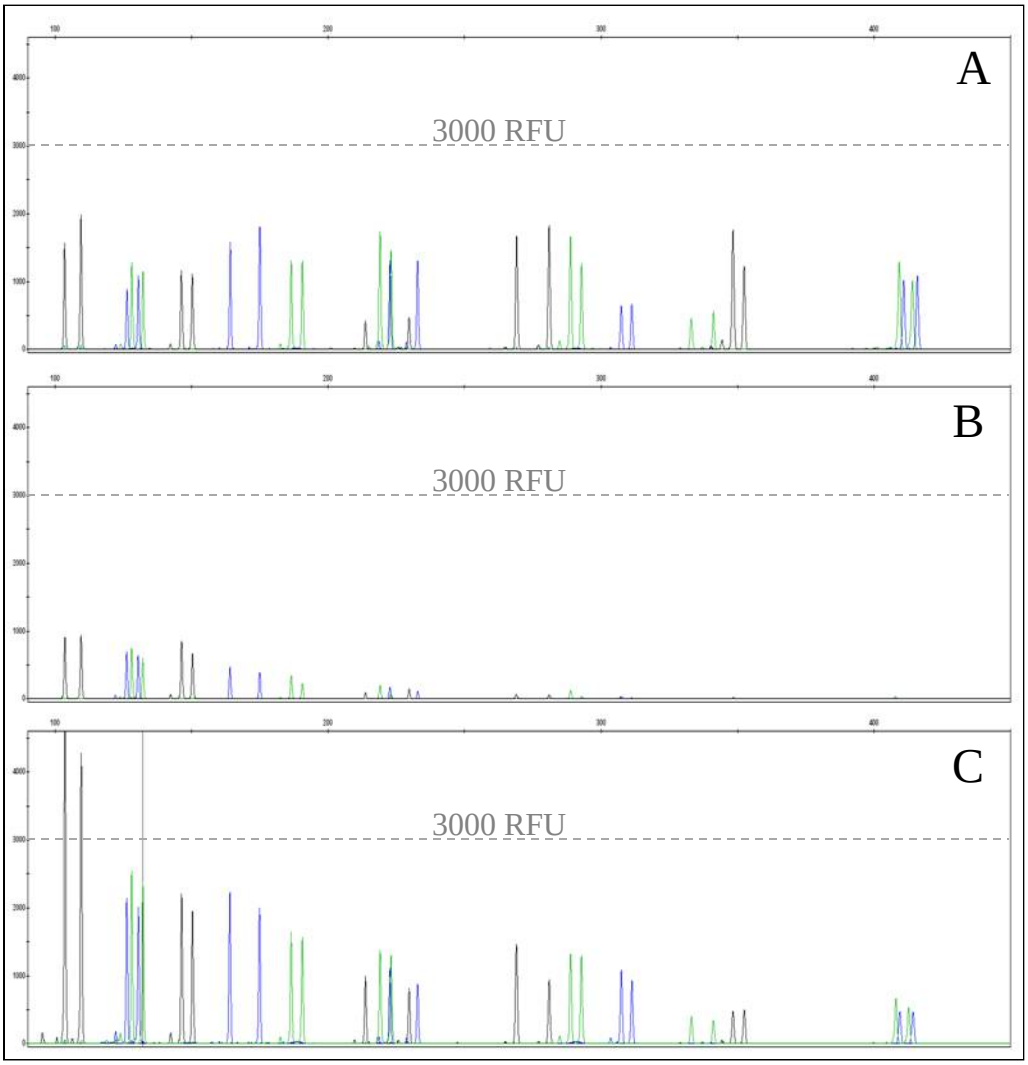

Figure 21: Representative electropherograms (EPGs) from three saliva samples tested during Phase I. (A) An untreated (control) saliva sample stored at RT for 0 months. (B) An untreated saliva sample that was stored at an accelerated temperature (AT) of 50°C for 225 days (equivalent to 5 years of RT storage). (C) A saliva sample that was treated with the fixative Zinc and stored at an AT of 50°C for 225 days (equivalent to 5 years of RT storage).

Semen

Across the 0 month, 2 month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, the untreated control semen samples generated a mean average peak height (PH) value of approximately 3000 RFU. At the subsequent times points, the mean average PH value decreased to approximately 1400 RFU.

The mean peak height values for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 22. Across the 0 month, 2 month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, mean average PH values that ranged from 2700 – 3300 RFU were observed for the semen samples treated with

Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. For all subsequent time points, the mean average PH values for the Sodium Azide treated, Parabens treated, Zinc treated, and Propyl Gallate treated semen samples ranged from 1300 – 1700 RFU, whereas the mean average PH for the EDTA treated samples was 2400 RFU. No statistically significant differences were observed between the untreated control semen samples and Sodium Azide treated, Parabens treated, EDTA treated, Zinc treated, and Propyl Gallate treated samples.

Figure 23 displays the mean PH results generated for the semen samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Across the 0 month, 2 month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, the semen samples treated with ATA, Actin, Nisin, Chitosan, Ascorbic Acid, and Lysozyme generated mean average PH values that ranged from 2700 - 3000 RFU, whereas the Bronopol treated semen samples generated mean average PH values of 1700 RFU. For all subsequent time points, the ATA treated, Actin treated, Nisin treated, Chitosan treated, and Ascorbic Acid treated blood samples generated mean average PH values that ranged from 1200 – 1800 RFU, whereas the Bronopol and Lysozyme treated blood samples generated mean average PH values of 550 RFU and 900 RFU, respectively. No statistically significant increases in peak height were observed between the untreated control semen samples and ATA treated, Actin treated, Nisin treated, Bronopol treated, Chitosan treated, Ascorbic Acid treated, and Lysozyme treated samples; however, statistically significant deceases were observed across most time points for the Bronopol treated samples.

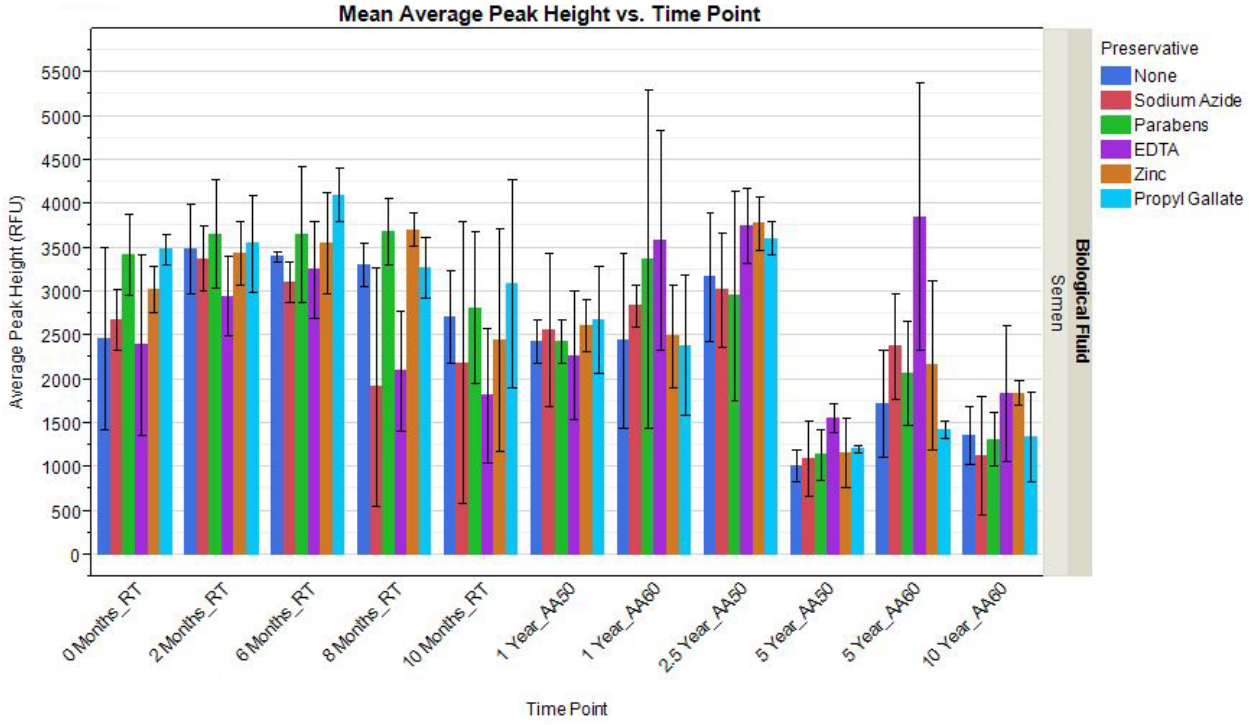

Figure 22: Mean average peak heights generated for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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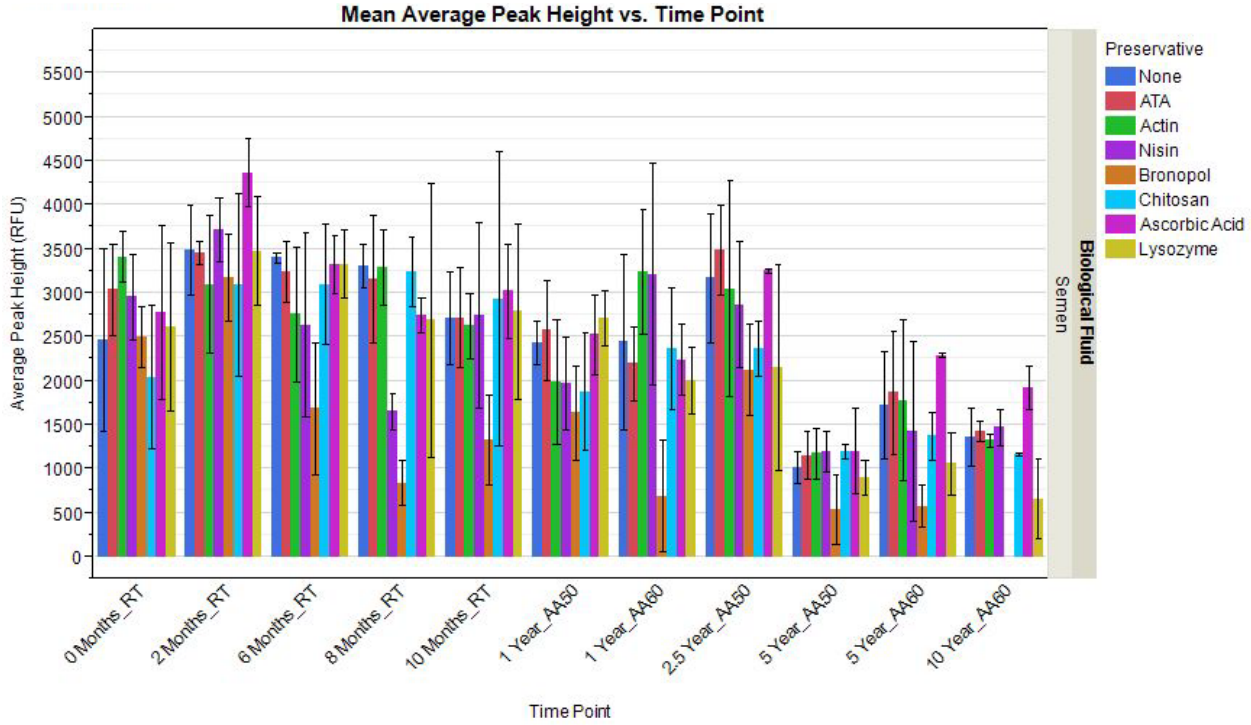

Figure 23: Mean average peak heights generated for the semen samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Vaginal Fluid

Across the 0 – 10 month time points, the untreated control vaginal fluid samples generated a mean average peak height (PH) value of approximately 2100 RFU. At the subsequent times points, the mean average PH value decreased to approximately 500 RFU.

The mean peak height values for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 24. The mean average peak height for the vaginal fluid samples treated with Sodium Azide averaged 2300 RFU at the 0 month, 2 month, 8 month, 10 month RT time points. For the subsequent time points, the mean average PH was 1100 RFU. At the 0 month, 2 month, 8 month, 10 month, 1 year RT equivalents at 50°C/60°C, and 2.5 year RT equivalent time points, the mean average PH values for the Parabens treated vaginal fluid samples averaged 1800 RFU. For the subsequent time points, the mean average PH was 700 RFU. Across all time points, the mean average PH values for the EDTA treated and Zinc treated vaginal fluid samples were 2000 RFU and 1700 RFU, respectively. Across the 0 month, 2 month, 8 month, 10 month, and 1 year RT equivalents at 50°C/60°C time points, the mean average peak heights for the vaginal fluid samples treated with Propyl Gallate was 2000 RFU. The mean average PH for all subsequent time points averaged 450 RFU. Statistically significant increases in average peak heights were observed between the untreated controls and the following treated saliva samples: Sodium Azide treated samples at the 6 month RT time point ($p = 0.0251$) and the 5 year RT equivalent at 50°C time point ($p = 0.0012$); EDTA treated samples at the 1 year RT equivalent at 60°C time point ($p = 0.0008$), the 2.5 year 50°C RT equivalent ($p = 0.0136$), and the 5 year RT

equivalent at 50°C (p = 0.00007); Zinc treated samples at the 1 year RT equivalent at 60°C time point (p = 0.0011) and the 5 year RT equivalent at 50°C (p = 0.0321); and Propyl Gallate treated samples at the 6 month time point (p = 0.0148).

Figure 25 displays the mean PH results generated for the vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Across the 0 - 10 month time points, mean average PH values of 1800 RFU, 2100 RFU, 1700 RFU, 800 RFU, and 1200 RFU were observed for the vaginal fluid samples treated with ATA, Actin, Nisin, Bronopol, and Chitosan, respectively. For the subsequent time points, mean average PH values of 200 RFU, 700 RFU, 100 RFU, 10 RFU, and 300 RFU were observed for the samples treated with ATA, Actin, Nisin, Bronopol, and Chitosan, respectively. Across the 0 month time point to the 1 year RT equivalent at 60°C time point, the Ascorbic Acid treated and Lysozyme treated vaginal fluid samples generated mean average PH values that ranged from 1700 – 2000 RFU. For the subsequent time points, the mean average PH values decreased to approximately 500 RFU. The vaginal fluid samples treated with Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme did not demonstrate any statistically significant differences in mean average peak heights when compared to those generated by the untreated control samples. Statistically significant decreases in peak heights were observed for the following preservatives when compared to the control samples: ATA treated samples at the 6 month time point (p = 0.0369) and 1 year RT equivalent at 50°C time point (p = 0.0282), and Actin treated samples at the 1 year RT equivalent at 60°C (p = 0.0127). Statistically significant decreases were also observed for the Bronopol treated samples across most of the time points.

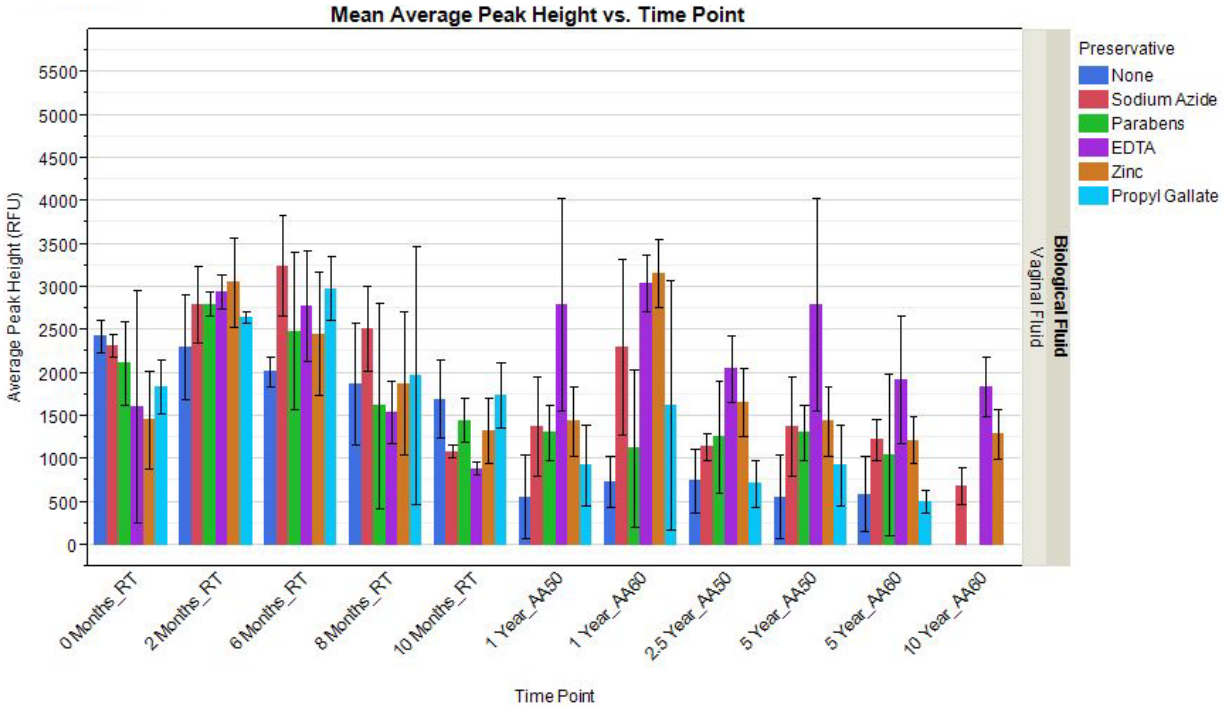

Figure 24: Mean average peak heights generated for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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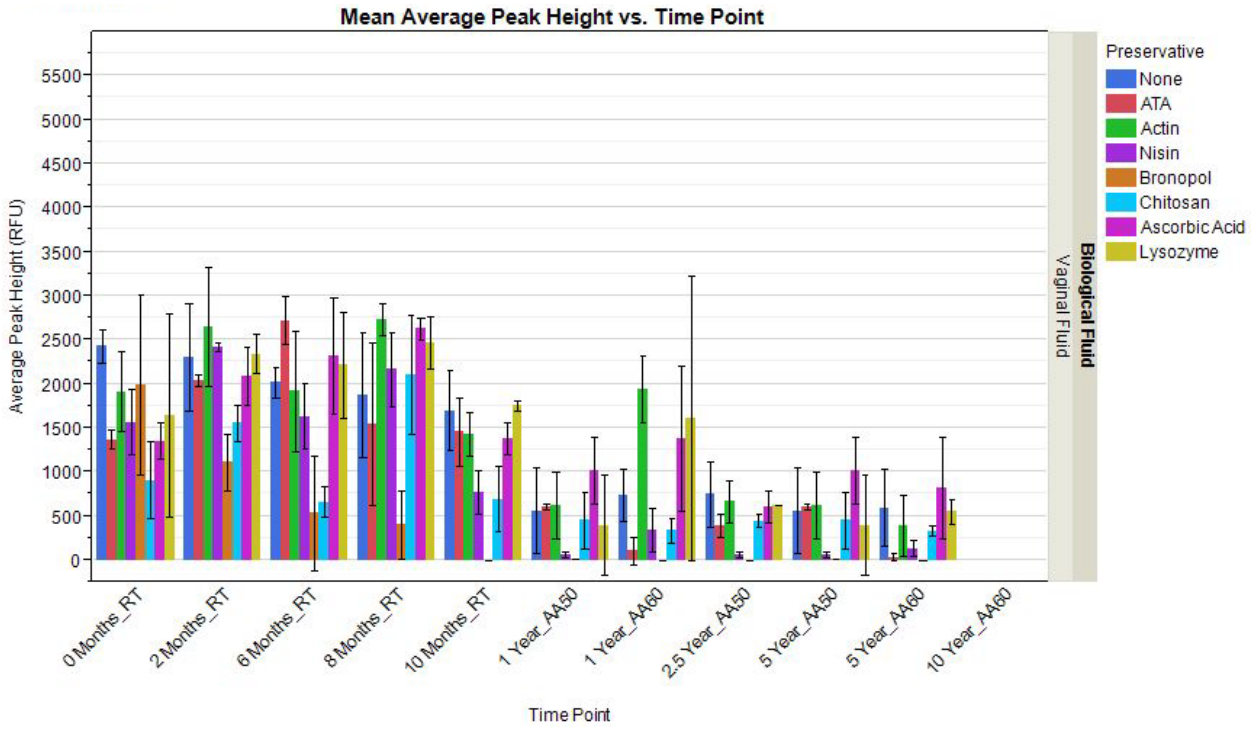

Figure 25: Mean average peak heights generated for the vaginal fluid samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Profile Balance Ratios

Profile balance ratios were only calculated for samples that generated full profiles.

Blood

For the blood samples, imbalanced profiles (Max PH/Min PH greater than 5.0) were generated at varying time points. On average, the untreated control blood samples generated balanced profiles up until the final two time points (5 year RT equivalent at 60°C and 10 year RT equivalent time points). Across the final two time points, a mean average profile balance ratio of 7.75 was observed.

The profile balance ratios for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 26. On average, the Sodium Azide treated, Parabens treated, Zinc treated, and Propyl Gallate treated blood samples generated balanced profiles up until the final two time points (5 year RT equivalent at 60°C and 10 year RT equivalent time points), whereas the EDTA treated samples generated balanced profiles until the 5 year RT

equivalent at 50°C time point. Across the balanced time points, all five preservatives generated mean average profile balance ratios that ranged from 2.65 – 2.81.

Figure 27 displays the profile balance ratios generated for the blood samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Profile imbalance was observed in the blood samples that were treated with ATA and Actin beginning at the 1 year RT equivalent at 60°C time point. On average, the Nisin treated, Chitosan treated, and Lysozyme treated samples generated balanced profiles up until the final three time points (5 year RT equivalents at 50°C/60°C and 10 year RT equivalent time points). Across the time points where balanced profiles were observed, the mean average profile balance ratios ranged from 2.73 - 3.41. Beginning at the 1 year RT equivalent at 50°C time point, profile imbalance was observed in the blood samples that were treated with Bronopol. The imbalanced Bronopol treated samples generated a mean average profile balance ratio of 56.61. The Ascorbic Acid treated blood samples generated balanced profiles with an average profile balance ration of 2.82 through the one year RT equivalent at 50°C time point. The mean average profile balance ratio for the remaining time points increased to 13.95.

Overall, the Zinc treated, Parabens treated, EDTA treated, and Propyl Gallate treated blood samples generated more balanced profiles than the untreated control samples (Table 9). These preservatives also generated the same number of full profiles at the untreated control samples. The samples treated with the remaining preservatives generated fewer balanced and full profiles than the untreated control samples. The fewest balanced profiles and full profiles were observed from the Bronopol treated samples.

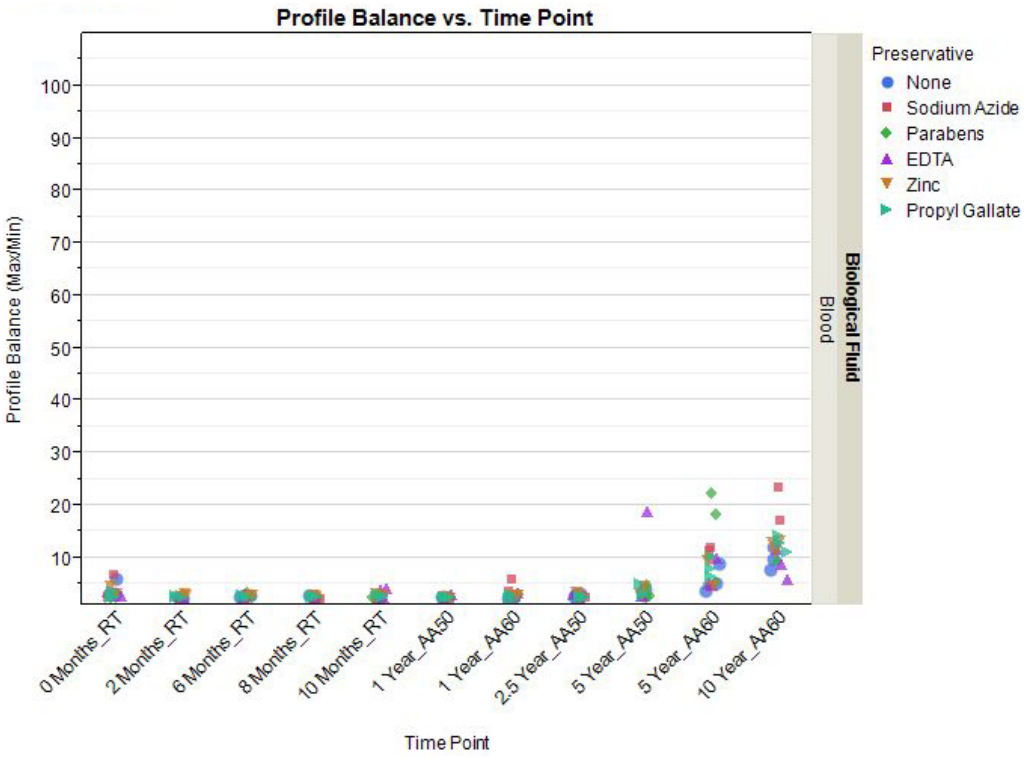

Figure 26: Overall profile balance generated for the blood samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None."

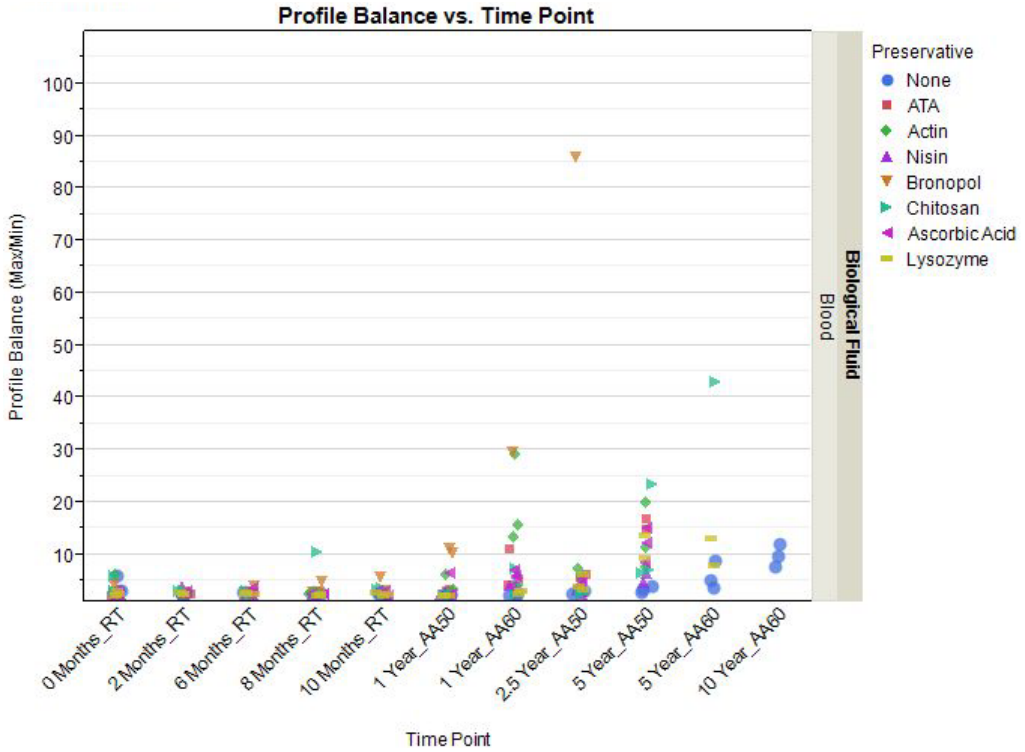

Figure 27: Overall profile balance generated for the blood samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None."

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Table 9: Total number of balance, imbalanced, and no/partial profiles generated by the treated and untreated blood samples

Biological Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/Partial
Blood	Zinc	33	28	5	0	84.8%	15.2%	0.0%
	Parabens	44	37	7	0	84.1%	15.9%	0.0%
	EDTA	33	27	6	0	81.8%	18.2%	0.0%
	Propyl Gallate	33	27	6	0	81.8%	18.2%	0.0%
	None	33	27	6	0	81.8%	18.2%	0.0%
	Sodium Azide	33	25	6	2	75.8%	18.2%	6.1%
	Nisin	33	24	7	2	72.7%	21.2%	6.1%
	Lysozyme	33	23	5	5	69.7%	15.2%	15.2%
	ATA	33	23	3	7	69.7%	9.1%	21.2%
	Actin	33	22	5	6	66.7%	15.2%	18.2%
	Chitosan	33	21	7	5	63.6%	21.2%	15.2%
	Ascorbic Acid	33	20	8	5	60.6%	24.2%	15.2%
	Bronopol	33	14	7	12	42.4%	21.2%	36.4%

Saliva

For the saliva samples, imbalanced profiles (Max PH/Min PH greater than 5.0) were generated at varying time points. The profile balance ratios obtained for the untreated control saliva samples were not consistent, with balanced profiles observed at 0 months, 6 months, and 10 months. Although balanced profiles were observed at the aforementioned time points, the mean average profile balance ratio across all time points for the untreated saliva samples was 10.03, suggesting imbalanced profiles.

The profile balance ratios for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 28. Across all time points, the mean average profile balance ratio generated for the Sodium Azide treated saliva samples was 9.50. On average, balanced profiles were observed at only the 2 month and 1 year RT equivalent at 60°C time points. Balanced profiles were observed from the Parabens treated saliva samples until the 2.5 year RT equivalent time point. Across the 2.5 year RT equivalent and subsequent time points, the mean average profile balance ratio increased from 3.98 to 24.49. The profile balance ratios obtained for the EDTA treated saliva samples were not consistent, with balanced profiles observed at 0 months, 6 months, 1 year RT equivalent at 50°C, 1 year RT equivalent at 60°C, 2.5 year RT equivalent, and 5 year RT equivalent at 50°C. The average profile balance ratio across all time points for the EDTA treated saliva samples was 6.67, suggesting imbalanced profiles. Until the 2.5 year RT equivalent time point, the average profile balance ratio for all Zinc treated saliva samples was 5.0, but the ratio increased to 17 across the remaining time points. On average, the profile balance ratios observed from the Propyl Gallate treated saliva samples indicated profile imbalance. Across all time points, the mean average profile balance ratio was 9.86.

Figure 29 displays the profile balance ratios generated for the saliva samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Across all time points, profile balance ratios of 24.33 and 31.67 were observed from the ATA treated and Bronopol treated saliva samples. Profile imbalance was observed at every time point. The Actin treated and Nisin treated saliva samples displayed profile imbalance across every time point except for the 2 month

time point. Overall, the mean average profile balance ratios were 12.11 and 15.86, respectively. At the 8 month time point and beyond, the Chitosan treated and Lysozyme treated samples displayed imbalanced profiles with mean average profile balance ratios of 11.88 and 20.52 across all time points. Imbalanced profiles were observed across all time points for the Ascorbic Acid treated saliva samples, which generated a mean average profile balance ratio of 10.47.

Overall, the EDTA treated, Actin treated, Parabens treated, Zinc treated, Lysozyme treated, and Chitosan treated saliva samples generated more balanced profiles than the untreated control samples (Table 10). The ATA treated saliva samples generated equivalent numbers of balanced and unbalanced profiles as the untreated control samples. The samples treated with the remaining preservatives generated fewer balanced profiles than the untreated control samples. The ATA treated samples generated the same number of full profiles as the control samples. Fewer full profiles were observed for the Bronopol treated and Ascorbic Acid treated samples. The Bronopol treated saliva samples generated the fewest balanced profiles and full profiles. The remaining treated saliva sample types generated more full profiles than the untreated control samples.

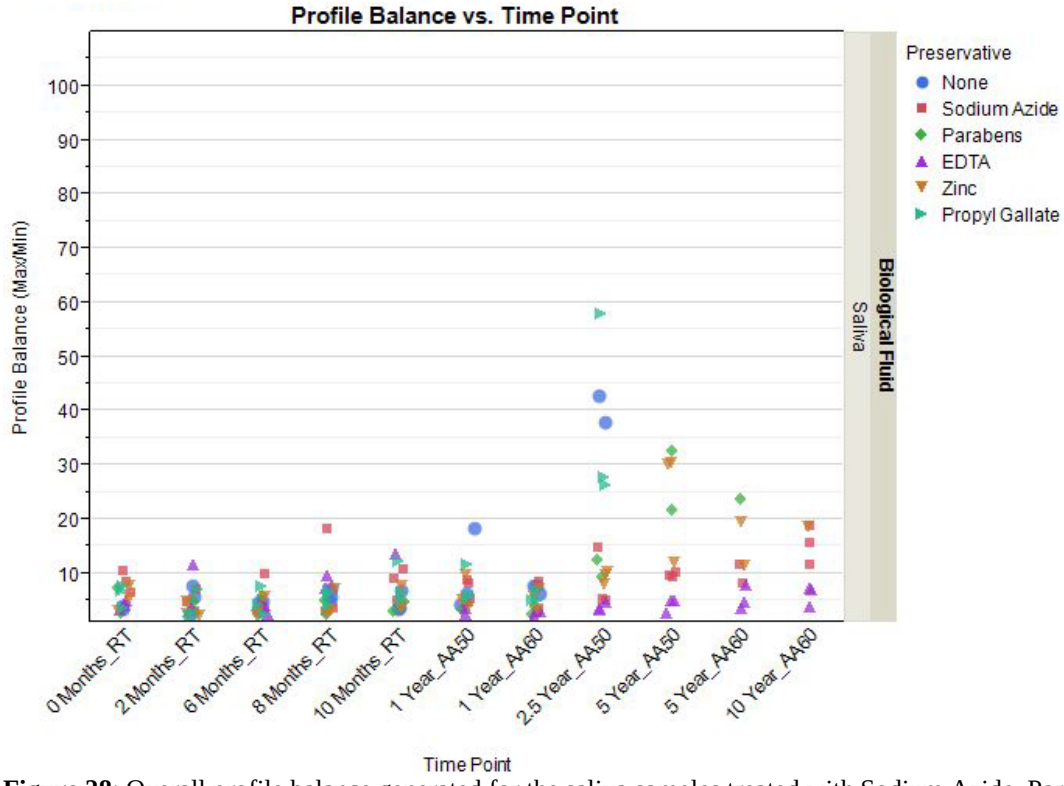

Figure 28: Overall profile balance generated for the saliva samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None."

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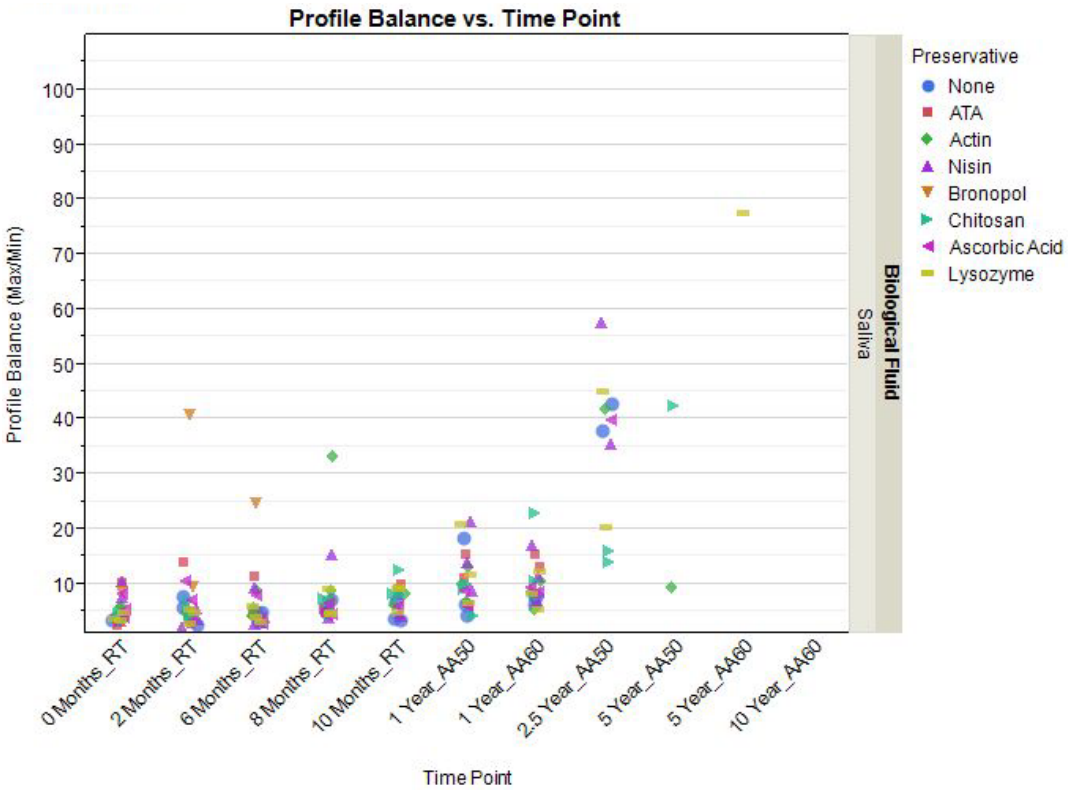

Figure 29: Overall profile balance generated for the saliva samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None."

Table 10: Total numbers of balanced, imbalanced, and no/partial profiles generated by the treated and untreated saliva samples

Biological Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/Partial
Saliva	EDTA	33	20	12	1	60.6%	36.4%	3.0%
	Actin	33	19	4	10	57.6%	12.1%	30.3%
	Parabens	22	11	9	2	50.0%	40.9%	9.1%
	Zinc	33	12	18	3	36.4%	54.5%	9.1%
	Lysozyme	33	10	15	8	30.3%	45.5%	24.2%
	Chitosan	33	10	14	9	30.3%	42.4%	27.3%
	ATA	33	10	12	11	30.3%	36.4%	33.3%
	None	33	10	12	11	30.3%	36.4%	33.3%
	Sodium Azide	33	9	24	0	27.3%	72.7%	0.0%
	Nisin	33	8	17	8	24.2%	51.5%	24.2%
	Propyl Gallate	33	8	16	9	24.2%	48.5%	27.3%
	Ascorbic Acid	33	6	14	13	18.2%	42.4%	39.4%
	Bronopol	33	2	6	25	6.1%	18.2%	75.8%

Semen

For the semen samples, imbalanced profiles (Max PH/Min PH greater than 5.0) were generated at varying time points. Balanced profiles were generated from all untreated control semen samples across all time points, except for the 5 and 10 year RT equivalents at 60°C where slight imbalance was observed. Across all time points, the mean average profile balance ratio was 4.98.

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The profile balance ratios for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 30. Across all time points, the Sodium Azide treated, Parabens treated, EDTA treated, Zinc treated, and Propyl Gallate treated semen samples generated mean average profile balance ratios that ranged from 4.00 – 4.86; however, imbalanced profiles were observed at varying time points for each preservative.

Figure 31 displays the profile balance ratios generated for the semen samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Balanced profiles were generated from all ATA treated, Actin treated, and Nisin treated semen samples across all time points, except for the 5 and 10 year RT equivalents at 60°C where slight imbalance was observed. The Bronopol treated semen samples generated a mean average profile balance ratio of 3.17 though the 6 month time point, after which the mean average profile balance ratio increased to 14.95. The Chitosan and Lysozyme treated semen samples generated full profiles up to the 2.5 year RT equivalent and 1 year RT equivalent at 60°C time points, respectively. The Ascorbic Acid treated semen samples generated a mean average profile balance ratio of 3.19 through the 2.5 year RT equivalent time point at 50°C, after which the mean average profile balance ratio increased to 10.14.

Overall, the ATA treated, EDTA treated, Actin treated, Zinc treated, Parabens treated, Propyl Gallate treated, and Nisin treated semen samples generated more balanced profiles than the untreated control samples (Table 11). The Sodium Azide treated semen samples generated equivalent numbers of balanced and imbalanced profiles as the untreated control samples. The samples treated with the remaining preservatives generated fewer balanced profiles than the untreated control samples. The EDTA treated, Actin treated, Zinc tread, Parabens treated, Sodium Azide treated, Ascorbic Acid treated, and Chitosan treated samples generated the same number of full profiles as the control samples. Fewer full profiles were observed for the remaining treated sample types. The fewest balanced profiles and full profiles were observed from the Bronopol treated samples.

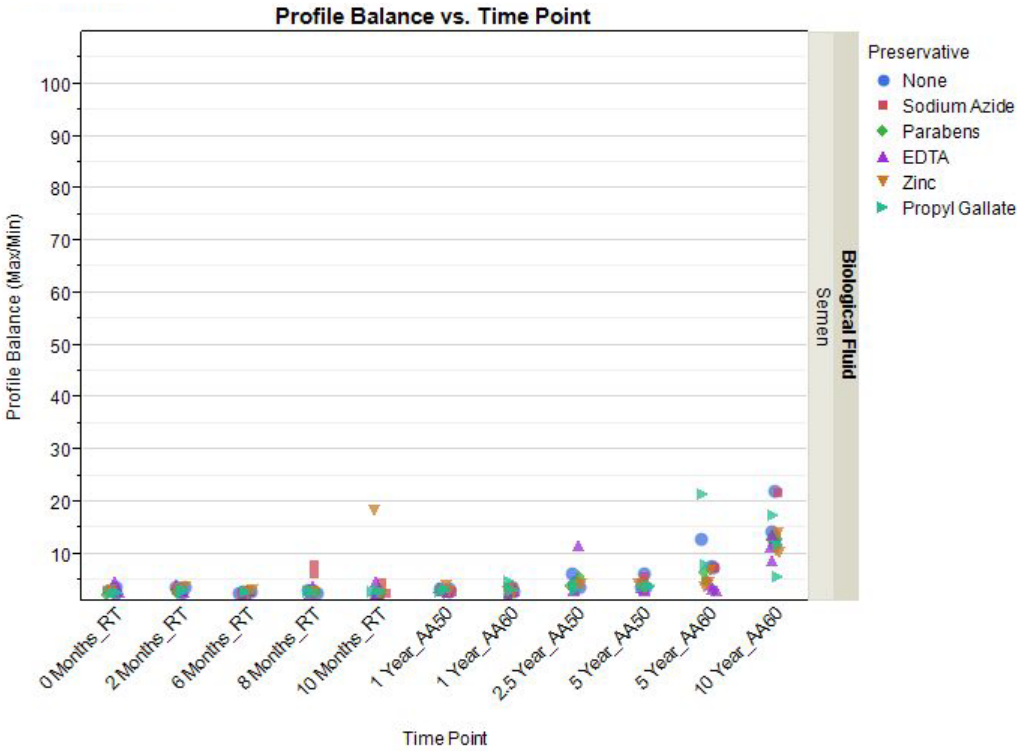

Figure 30: Overall profile balance generated for the semen samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None."

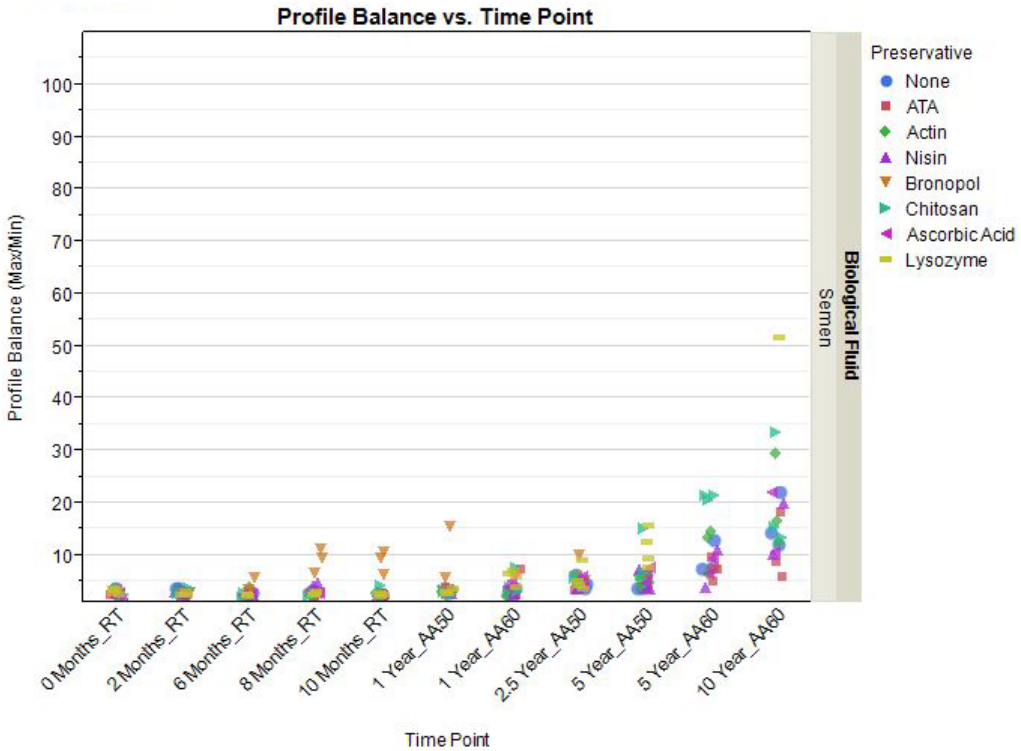

Figure 31: Overall profile balance generated for the semen samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None."

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Table 11: Total numbers of balanced, imbalanced, and no/partial profiles generated by the treated and untreated semen samples

Biological Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/Partial
Semen	ATA	44	42	1	1	95.5%	2.3%	2.3%
	EDTA	33	29	4	0	87.9%	12.1%	0.0%
	Actin	33	28	5	0	84.8%	15.2%	0.0%
	Zinc	33	28	5	0	84.8%	15.2%	0.0%
	Parabens	33	27	6	0	81.8%	18.2%	0.0%
	Propyl Gallate	33	27	5	1	81.8%	15.2%	3.0%
	Nisin	33	27	5	1	81.8%	15.2%	3.0%
	None	33	25	8	0	75.8%	24.2%	0.0%
	Sodium Azide	33	25	8	0	75.8%	24.2%	0.0%
	Ascorbic Acid	22	15	7	0	68.2%	31.8%	0.0%
	Chitosan	33	22	11	0	66.7%	33.3%	0.0%
	Lysozyme	44	27	13	4	61.4%	29.5%	9.1%
	Bronopol	33	9	15	9	27.3%	45.5%	27.3%

Vaginal Fluid

For the vaginal fluid samples, imbalanced profiles (Max PH/Min PH greater than 5.0) were generated at varying time points. For the untreated control vaginal fluid samples, the observed profiles were balanced through the 10 month time point. After the 10 month time point, the mean average profile balance ratio increased to 17.56.

The profile balance ratios for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate are displayed in Figure 32. Across the 0 month – 1 year RT equivalent at 60°C time points, the vaginal fluid samples treated with Sodium Azide generated a mean average profile balance ratios of 3.47. Beginning at the 2.5 year RT equivalent time point, the mean average profile balance ratio increased to 8.53. For the Parabens treated vaginal fluid samples, the observed profiles were balanced through the 10 month time point. After the 10 month time point, the mean average profile balance ratio increased to 14.24. For the EDTA treated vaginal fluid samples, the average profile balance ratio was 4.0 across all time points, suggesting balanced profiles. The average profile balance ratio for all Zinc treated vaginal fluid samples was 5.0 up until the 2.5 year RT equivalent time point, but the ratio increased to 13 across the remaining time points. For the 0 – 10 month time points, the Propyl Gallate treated vaginal fluid samples generated a mean average profile balance ratio of 2.57. Across the remaining time points, the mean average profile balance ratio increased to 22.59.

Figure 33 displays the profile balance ratios generated for the vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. Over time, few full profiles were generated from the ATA treated, Actin treated, Nisin treated, Chitosan treated, and Ascorbic Acid treated vaginal fluid samples, but for those profiles, overall profile imbalance was observed beginning at the 1 year RT equivalent at 50°C time point. Balanced profiles were observed at only the 0 month time point for the Bronopol treated vaginal fluid samples. The Lysozyme treated vaginal fluids produced balanced profiles through the 1 year RT equivalent at 50°C time point. After this time point, profile imbalance was observed in the only sample that generated a full profile. For the balanced time points, the mean average profile balance

ratios for the vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme ranged from 2.4 – 4.0. For the time points that demonstrated imbalance, the mean average profile balance ratios ranged from 5.5 – 21.3. Fewer full profiles were generated for the ATA treated, Actin treated, Nisin treated, Bronopol treated, Chitosan treated, Ascorbic Acid treated, and Lysozyme treated vaginal fluid samples than were generated for the other preservative treated samples. Due to this, no profile balance data were available at some time points. As a result, some of the mean average profile balance ratios for these samples appeared lower than the ratios calculated for the previously discussed preservatives.

Overall, the EDTA treated, Sodium Azide treated, Zinc treated, Actin treated, Lysozyme treated, Propyl Gallate treated, and Parabens treated vaginal fluid samples generated more balanced profiles than the untreated control samples (Table 12). The samples treated with the remaining preservatives generated fewer balanced profiles than the untreated control samples. The Ascorbic Acid treated samples and untreated control samples generated the same proportion of full profiles. The EDTA treated, Zinc treated, Sodium Azide treated, Parabens treated, and Propyl Gallate treated samples generated more full profiles than the untreated control samples. Fewer full profiles were generated from the Actin treated, Lysozyme treated, Chitosan treated, Nisin treated, ATA treated, and Bronopol treated samples than were generated by the untreated control samples. The fewest balanced profiles and full profiles were observed from the Bronopol treated samples.

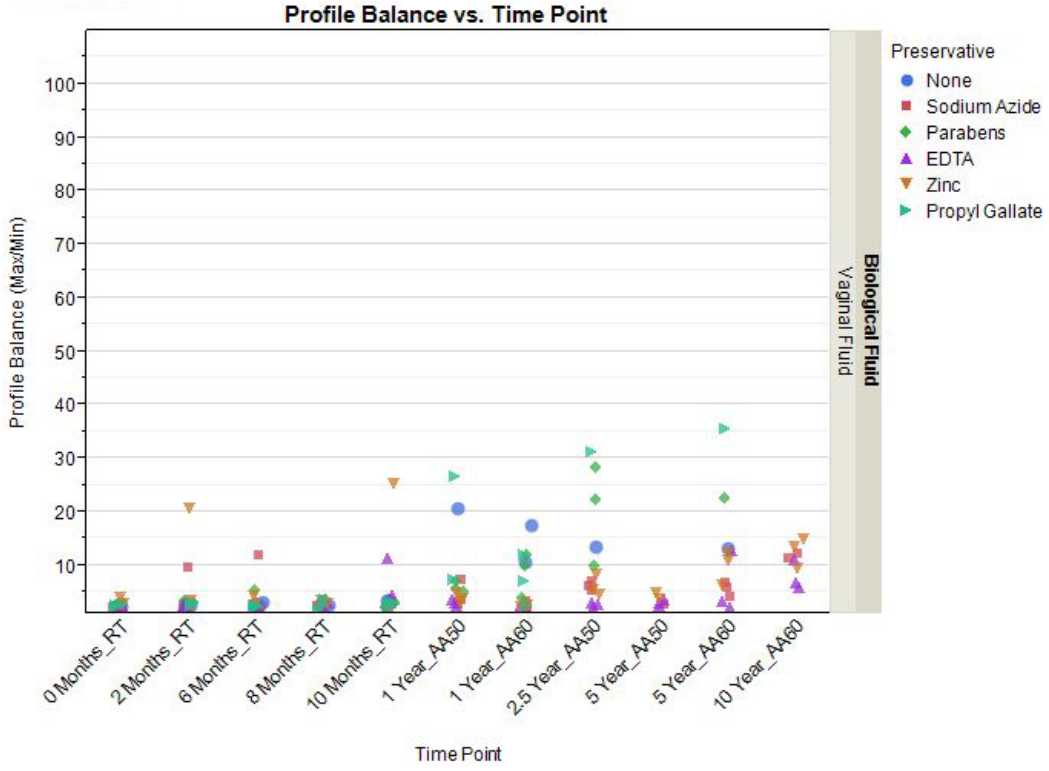

Figure 32: Overall profile balance generated for the vaginal fluid samples treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None."

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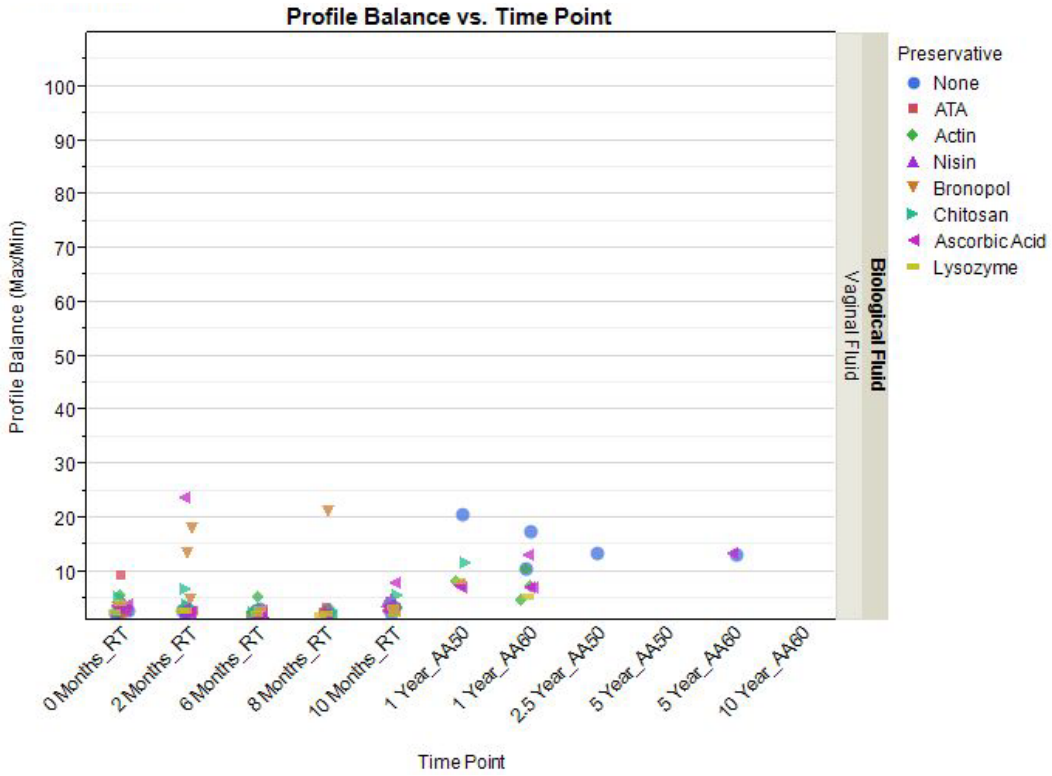

Figure 33: Overall profile balance generated for the vaginal fluid samples treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None."

Table 12: Total numbers of balanced, imbalanced, and no/partial profiles generated by the treated and untreated vaginal fluid samples

Biological Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/Partial
Vaginal Fluid	EDTA	33	26	6	1	78.8%	18.2%	3.0%
	Sodium Azide	33	19	11	3	57.6%	33.3%	9.1%
	Zinc	33	18	13	2	54.5%	39.4%	6.1%
	Actin	33	18	2	13	54.5%	6.1%	39.4%
	Lysozyme	22	12	1	9	54.5%	4.5%	40.9%
	Propyl Gallate	33	16	7	10	48.5%	21.2%	30.3%
	Parabens	33	15	10	8	45.5%	30.3%	24.2%
	None	33	15	6	12	45.5%	18.2%	36.4%
	ATA	22	10	0	12	45.5%	0.0%	54.5%
	Nisin	33	15	0	18	45.5%	0.0%	54.5%
	Ascorbic Acid	44	18	10	16	40.9%	22.7%	36.4%
	Chitosan	33	11	7	15	33.3%	21.2%	45.5%
	Bronopol	33	4	4	25	12.1%	12.1%	75.8%

Forensic Index

Calibration Data

The 0 month, 10 month, and 1 year RT equivalent at 50°C samples were used to provide data for the Forensic Index calibration set. For each sample in the calibration set, *TPH*, *MLB*, and *SH* were calculated using the aforementioned formulas. For all samples containing dropout, the MLB was

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considered to be 0.4. *TPH*, *MLB*, and *SH* values were then standardized (*tph*, *mlb*, *sh*) using the sample means and sample standard deviations (Table 13).

$$tph = \frac{TPH - \overline{TPH}}{\sigma_{TPH}}$$

$$mlb = \frac{MLB - \overline{MLB}}{\sigma_{MLB}}$$

$$sh = \frac{SH - \overline{SH}}{\sigma_{SH}}$$

Principal component analysis was then performed using StatistiXL 1.8 software, and component loading values (α_1, α_2, α_3) were obtained (Table 14). The following formula was used to combine *tph*, *mlb*, and *sh* into one single factor, the principal component (*pc*):

$$pc_i = a_1 * tph_i + a_2 * mlb_i + a_3 * sh_i\ ,\ i = 1,\ \ldots n$$

To rank each sample in the calibration set, the *pc* was plotted against a profile grading (*prg*) scale; however, the scale presented by Hedman was based on the 10 loci (AmpF*l*STR® SGM Plus® kit), whereas this study examined 15 STR loci (PowerPlex 16 kit). Because of this, Hedman's manual grading scale was adjusted by multiplying each interval range by 1.5 (Table 15). A *prg* was assigned to each sample in the calibration set. Next, the *d* score for each sample was calculated as follows:

$$d = \begin{cases} \dfrac{1 - MLB}{Range(MLB)} & \text{if } MLB > SH/\ln(10) \\ \dfrac{\ln(10) - SH}{Range(SH)} & \text{if } MLB \geq SH/\ln(10) \end{cases}$$

The *d* score was added to the *prg* for each sample. After this, the combined *prg* + *d* were adjusted to a 0.05 – 10 point scale, so that the samples with the lowest TPH had the lowest adjusted *prg* + *d*. At this point, the adjusted *prg* + *d* score was considered the forensic index (*FI*) ranking.

$$FI = \frac{20 - (prg + d)}{2}$$

Finally, the *pc* was plotted against the adjusted *prg* + *d* scale to obtain the equation of the line (Figure 34).

$$y = 0.7208x + 7.4052$$

Table 13: TPH, MLB, and SH means and standard deviations obtained for the calibration set.

Variable	Mean	Standard Deviation
TPH	45932.21	31796.43
MLB	0.865006	0.07351
SH	2.506644	0.257133

Table 14: TPH, MLB, and SH Eigenvalues and component loadings obtained from the PCA for the calibration set.

Variable	Eigenvalues	Component Loading (α)
TPH	2.445	0.823
MLB	0.464	0.962
SH	0.091	0.918

Table 15: Profile grading scales for AmpF*l*STR SGM Plus and PowerPlex 16.

AmpF*l*STR SGM Plus		**PowerPlex 16**	
Interval	**Profile Grade**	**Interval**	**Profile Grade**
50000 ≤ TPH	1	75001 ≤ TPH	1
40000 ≤ TPH < 50000	2	60001 ≤ TPH ≤ 75000	2
30000 ≤ TPH < 40000	3	45001 ≤ TPH ≤ 60000	3
25000 ≤ TPH < 30000	4	37501 ≤ TPH ≤ 45000	4
20000 ≤ TPH < 25000	5	30001 ≤ TPH ≤ 37500	5
15000 ≤ TPH < 20000	6	22501 ≤ TPH ≤ 30000	6
12500 ≤ TPH < 15000	7	18751 ≤ TPH ≤ 22500	7
10000 ≤ TPH < 12500	8	15001 ≤ TPH ≤ 18750	8
7500 ≤ TPH < 10000	10	11251 ≤ TPH ≤ 15000	10
5000 ≤ TPH < 7500	12	7501 ≤ TPH ≤ 11250	12
2500 ≤ TPH < 5000	14	3751 ≤ TPH ≤ 7500	14
1000 ≤ TPH < 2500	16	1501 ≤ TPH ≤ 3750	16
500 ≤ TPH < 1000	18	751 ≤ TPH ≤ 1500	18
0 ≤ TPH < 500	19	1 ≤ TPH ≤ 750	19
TPH = 0	20	TPH = 0	20

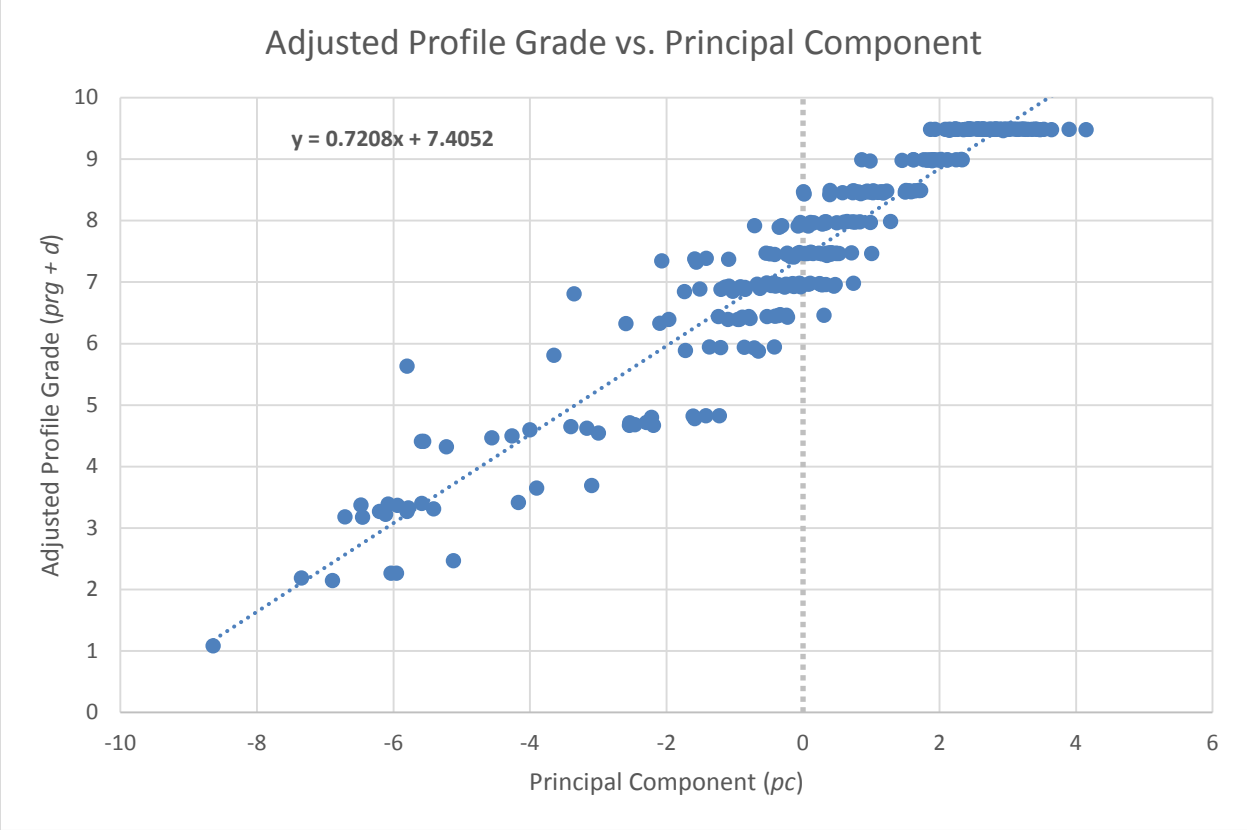

Figure 34: The *pc* values from the calibration set were plotted against the adjusted *prg + d* (Forensic Index) to obtain the equation of the line.

Phase I Sample Data

For each sample in Phase I, *TPH*, *MLB*, and *SH* were calculated using the aforementioned formulas. For all samples containing dropout, the MLB was considered to be 0.4. *TPH*, *MLB*, and *SH* values were then standardized (*tph*, *mlb*, *sh*) using the aforementioned equations and the sample means and sample standard deviations from the calibration set (Table 13). Principal components (*pc*) were calculated using *tph*, *mlb*, and *sh* values from each sample and the component loading values (α_1, α_2, α_3) from the calibration set (Table 14). Finally, to obtain the *FI*, the *pc* values were plotted against the equation of the line obtained from the calibration set. *FI* data from Phase I is listed in Appendix A.

FI values decreased over time for each biological fluid type and preservative (Figure 35 and Figure 36). As previously observed, the blood and saliva samples were the most stable over time. The following treated blood samples demonstrated statistically significant increases in *FI* when compared to the untreated control samples: Parabens treated blood samples at the 2.5 year RT equivalent at 50°C time points ($p = 0.02722$) and Propyl Gallate treated blood samples at the 2.5 year RT equivalent at 50°C time point ($p = 0.03876$). The following treated saliva samples demonstrated statistically significant increases in *FI* when compared to the untreated control samples: Sodium Azide treated saliva samples at the 5 year RT equivalents at 50°C/60°C time points ($p = 0.00086$, $p = 0.00389$); Parabens treated saliva samples at the 8 month, 2.5 year RT

equivalent at 50°C and 5 year RT equivalents at 50°C/60°C time points (p = 0.03752, p = 0.01175, p = 0.00712, p = 0.02991); EDTA treated saliva samples at the 2.5 year RT equivalent at 50°C and 5 year RT equivalents at 50°C/60°C time points (p = 0.00349, p = 0.00600, p = 0.00126); Zinc treated saliva samples at the 2.5 year RT equivalent at 50°C and 5 year RT equivalents at 50°C/60°C time points (p = 0.00689, p = 0.00414, p = 0.02753); and Propyl Gallate treated saliva samples at the 8 month and 5 year RT equivalent at 50°C time points (p = 0.01611, p = 0.02128). The following treated semen samples demonstrated statistically significant increases in *FI* when compared to the untreated control samples: EDTA treated semen samples at the 2.5 year RT equivalent at 50°C and 5 year RT equivalent at 50°C time points (p = 0.04423, p = 0.01980); Propyl Gallate treated semen samples at the 6 month time point (p = 0.04414); and Zinc treated semen samples at the 8 month time point (p = 0.04927). The following vaginal fluid treated samples demonstrated statistically significant increases in *FI* when compared to the untreated control samples: Sodium Azide treated vaginal fluid samples at the 1 year RT equivalent at 60°C and the 5 year RT equivalent at 50°C time points (p = 0.02407, p = 0.00216); EDTA treated vaginal fluid samples at the 1 year RT equivalent at 60°C, and 5 year RT equivalents at 50°C/60°C time points (p = 0.01935, p = 0.00381, p = 0.01813); Zinc treated vaginal fluid samples at the 1 year RT equivalent at 60°C time point (p = 0.00297); and Actin treated vaginal fluid samples at the 1 year RT equivalent at 60°C time point (p = 0.02728).

Electropherograms (EPGs) representing profiles that were assigned high, medium, and low FI rankings are depicted in Figure 37, Figure 38, and Figure 39. Figure 37 represents the full profile generated for an Actin treated blood sample at the 2 month time point. The FI ranking was 9.17, which indicated a high quality profile. The quantification value for this sample was 1.59 ng/µl, and the average peak height value was approximately 4100 RFU. The profile balance ratio was 2.68, indicating a well balanced profile. Figure 38 represents the full profile generated for an Actin treated blood sample at the 1 year RT equivalent at 60°C time point. The FI ranking was 6.21, which indicated a profile of moderate quality. The quantification value for this sample was 0.261 ng/µl, and the average peak height value was approximately 1900 RFU. The profile balance ratio was 19.49, indicating an imbalanced profile. Figure 39 represents a partial profile generated for an Actin treated blood sample at the 5 year RT equivalent at 60°C time point. The FI ranking was 1.78, which indicated a low quality profile. The quantification value for this sample was 0.037 ng/µl, and the average peak height value was approximately 200 RFU. Because this was a partial profile (62.5% profile), no profile balance ratio was calculated.

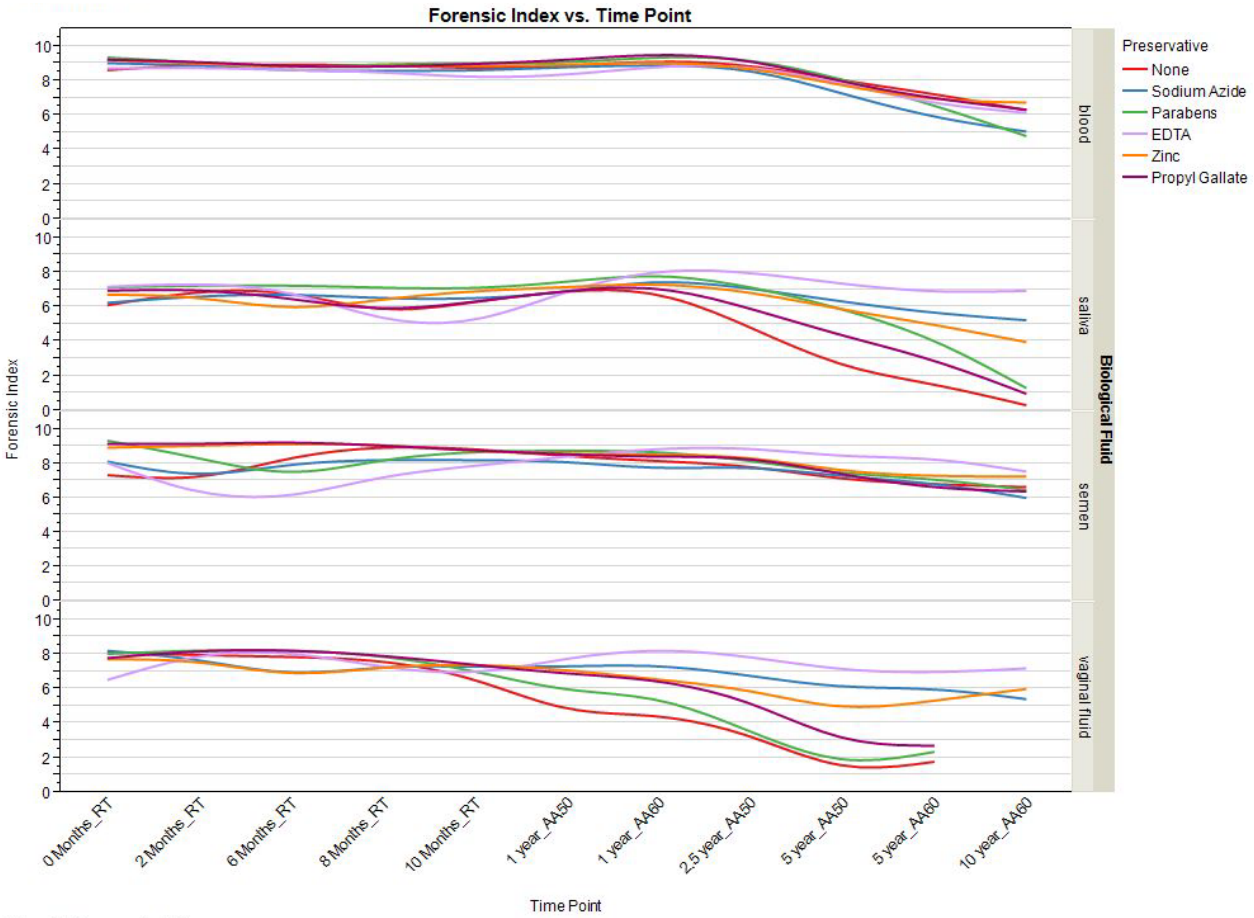

Figure 35: FI was plotted against time points for blood, saliva, semen, and vaginal fluid samples that were treated with Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate. The untreated control samples are represented as "None." For some samples, 10 year RT equivalent at 60°C time point data was unavailable due to an amplification issue.

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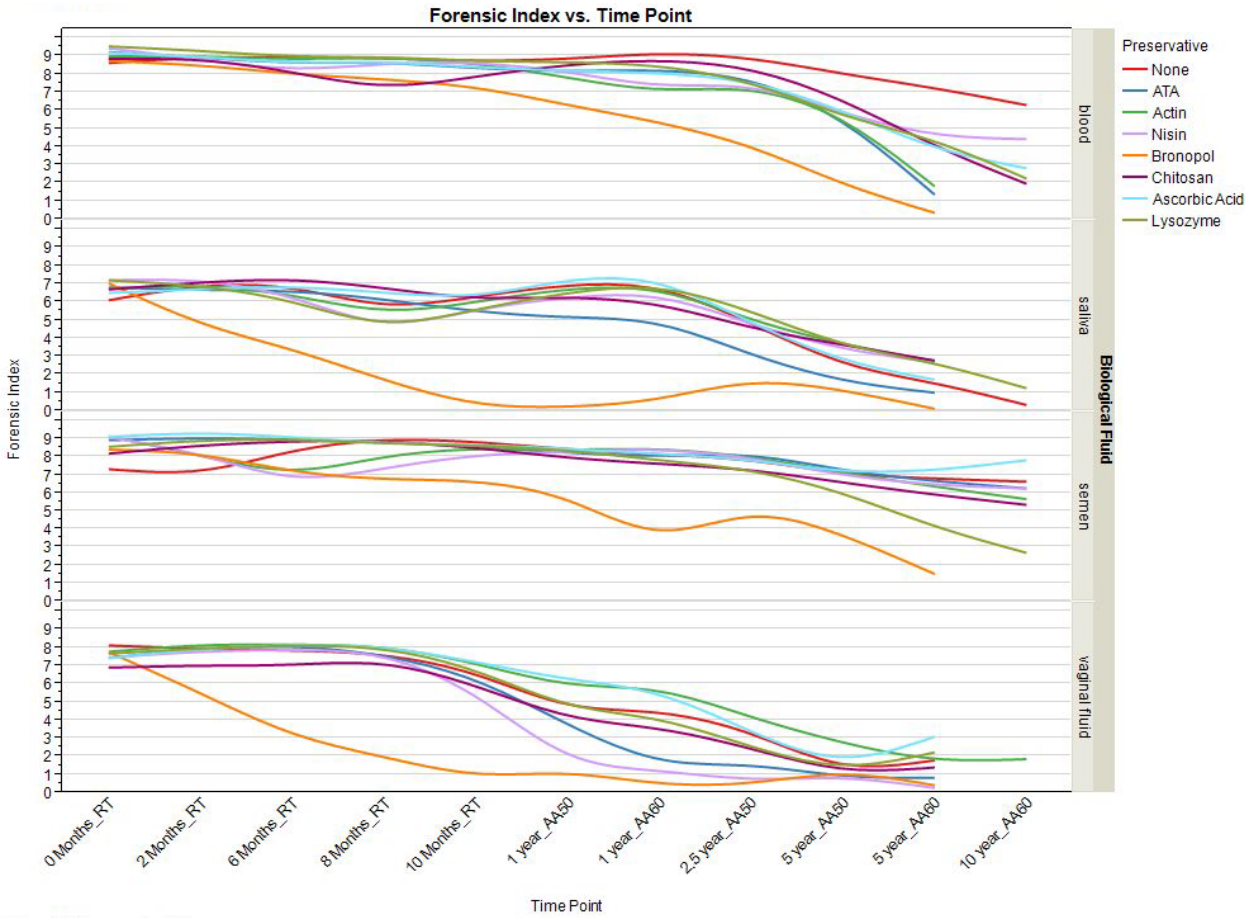

Figure 36: FI was plotted against time points for blood, saliva, semen, and vaginal fluid samples that were treated with ATA, Actin, Nisin, Bronopol, Chitosan, Ascorbic Acid, and Lysozyme. The untreated control samples are represented as "None." For some samples, 10 year RT equivalent at 60°C time point data was unavailable due to an amplification issue.

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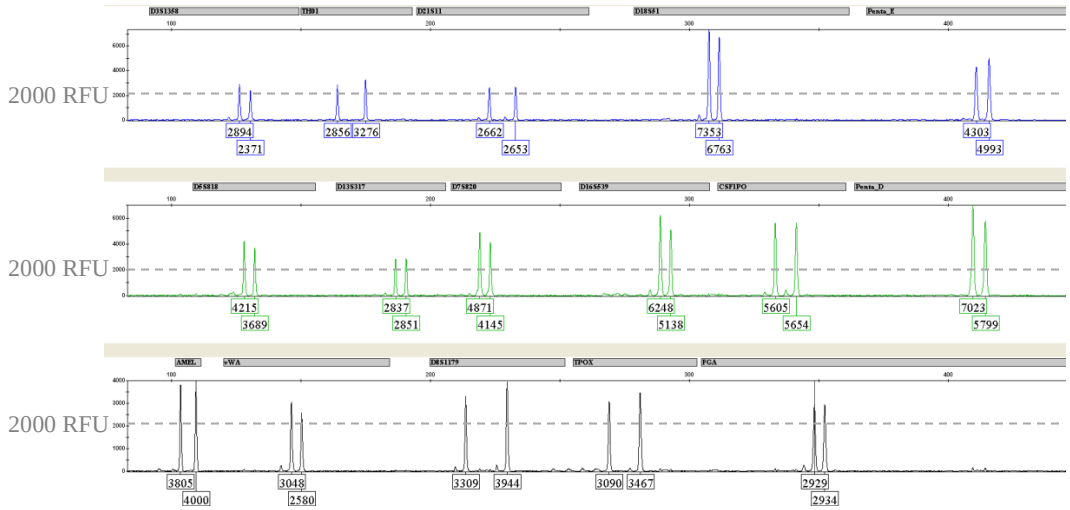

Figure 37: A full profile was generated for an Actin treated blood sample at the 2 month time point. This sample had a FI ranking of 9.17, a quantification value of 1.59 ng/µl, an average peak height value of 4100 RFU, and a profile balance ratio of 2.68.

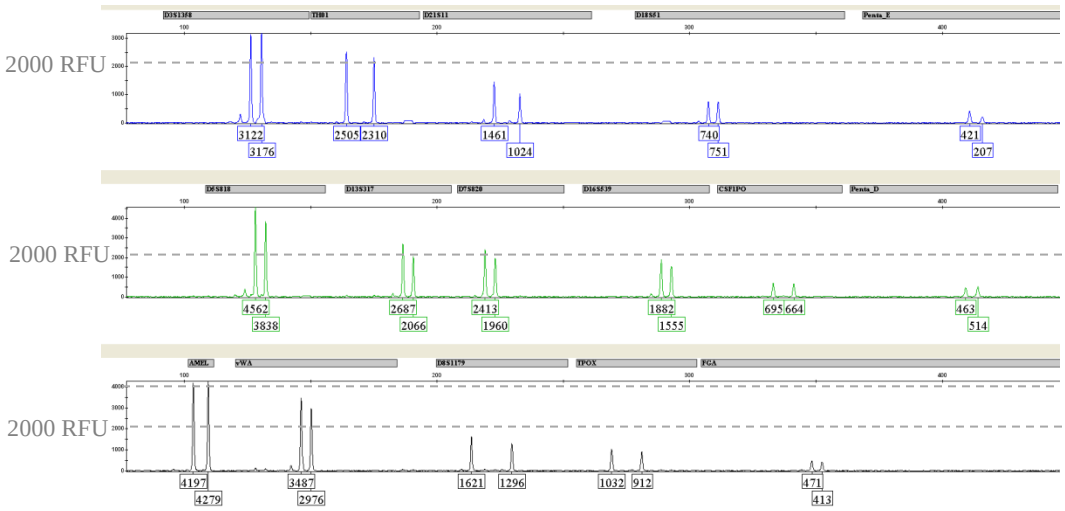

Figure 38: A full profile was generated for an Actin treated blood sample at the 1 year RT equivalent at 60°C time point. The sample had a FI ranking was 6.21, a quantification value of 0.261 ng/µl, an average peak height value of approximately 1900 RFU, and a profile balance ratio of 19.49.

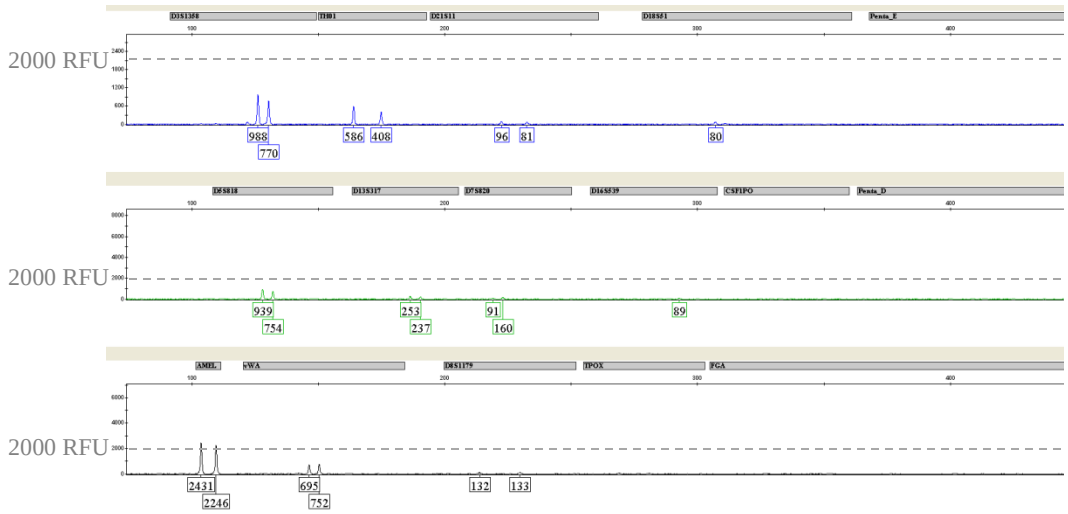

Figure 39: A partial profile with a percent profile of 62.5% was generated for an Actin treated blood sample at the 5 year RT equivalent at 60°C time point. This sample had a FI ranking of 1.78, a quantification value of 0.037 ng/µl, an average peak height value of approximately 200 RFU. Because this was a partial profile, no profile balance ratio was calculated

Phase II

Preservative Combinations

Mean Quantification Values

Figure 40 displays the mean quantification results (ng/µl) for all untreated control and treated blood, saliva, and vaginal fluid samples tested during Phase II. Consistent quantification results were observed for each set of treated samples within the specified time points. Over time, decreasing quantification values were observed for each fluid's treated samples and untreated control samples; however, the 1 year RT equivalent blood samples generated higher mean average quantification values than the 0 month samples. The cause for this is unknown.

Figure 41 displays the mean quantification results (ng/µl) for all untreated control and treated semen samples tested during Phase II. As with the blood, saliva, and vaginal fluid samples, consistent quantification results were generated for each set of treated samples within the specified time points. Similar to the results obtained for the blood, saliva and vaginal fluid samples, decreasing quantification values were obtained for the treated samples over time; however, the samples treated with the Zinc-EDTA and the Zinc-EDTA-Sodium Azide combinations produced quantification values that were more consistent with the earlier time points.

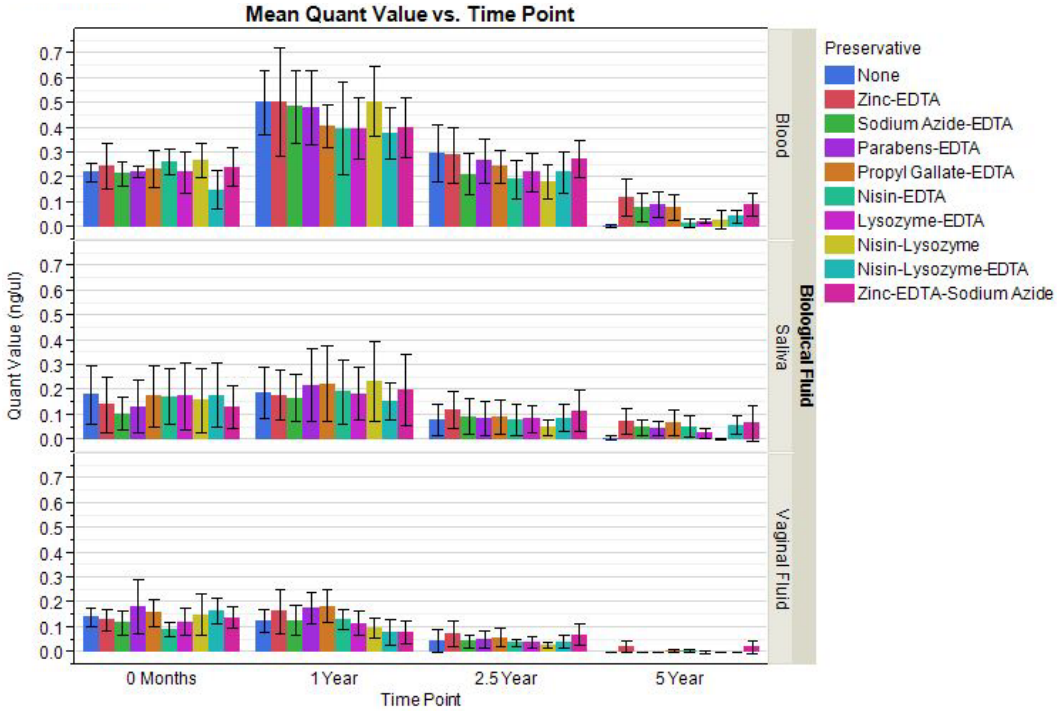

Figure 40: Mean Quantifiler Duo Human results (ng/µl) for the blood, saliva and vaginal fluid samples that were treated with a variety of preservative combinations. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

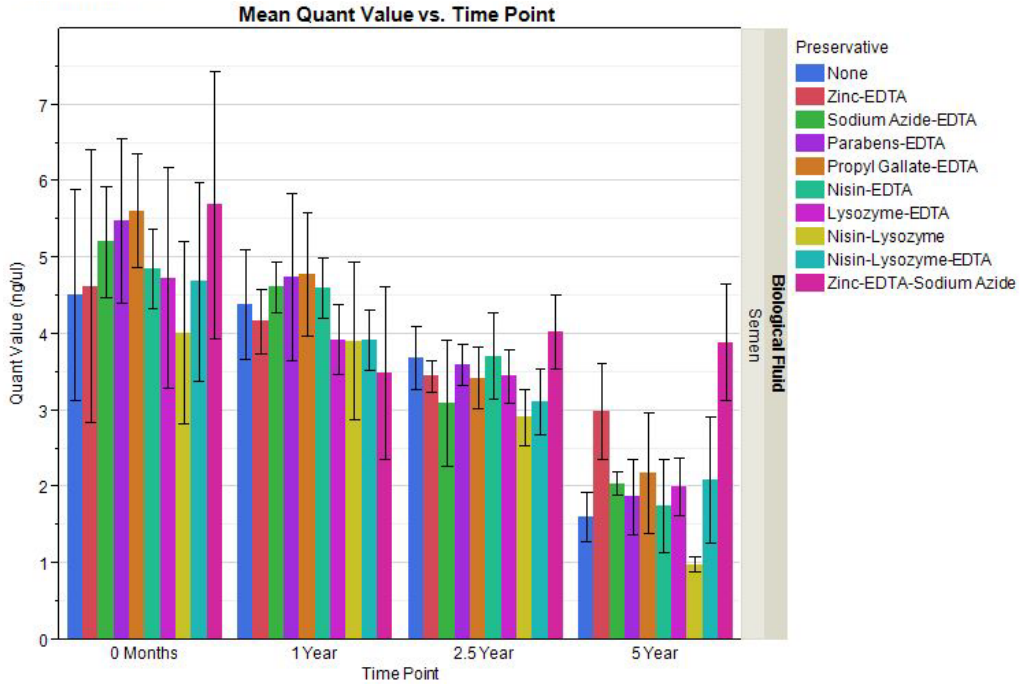

Figure 41: Mean Quantifiler Duo Human results (ng/µl) for the semen samples that were treated with a variety of preservative combinations. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Mean Percent Profile

The mean percent profiles obtained for all blood, saliva, semen and vaginal fluid samples are displayed in Figure 42. Full to high partial profiles were generated for all treated and untreated blood samples processed at the 0 month, 1 year RT equivalent (stored at 50°C for 52 days) and 2.5 year RT equivalent (stored at 50°C for 131 days) time points. At the 5 year RT equivalent time point, average percent profiles greater than 80% were generated for blood samples treated with all preservative combinations except for Nisin-EDTA and Nisin-Lysozyme. The untreated control produced an average percent profile of 52%. At the 5.0 year RT equivalent time point, a statistically significant increase in mean percent profile over the untreated control samples was generated for the following preservative combinations: Zinc-EDTA ($p = 0.0001$), Sodium Azide-EDTA ($p = 0.0003$), Parabens-EDTA ($p = 0.00289$), Propyl Gallate-EDTA ($p = 0.0001$), Lysozyme-EDTA ($p = 0.00007$), Zinc-EDTA-Sodium Azide ($p = 0.0000045$) and Nisin-Lysozyme-EDTA ($p = 0.0000615$).

With the exception of the Sodium Azide-EDTA treated saliva samples, full to high partial profiles were generated for all treated and untreated saliva samples processed at the 0 month and 1 year RT equivalent time points. Greater than 80% average percent profiles were achieved for all samples associated with the 2.5 year RT equivalent time point. All treated 5 year RT equivalent samples generated average percent profiles greater than 80% except for the samples treated with the Nisin/Lysozyme preservative combination. The untreated control saliva samples produced an average percent profile of 61%. At the 5.0 year RT equivalent time point, a statistically significant increase in mean percent profile over the untreated control samples was generated for the following preservative combinations: Zinc-EDTA ($p = 0.000016$), Sodium Azide-EDTA ($p = 0.00002$), Parabens-EDTA ($p = 0.00001$), Propyl Gallate-EDTA ($p = 0.00007$), Nisin-EDTA ($p = 0.000002$), Lysozyme-EDTA ($p = 0.000004$), Zinc-EDTA-Sodium Azide ($p = 0.00002$) and Nisin-Lysozyme-EDTA ($p = 0.00002$).

Full to high partial profiles were generated for all treated and untreated semen samples processed at the 0 month, 1 Year RT equivalent, 2.5 Year RT equivalent and 5 year RT equivalent time points. No significance increases in mean percent profile were generated for the treated semen samples when compared to the untreated control samples.

Full to high partial profiles were generated for all treated and untreated vaginal fluid samples processed at the 0 month, 1 Year RT equivalent and 2.5 Year RT equivalent time points. At the 5 year RT equivalent time point, average percent profiles > 80% were generated for the samples that were treated with the Zinc-EDTA and Zinc-EDTA-Sodium Azide preservative combinations. The untreated control failed to produce any profiles. At the 5.0 year RT equivalent time point, a statistically significant increase in mean percent profile over the untreated control samples was generated for the following preservative combinations: Zinc-EDTA ($p = 2.4 \times 10^{-9}$), Sodium Azide-EDTA ($p = 0.0011$), Parabens-EDTA ($p = 0.0003$), Propyl Gallate-EDTA ($p = 0.0016$), Nisin-EDTA ($p = 0.0381$), Lysozyme-EDTA ($p = 0.0248$), Zinc-EDTA-Sodium Azide ($p = 9.5 \times 10^{-5}$), and Nisin-Lysozyme-EDTA ($p = 0.0183$).

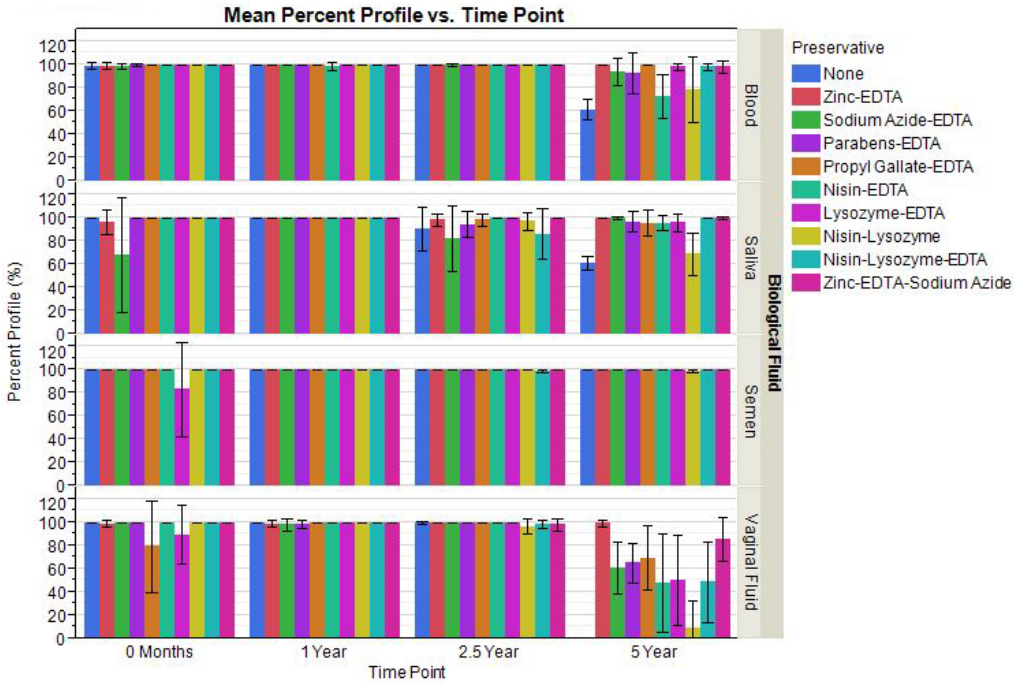

Figure 42: Mean percent profile (%) results for the blood, saliva, semen, and vaginal fluid samples that were treated with a variety of preservative combinations. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Mean Peak Height Values

The mean average peak heights (PH) obtained for all blood, saliva, semen and vaginal fluid samples are displayed in Figure 43. The mean average peak height for the treated and untreated blood samples at the 0 month, 1 year RT equivalent and 2.5 year RT equivalent time points ranged from 1,000 – 3,000 RFU. Mean average peak heights of 1,000 RFU were generated by the majority of treated samples at the 5 year RT equivalent time point, and a mean average peak height of 500 RFU was generated by the untreated controls. At the 5 year RT equivalent time point, a statistically significant increase in peak heights was generated for the following preservative combinations when compared to the control samples: Zinc-EDTA ($p = 0.010$), Sodium Azide-EDTA ($p = 0.035$), Propyl Gallate-EDTA ($p = 0.003$), Lysozyme-EDTA ($p = 0.00006$), Zinc-EDTA-Sodium Azide ($p = 0.0062$), and Nisin-Lysozyme-EDTA ($p = 0.014$).

The mean average peak height for the treated saliva samples was 1,000 RFU across all time points. The untreated control samples generated mean average peak heights of 900 RFU until the 5 year RT equivalent time point, where an average peak height of 400 RFU was generated. At the 5 year RT equivalent time point, a statistically significant increase in peak height was generated for the following preservative combinations when compared to the control samples: Zinc-EDTA ($p = 0.0002$), Sodium Azide-EDTA ($p = 0.0261$), Parabens-EDTA ($p = 0.0141$), Propyl Gallate-EDTA ($p = 0.0232$), Nisin-EDTA ($p = 0.0134$), Lysozyme-EDTA ($p = 0.0019$), Nisin-Lysozyme-EDTA ($p = 0.0001$), and Zinc-EDTA-Sodium Azide ($p = 0.00862$).

The mean average peak heights for the treated and untreated semen samples at the 0 month, 1 year RT equivalent and 2.5 year RT equivalent time points ranged from 2,600 – 4,100 RFU. Mean average peak heights of 1,700 RFU were generated by the samples at the 5 year RT equivalent time point. No statistically significant differences in peak heights were generated for any of the treated samples when compared to the control samples across all time points.

The mean average peak heights for the treated and untreated vaginal fluid samples at the 0 month, 1 year RT equivalent and 2.5 year RT equivalent time points ranged from 1400 – 2000 RFU. Mean average peak heights of 500 RFU were generated from the majority of treated vaginal fluid samples at the 5 year RT equivalent time point, whereas a decrease in mean average RFU was observed for the untreated control. A statistically significant increase in mean peak heights was generated for the following preservative combinations when compared to the control samples: Zinc-EDTA ($p = 0.000000002$), Sodium Azide-EDTA ($p = 0.001$), Parabens-EDTA ($p = 0.00024$), Propyl Gallate-EDTA ($p = 0.0015$), Nisin-EDTA ($p = 0.038$), Lysozyme-EDTA ($p = 0.024$), Nisin-Lysozyme-EDTA ($p = 0.000095$), and Zinc-EDTA-Sodium Azide ($p = 0.018$).

Figure 44 displays three electropherograms (EPGs) from untreated and treated saliva samples during Phase II. The first EPG (A) represents an untreated saliva sample stored at RT for 0 months. The second EPG (B) represents an untreated saliva sample that was stored at an accelerated temperature (AT) of 50°C for 225 days (equivalent to 5 years of RT storage). The third EPG (C) represents a saliva sample that was treated with the preservative combination Zinc-EDTA and was stored at an AT of 50°C for 225 days (equivalent to 5 years of RT storage).

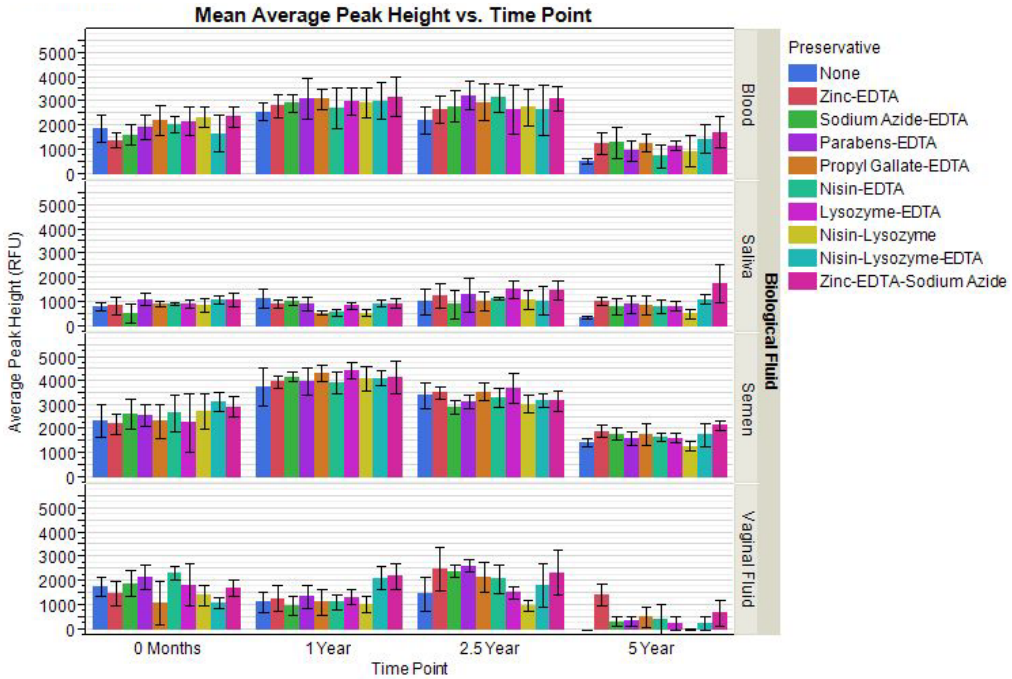

Figure 43: Mean average peak height results for the blood, saliva, semen, and vaginal fluid samples that were treated with a variety of preservative combinations. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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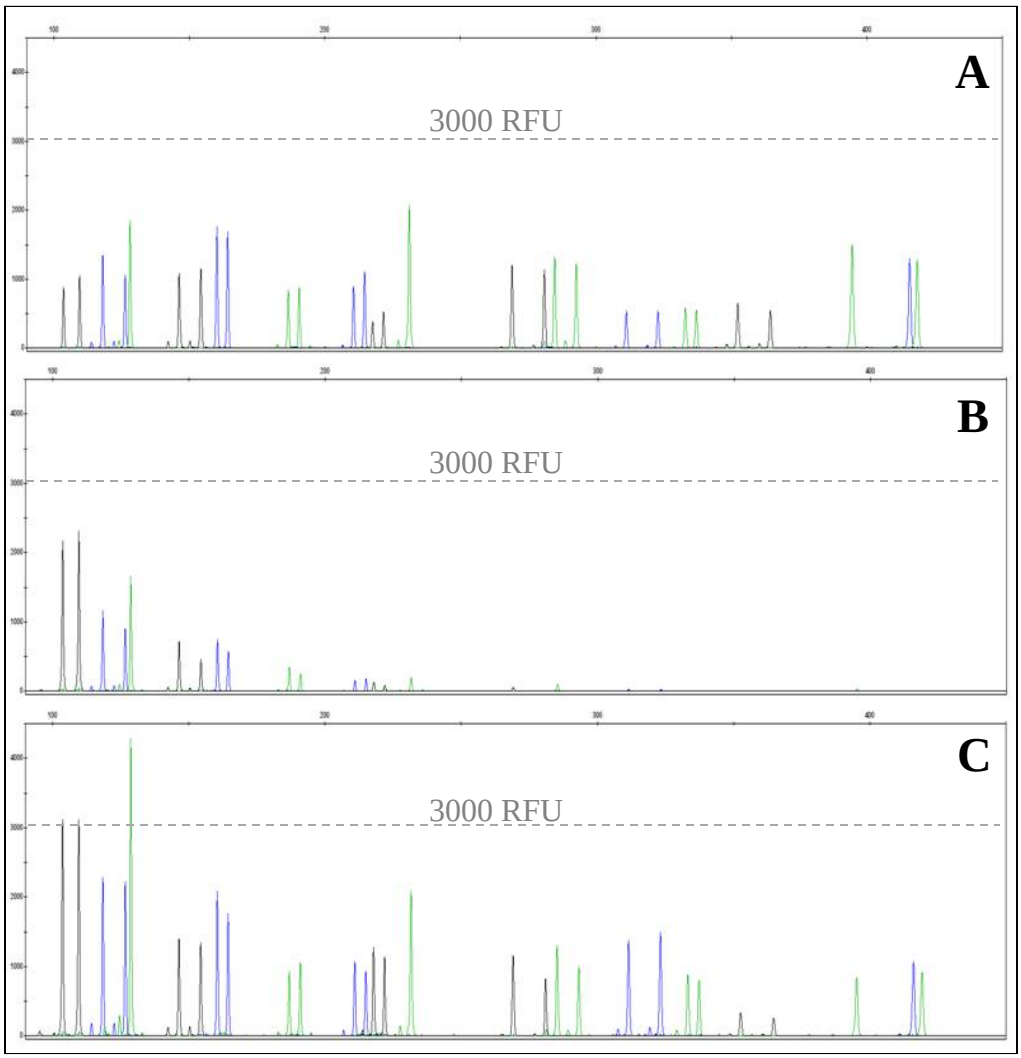

Figure 44: Representative electropherograms (EPGs) from three saliva samples tested during Phase I. (A) An untreated (control) saliva sample stored at RT for 0 months. (B) An untreated saliva sample that was stored at an accelerated temperature (AT) of 50°C for 225 days (equivalent to 5 years of RT storage). (C) A saliva sample that was treated with the preservative combination Zinc-EDTA and was stored at an AT of 50°C for 225 days (equivalent to 5 years of RT storage).

Profile Balance Ratios

Figure 45 displays the profile balance ratios for all treated and untreated blood, saliva, semen and vaginal fluid samples at the 0 month, 1 year RT equivalent, 2.5 year RT equivalent, and 5 year RT equivalent time points during Phase II. Only samples that generated full profiles are represented. Untreated controls that generated full profiles are also included. For each fluid tested, imbalanced profiles were generated at varying time points. For the Lysozyme-EDTA treated, Nisin-Lysozyme-EDTA treated, and untreated blood samples, imbalanced or no profiles were generated at the 0 month, 2.5 year RT equivalent, and 5 year RT equivalent time points. For the Zinc-EDTA treated and Sodium Azide-EDTA treated blood samples, imbalanced profiles were observed at the 0 months and 5 year RT equivalent time points. For the Parabens-EDTA treated, Propyl Gallate-

EDTA treated, Nisin-EDTA, Nisin-Lysozyme, and Zinc-EDTA-Sodium Azide treated blood samples, balanced profiles were observed until the 5 year RT equivalent time point, at which point the mean average profile balance ratios increased from approximately 3 to 12.69, 81.56, 10.10, and 8.34, respectively.

More imbalanced profiles were observed from the saliva samples than from any other fluid type. On average, the Zinc-EDTA treated, Sodium Azide-EDTA treated, and Nisin-Lysozyme treated saliva samples generated imbalanced profiles at every time point. Balanced profiles were observed at only the 0 month time point for the Propyl Gallate-EDTA treated, Nisin-EDTA treated, Lysozyme-EDTA treated, and untreated saliva samples. The Parabens-EDTA treated saliva samples displayed balanced profile at the 0 month and 2.5 year RT equivalent time points, whereas the Nisin-Lysozyme-EDTA treated and Zinc-EDTA-Sodium Azide treated saliva samples displayed balanced profiles at the 0 month and 1 year RT equivalent time points.

For the semen samples, all treated and untreated samples generated balanced profiles until the 5 year RT equivalent time point. The Zinc-EDTA-Sodium Azide treated samples also generated balanced profiles at the 5 year RT equivalent time point.
On average, the Nisin-Lysozyme treated and untreated vaginal fluid samples generated imbalanced profiles at every time point, with mean average profile balance ratios of 15.19 and 9.23, respectively. Imbalanced profiles were observed from the Sodium Azide-EDTA treated and Lysozyme-EDTA treated vaginal fluid samples at every time point except for the 2.5 year R equivalent time point. Balanced profiles were observed from the Zinc-EDTA treated and Nisin-Lysozyme-EDTA treated vaginal fluid samples at only the 1 year RT equivalent and 2.5 year RT equivalent time points. The Parabens-EDTA treated, Propyl Gallate-EDTA treated, Nisin-EDTA treated, and Zinc-EDTA-Sodium Azide treated vaginal fluid samples generated balanced profile through the 2.5 year RT equivalent time point, after which all full profiles demonstrated imbalance.

Overall, all of the preservative combination treated blood samples generated more balanced profiles than the untreated control samples, except for the Sodium Azide-EDTA treated blood samples, which generated the same number of balanced profiles as the untreated controls (Table 16). Furthermore, all of the preservative combination treated blood samples, except for the Nisin-EDTA treated samples, generated more full profiles than the untreated control samples. The Nisin-EDTA treated samples generated the same number of full profiles as the control samples. More balanced profiles were generated by Zinc-EDTA-Sodium Azide treated and Parabens-EDTA treated saliva samples than were generated by the untreated control saliva samples (Table 17). The remaining preservative combinations generated fewer balanced profiles; however, all of the preservative combination treated samples generated more full profiles than the untreated control samples. For the semen samples, the number of balanced profiles generated by the Zinc-EDTA-Sodium Azide treated, Zinc-EDTA treated, Propyl Gallate-EDTA treated, and Parabens-EDTA treated samples exceeded the number generated by the untreated control semen samples. The untreated control samples and the samples treated with Sodium Azide-EDTA, Nisin-EDTA, and Nisin-Lysozyme generated the same quantity of balanced profiles (Table 18). The remaining preservative combinations generated fewer balanced profiles than the control samples. All preservative combinations, except for Nisin-Lysozyme, Lysozyme-EDTA, and Nisin-Lysozyme-EDTA, generated the same number of full profiles as the untreated control samples. The Nisin-

Lysozyme treated, Lysozyme-EDTA treated, and Nisin-Lysozyme-EDTA treated semen samples generated fewer full profiles than the untreated control samples. For the vaginal fluid samples, a greater number of balanced profiles were generated by all of the preservative combination treated samples, except those treated with Nisin-Lysozyme, than the untreated control samples. The Nisin-Lysozyme treated vaginal fluid samples generated fewer balanced profiles (Table 19). Only three of the preservative combinations (Nisin-EDTA, Zinc-EDTA-Sodium Azide, and Zinc-EDTA) generated a greater number of full profiles than the untreated control vaginal fluids samples. Comparable numbers of full profiles were generated by the untreated, Parabens-EDTA treated, Sodium Azide-EDTA treated, Nisin-Lysozyme-EDTA treated, and Lysozyme-EDTA treated vaginal fluid samples, whereas the Propyl Gallate-EDTA treated and Nisin-Lysozyme treated samples generated fewer full profiles than the untreated control vaginal fluid samples.

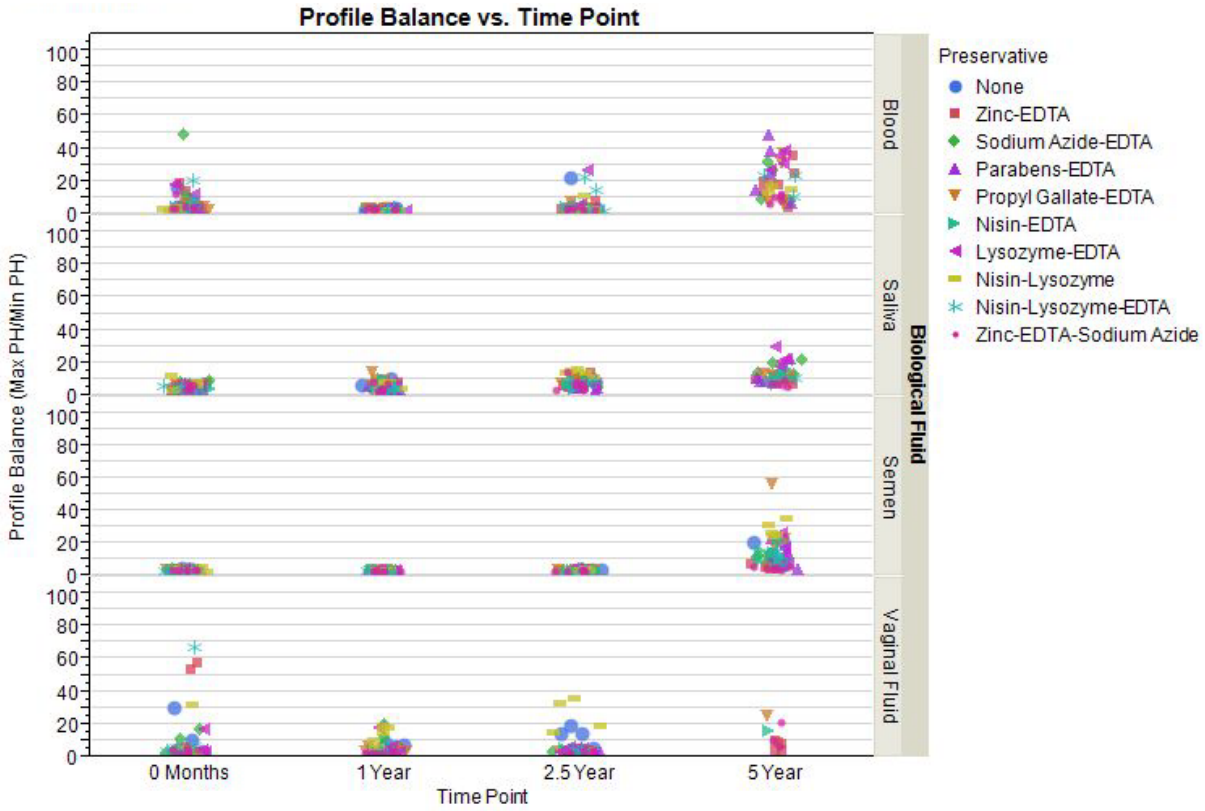

Figure 45: Profile balance (Maximum PH/Minimum PH) results for the blood, saliva, semen, and vaginal fluid samples that were treated with a variety of preservative combinations. The untreated control samples are represented as "None**."**

Table 16: Total number of balanced, imbalanced, and partial/no profiles generated for each preservative combination applied to blood samples.

Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/ Partial
Blood	Zinc-EDTA-Sodium Azide	24	17	6	1	70.8%	25.0%	4.2%
	Nisin-Lysozyme	24	17	4	3	70.8%	16.7%	12.5%
	Nisin-EDTA	24	17	0	7	70.8%	0.0%	29.2%
	Propyl Gallate-EDTA	24	16	8	0	66.7%	33.3%	0.0%
	Parabens-EDTA	24	16	6	2	66.7%	25.0%	8.3%
	Zinc-EDTA	24	14	9	1	58.3%	37.5%	4.2%
	Lysozyme-EDTA	24	14	8	2	58.3%	33.3%	8.3%
	Nisin-Lysozyme-EDTA	24	14	7	3	58.3%	29.2%	12.5%
	Sodium Azide-EDTA	24	13	6	5	54.2%	25.0%	20.8%
	None	24	13	4	7	54.2%	16.7%	29.2%

Table 17: Total number of balanced, imbalanced, and partial/no profiles generated for each preservative combination applied to saliva samples.

Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/ Partial
Saliva	Zinc-EDTA-Sodium Azide	24	10	13	1	41.7%	54.2%	4.2%
	Parabens-EDTA	24	10	11	3	41.7%	45.8%	12.5%
	None	24	7	9	8	29.2%	37.5%	33.3%
	Zinc-EDTA	24	6	16	2	25.0%	66.7%	8.3%
	Lysozyme-EDTA	24	6	16	2	25.0%	66.7%	8.3%
	Nisin-Lysozyme-EDTA	24	6	16	2	25.0%	66.7%	8.3%
	Nisin-EDTA	24	5	16	3	20.8%	66.7%	12.5%
	Nisin-Lysozyme	24	5	12	7	20.8%	50.0%	29.2%
	Propyl Gallate-EDTA	24	4	18	2	16.7%	75.0%	8.3%
	Sodium Azide-EDTA	24	3	16	5	12.5%	66.7%	20.8%

Table 18: Total number of balanced, imbalanced, and partial/no profiles generated for each preservative combination applied to semen samples.

Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/ Partial
Semen	Zinc-EDTA-Sodium Azide	24	23	1	0	95.8%	4.2%	0.0%
	Zinc-EDTA	24	20	4	0	83.3%	16.7%	0.0%
	Propyl Gallate-EDTA	24	20	4	0	83.3%	16.7%	0.0%
	Parabens-EDTA	24	19	5	0	79.2%	20.8%	0.0%
	Sodium Azide-EDTA	24	18	6	0	75.0%	25.0%	0.0%
	Nisin-EDTA	24	18	6	0	75.0%	25.0%	0.0%
	None	24	18	6	0	75.0%	25.0%	0.0%
	Nisin-Lysozyme	24	18	5	1	75.0%	20.8%	4.2%
	Lysozyme-EDTA	24	17	6	1	70.8%	25.0%	4.2%
	Nisin-Lysozyme-EDTA	24	17	6	1	70.8%	25.0%	4.2%

Table 19: Total number of balanced, imbalanced, and partial/no profiles generated for each preservative combination applied to semen samples.

Fluid	Preservative	Total Samples	Balanced Profiles	Imbalanced Profiles	No/Partial Profiles	% Balanced	% Imbalanced	% No/ Partial
Vaginal Fluid	Nisin-EDTA	24	17	2	5	70.8%	8.3%	20.8%
	Zinc-EDTA-Sodium Azide	24	16	4	4	66.7%	16.7%	16.7%
	Zinc-EDTA	24	15	6	3	62.5%	25.0%	12.5%
	Parabens-EDTA	24	15	2	7	62.5%	8.3%	29.2%
	Propyl Gallate-EDTA	24	14	2	8	58.3%	8.3%	33.3%
	Sodium Azide-EDTA	24	13	4	7	54.2%	16.7%	29.2%
	Nisin-Lysozyme-EDTA	24	13	4	7	54.2%	16.7%	29.2%
	Lysozyme-EDTA	24	12	5	7	50.0%	20.8%	29.2%
	None	24	7	10	7	29.2%	41.7%	29.2%
	Nisin-Lysozyme	24	6	10	8	25.0%	41.7%	33.3%

PowerPlex Fusion

In addition to the necessary sample sets for Phase II, an extra set of experimental samples was prepared concurrently and stored under equivalent accelerated aging conditions at 50°C. The additional sample set was processed alongside the Phase II 5 year accelerated aging time point so that a direct comparison of the data generated from the PowerPlex 16 and PowerPlex Fusion systems could be performed.

Mean Quantification Values

Figure 46 displays the mean quantification values (ng/µl) obtained from the treated and untreated (control) blood, saliva and vaginal fluid sample sets that were stored at 50°C for 262 days (equivalent to 5 years of RT storage). Although the sample sets were prepared concurrently, the quantification values associated with the PowerPlex Fusion sample set were higher for all biological fluids than those obtained from the PowerPlex 16 sample set. This difference can only be attributed to chemistry of the Quantifiler Duo Quantification kit. As the sample sets were prepared concurrently, the average quantification results from the treated and untreated PowerPlex Fusion blood samples ranged from 0.07 ng/µl – 0.22 ng/µl. The average quantification results from the treated and untreated PowerPlex 16 blood samples ranged from 0.01 ng/µl – 0.12 ng/µl. For the treated and untreated saliva samples, the average PowerPlex Fusion quantification values ranged from 0.03 ng/µl – 0.13 ng/µl, and the average PowerPlex 16 quantification values ranged from 0.004 ng/µl – 0.08 ng/µl. The treated and untreated vaginal fluid samples generated the lowest average quantification values: 0.01 ng/µl – 0.08 ng/µl from the PowerPlex Fusion samples and 0.00 ng/µl – 0.02 ng/µl from the PowerPlex 16 samples. On the other hand, the treated and untreated semen samples generated the highest average quantification values: 2.04 ng/µl – 4.00 ng/µl from the PowerPlex Fusion samples and 0.99 ng/µl – 3.90 ng/µl from the PowerPlex 16 samples (Figure 47).

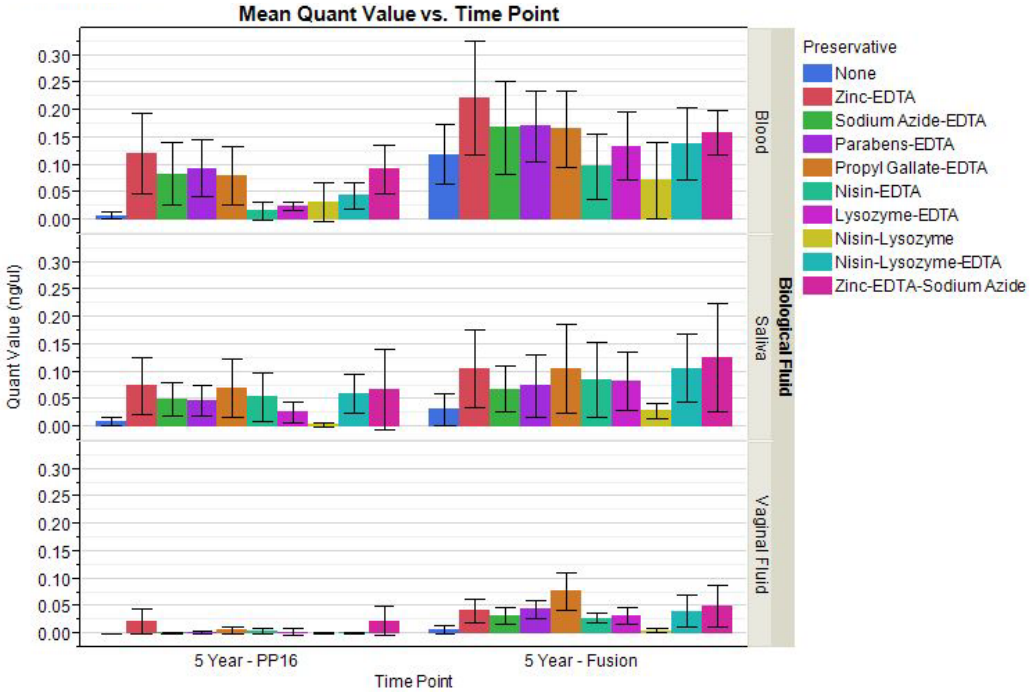

Figure 46: Mean quantification values (ng/µl) generated for all chemically treated blood, saliva, and vaginal fluid samples tested at the 5 year RT equivalent at 50°C time point followed by amplification with PowerPlex 16 and PowerPlex Fusion. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

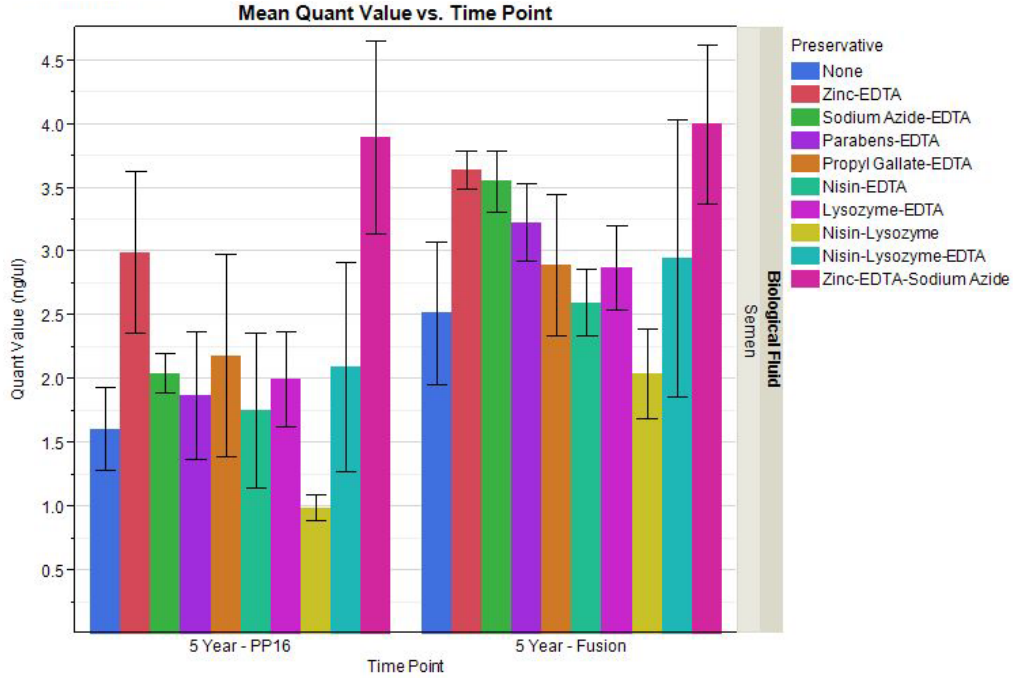

Figure 47: Mean quantification values (ng/µl) generated for all chemically treated semen samples tested at the 5 year RT equivalent at 50°C time point followed by amplification with PowerPlex 16 and PowerPlex Fusion. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

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Mean Percent Profile

Figure 48 summarizes the percent profile data generated by the two sample sets amplified with PowerPlex 16 and PowerPlex Fusion at the 5 year RT equivalent time points. For the blood samples, 62 full profiles were obtained with PowerPlex Fusion, whereas 36 full profiles were obtained with PowerPlex 16. Specifically, the Sodium Azide-EDTA treated, Parabens-EDTA treated, Nisin-EDTA treated, Lysosome-EDTA treated, Nisin-Lysozyme-EDTA treated, and untreated blood samples generated more full profiles when amplified with PowerPlex Fusion than with PowerPlex 16. For the saliva samples, 47 full profiles were obtained with PowerPlex Fusion, whereas 39 profiles were obtained with PowerPlex 16. The PowerPlex Fusion amplified Sodium Azide-EDTA treated, Parabens-EDTA treated, Propyl Gallate-EDTA treated, Nisin-EDTA treated, Lysozyme EDTA treated, and Zinc-EDTA-Sodium Azide treated saliva samples generated more full profiles than those amplified with PowerPlex 16. Regardless of the temperature or length of storage, all of the semen samples (chemically treated and control samples) generated full DNA profiles when amplified with either the PowerPlex 16 or PowerPlex Fusion Kits, except for one Nisin-Lysozyme treated sample that was amplified with PowerPlex 16. Upon observing the data generated from the vaginal fluid samples across most preservative combinations, a greater number of full DNA profiles were generated from the samples that were amplified with the PowerPlex Fusion Kit (Sodium Azide-EDTA, Parabens-EDTA, Propyl Gallate-EDTA, Lysozyme-EDTA and Nisin-Lysozyme-EDTA). Forty-seven full profiles were generated from the vaginal samples amplified with PowerPlex Fusion, whereas 5 full profiles were generated from the vaginal samples amplified with PowerPlex 16. These results suggest that the PowerPlex Fusion System is a more robust and sensitive kit than PowerPlex 16. With its increased sensitivity, PowerPlex Fusion has the ability to provide DNA analysts with more information by generating DNA profiles of increased quality.

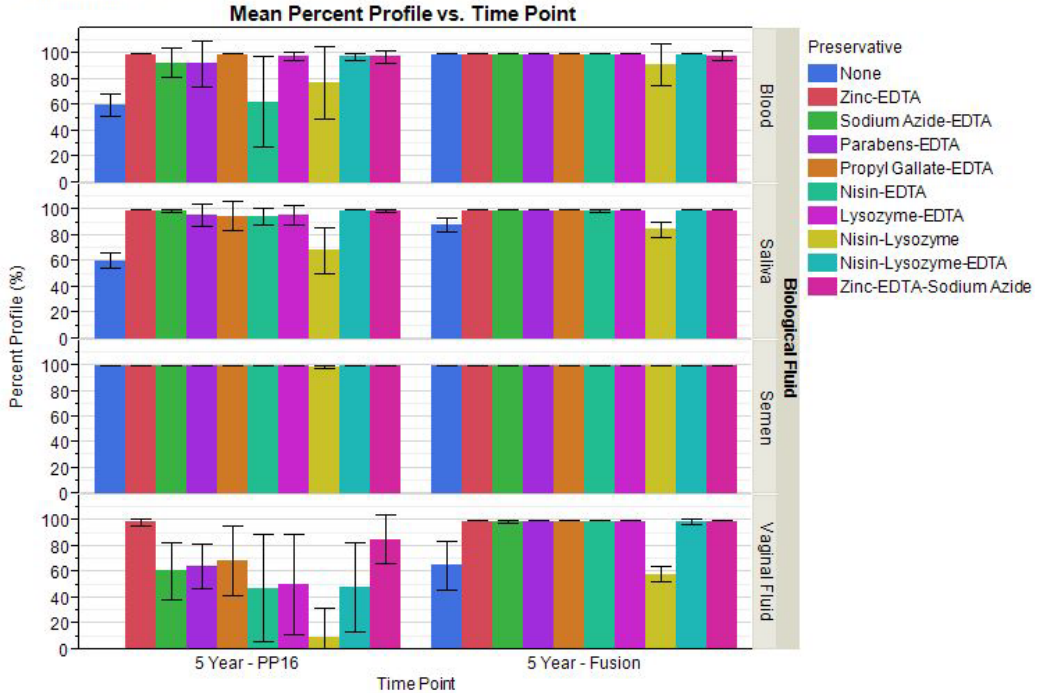

Figure 48: Mean percent profiles (%) generated for all chemically treated blood, saliva, semen and vaginal fluid samples tested at the 5 year RT equivalent time point (stored at 50°C for 262 days) followed by amplification with PowerPlex 16 and PowerPlex Fusion. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Mean Peak Height Values

Figure 49 summarizes the average peak height (PH) data for all preservative combination treated and untreated blood, saliva, semen, and vaginal fluid samples amplified with PowerPlex 16 and PowerPlex Fusion at the 5 year RT equivalent time point. Across all preservative combinations and biological fluids tested, greater average peak heights were observed for the majority of samples that were amplified with PowerPlex Fusion System. Regardless of the amplification kit used, lower average peak height values were observed from the saliva and vaginal samples than from the blood and semen samples. These reduced average peak height values were expected as the majority of the saliva and vaginal fluid samples were concentrated prior to amplification due to low quantification values (< 0.15 ng/µl).

When comparing a PowerPlex 16 amplified untreated saliva sample that was stored at 50°C for 262 days (equivalent to 5 years of RT storage) and PowerPlex 16 or PowerPlex Fusion amplified Nisin-EDTA treated saliva samples that were stored at 50°C for 262 days, it was observed that the PowerPlex 16 amplified untreated and treated saliva samples generated comparable RFU values, whereas the PowerPlex Fusion amplified Nisin-EDTA treated saliva sample generated significantly higher RFU values (Figure 50).

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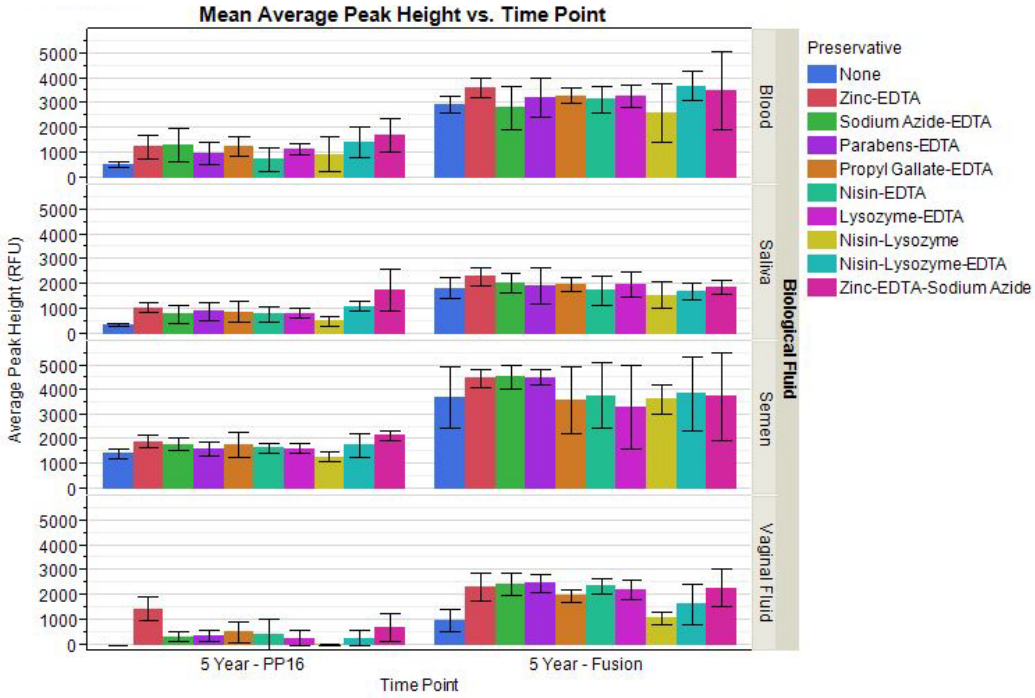

Figure 49: Mean average peak heights generated for all chemically treated blood, saliva, semen and vaginal fluid samples tested at the 5 year RT equivalent time point (stored at 50°C for 262 days) followed by amplification with PowerPlex 16 and PowerPlex Fusion. The profile balance for the untreated control samples is represented as "None." Each error bar is constructed using one standard deviation from the mean.

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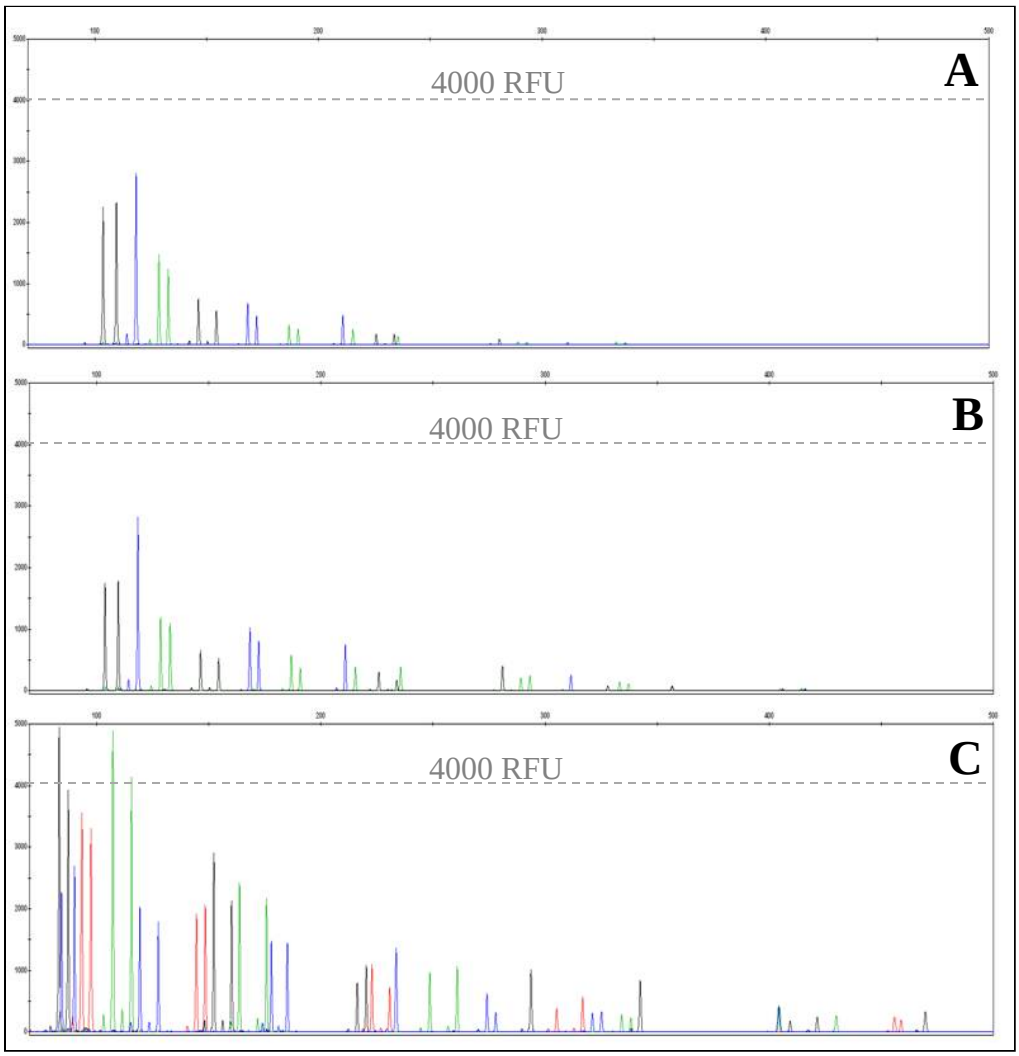

Figure 50: Representative electropherograms (EPGs) from three saliva samples tested during Phase II. (A) A PowerPlex 16 amplified untreated (control) saliva sample stored at 50°C for 262 days (equivalent to 5 years of RT storage). (B) A PowerPlex 16 amplified Nisin-EDTA treated saliva sample stored at 50°C for 262 days (equivalent to 5 years of RT storage). (C) A PowerPlex Fusion amplified Nisin-EDTA treated saliva sample stored at 50°C for 262 days (equivalent to 5 years of RT storage).

Profile Balance Ratios

On average, the profiles generated by both PowerPlex Fusion and PowerPlex 16 demonstrated imbalance across all samples types and preservative combinations (Figure 51). The PowerPlex Fusion blood, saliva, and vaginal fluid samples were generally more imbalanced than the PowerPlex 16 samples; however, as mentioned above, more full profiles were generated with PowerPlex Fusion. The PowerPlex Fusion amplified blood, saliva, and vaginal fluid samples generated mean average profile balance ratios of 27.40, 29.70, and 27.14, respectively, whereas the PowerPlex 16 amplified blood, saliva, and vaginal samples generated mean average profile balance ratios of 19.89, 12.75, and 15.05, respectively. On the other hand, more balanced profiles were generated from the PowerPlex Fusion amplified semen samples than from the PowerPlex 16

amplified semen samples. Of the semen samples amplified with PowerPlex Fusion, the mean average profile balance ratio was 9.54, and 20% of the samples generated balanced profiles. Of the semen samples amplified with PowerPlex 16, the mean average profile balance ratio was 12.77, and 17% of the samples generated balanced profiles.

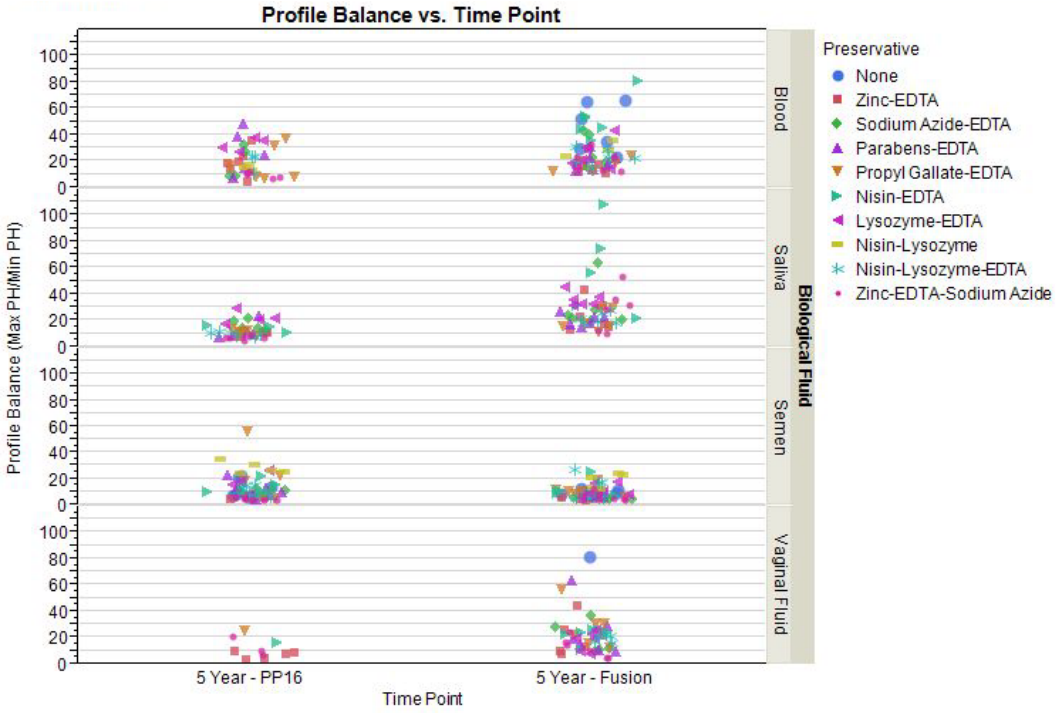

Figure 51: Profile balances generated for all untreated and treated blood, saliva, semen and vaginal fluid samples tested at the 5 year RT equivalent time point (stored at 50°C for 262 days) followed by amplification with PowerPlex 16 and PowerPlex Fusion. The untreated control samples are represented as "None."

Phase III: Direct Amplification

During this phase, the preservatives Zinc and Zinc-EDTA were tested in conjunction with alternative collection substrates that lend themselves to faster processing with the use of direct amplification. Blood on FTA paper, saliva on indicating FTA paper, and saliva on Buccal DNA Collectors are the sample types of interest.

Blood

Mean Percent Profile

The mean percent profiles generated for the blood on FTA samples are demonstrated in Figure 52. Across all time points, the mean percent profile for the untreated control samples was 100%. Full to high partial DNA profiles were generated for all blood samples treated with Zinc or Zinc-EDTA across all time points. No statistically significant differences in percent profile were observed between any of the samples at any time point.

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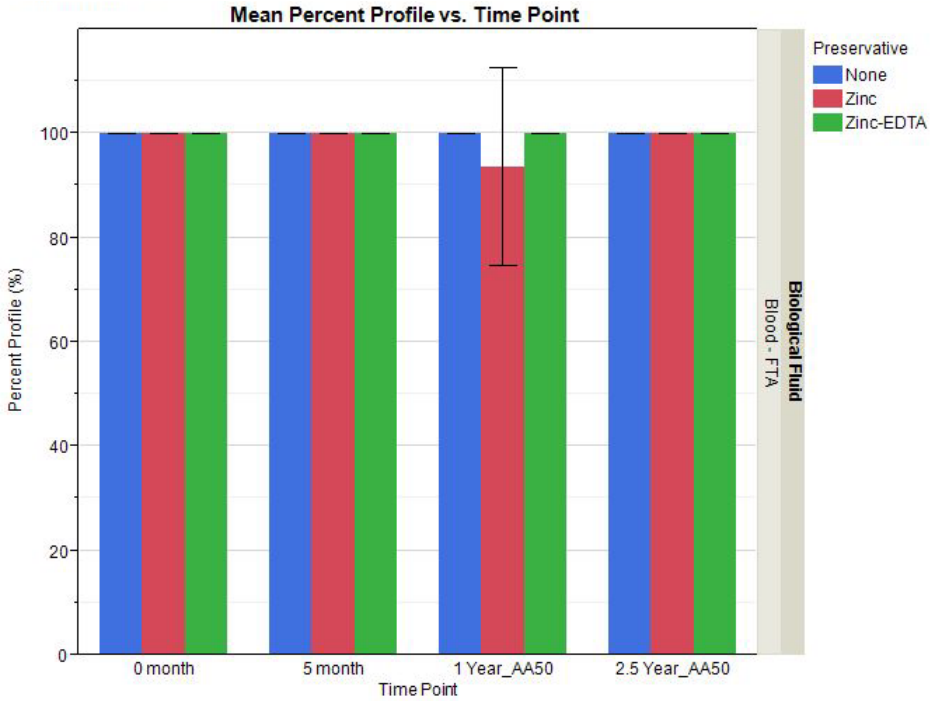

Figure 52: Mean percent profile (%) generated for all blood samples on FTA paper that were treated with Zinc and Zinc-EDTA across all Phase III time points.. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Mean Peak Height Values

The mean average peak heights generated for the untreated blood on FTA samples are demonstrated in Figure 53. Across all time points, the mean average peak height (PH) for the untreated control samples on FTA paper ranged from 4700 - 5900 RFU. The mean average peak height values for the Zinc treated blood samples on FTA paper ranged from 4200 – 6100 RFU, whereas the mean average peak height values for the Zinc-EDTA treated blood samples on FTA paper ranged from 3100 – 4000 RFU. No statistically significant differences were observed between the peak heights values generated by the Zinc treated samples and the untreated control samples. Across all time points, statistically significant decreases in peak height were observed when comparing the Zinc-EDTA treated samples to both the untreated control samples and the Zinc treated samples (Table 20).

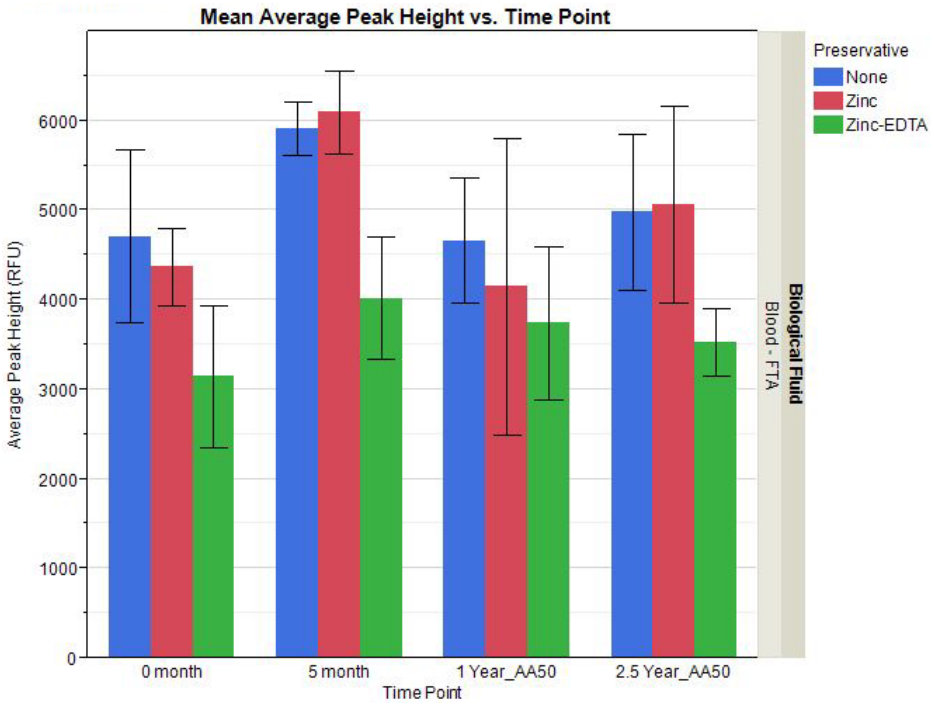

Figure 53: Mean average peak heights generated for all Zinc treated and Zinc-EDTA treated blood samples on FTA paper across all Phase III time points. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Table 20: P-values indicating statistically significant differences between the peak heights generated by the untreated, Zinc treated, and Zinc-EDTA treated control blood on FTA samples. P-values less than 0.05 indicate a statistically significant difference.

Blood on FTA Sample Types	Peak Height p-Value			
	0 Month	5 Month	1 Year_AA50	2.5 Year_AA50
Zinc Treated & Untreated	0.3491	0.8611	0.4069	0.348
Zinc-EDTA Treated & Untreated	0.0017	0.0003	0.0235	9.78×10^{-7}
Zinc-EDTA Treated & Zinc Treated	0.0009	0.0010	0.5174	1.10×10^{-6}

Profile Balance Ratios

Figure 54 displays the overall profile balance results that were generated for the treated blood on FTA samples that produced full profiles. Across all time points, the untreated control and the Zinc treated samples produced mean average profile balance ratios less than 8.0, suggesting some imbalance, whereas the Zinc-EDTA treated samples a mean average profile balance ratio of 26.0 across all time points. A slight increase in overall profile imbalance was observed for all treated and untreated samples at the 1 year RT equivalent at 50°C time point. No statistically significant differences in profile balances ratios were observed between the Zinc treated and untreated control

samples. Across all time points, statistically significant increases in profile balance ratios were observed when comparing the Zinc-EDTA treated samples to both the untreated control samples and the Zinc treated samples (Table 21).

PowerPlex Fusion STR profiles from seven blood on FTA samples are displayed in Figure 55. Untreated blood on FTA sample at the 0 month time point; Zinc treated blood on FTA samples at the 5 month, 1 year RT equivalent at 50°C, and 2 year RT equivalent at 50°C time points; and Zinc-EDTA treated blood on FTA samples at the 5 month, 1 year RT equivalent at 50°C, and 2.5 year RT equivalent at 50°C time points are displayed. Decreasing profile balance was observed over time.

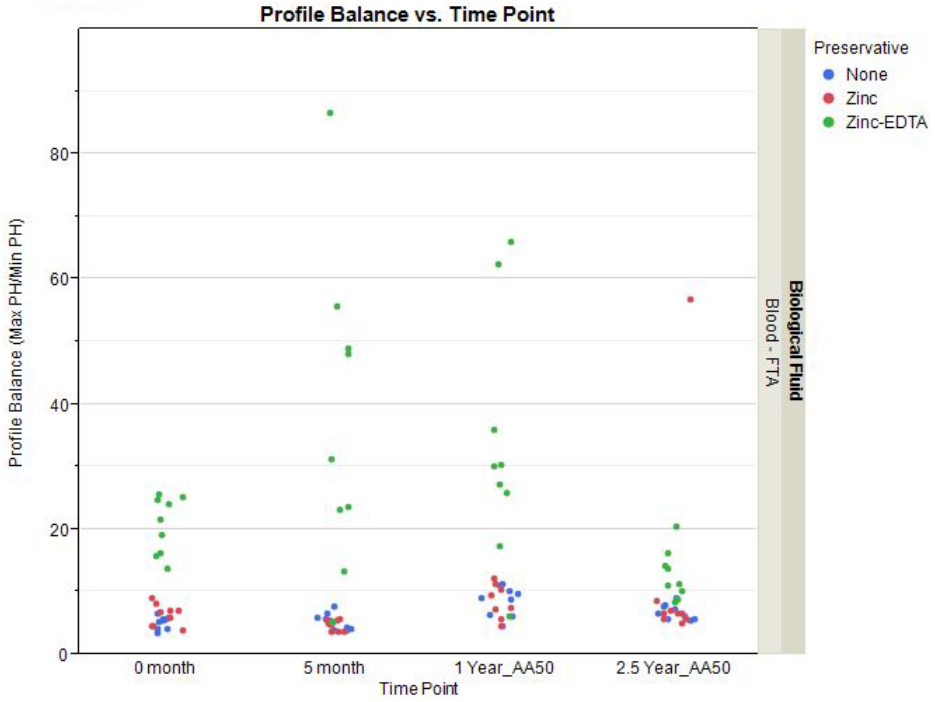

Figure 54: Overall profile balance generated for all blood samples on FTA paper that were treated with Zinc and Zinc-EDTA across all Phase III time points. The untreated control samples are represented as "None."

Table 21: P-values indicating statistically significant differences between the profile balance ratios generated by the untreated, Zinc treated, and Zinc-EDTA treated control blood on FTA samples. P-values less than 0.05 indicate a statistically significant difference.

Blood Sample Types	Profile Balance p-Value			
	0 Month	5 Month	1 Year_AA50	2.5 Year_AA50
Zinc Treated & Untreated	0.0994	0.3496	0.5365	0.3707
Zinc-EDTA Treated & Untreated	3.125×10^{-8}	0.0004	0.0015	0.0015
Zinc-EDTA Treated & Zinc Treated	1.553×10^{-7}	0.9185	0.0012	0.0012

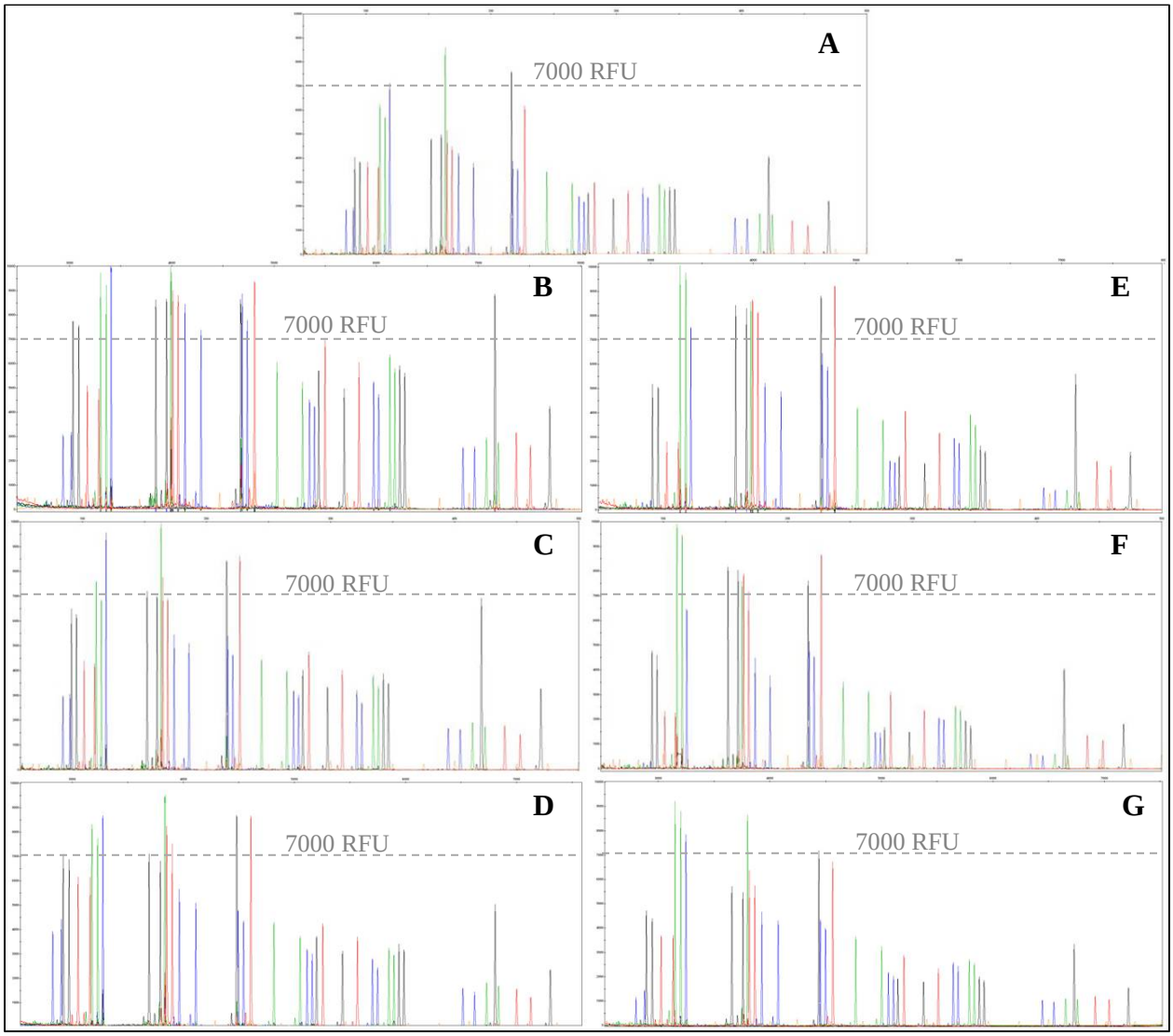

Figure 55: Representative PowerPlex Fusion STR profiles are displayed from the following samples that were subjected to direct amplification: (A) untreated control blood on FTA sample at the 0 month time point, (B) Zinc treated blood on FTA sample at the 5 month time point, (C) Zinc treated blood on FTA sample at the 1 year RT equivalent at 50°C time point, (D) Zinc treated blood on FTA samples at the 2.5 year RT equivalent at 50°C time point, (E) Zinc-EDTA treated blood on FTA sample at the 5 month time point, (F) Zinc-EDTA treated blood on FTA sample at the 1 year RT equivalent at 50°C time point, (G) Zinc-EDTA treated blood on FTA sample at the 2.5 year RT equivalent at 50°C time point.

Saliva

Mean Percent Profile

The mean percent profiles generated for the saliva on indicating FTA paper and Buccal DNA Collectors are demonstrated in Figure 56. At the 0 month and 1 year RT equivalent at 50°C time points, the mean percent profile for the untreated control samples ranged from 70 – 100% complete profiles. Regardless of the substrate, all saliva samples that contained the Zinc preservative did not

generated any profiles when subjected to direct amplification with PowerPlex Fusion. A mean percent profile of 10% was generated for the saliva samples treated with Zinc-EDTA on indicating FTA paper. Full DNA profiles were generated by only one out of nine Zinc-EDTA treated saliva on FTA samples. A lack of preservative on the area where the sample was punched most likely contributed to this result. Overall, these results suggest that the preservatives at their present concentrations are inhibiting the amplification reaction. Based on the results generated by the treated saliva samples at the 0 month and 1 year RT equivalent time points, the remaining saliva sample time points were not processed as the treated samples were not anticipated to generate profiles.

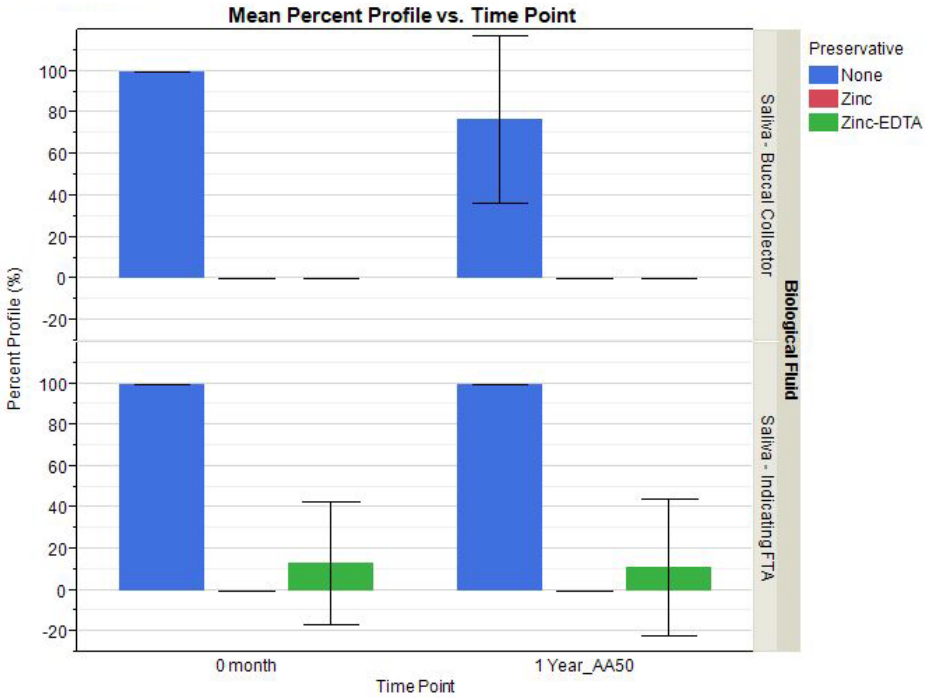

Figure 56: Mean percent profile (%) generated for all saliva on indicating FTA and saliva on Buccal DNA Collector samples treated with Zinc and Zinc-EDTA across all Phase III time points. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Mean Peak Height Values

The mean average peak heights generated for the saliva on indicating FTA paper and Buccal DNA Collectors are demonstrated in Figure 57. At the 0 month time point, the mean average peak heights (PH) for the untreated control samples on Buccal DNA Collectors and indicating FTA paper were approximately 4,000 RFU. At the 1 year RT equivalent at 50°C time points, the average peak heights generated were approximately 1500 RFU regardless of substrate tested. As noted above, no profiles were generated from the samples that were treated with Zinc. Mean average peak heights of 12 RFU were generated from the saliva on FTA samples that were treated with the Zinc-EDTA combination. As noted above, only 1 out of 9 total Zinc-EDTA treated saliva samples produced a DNA profile.

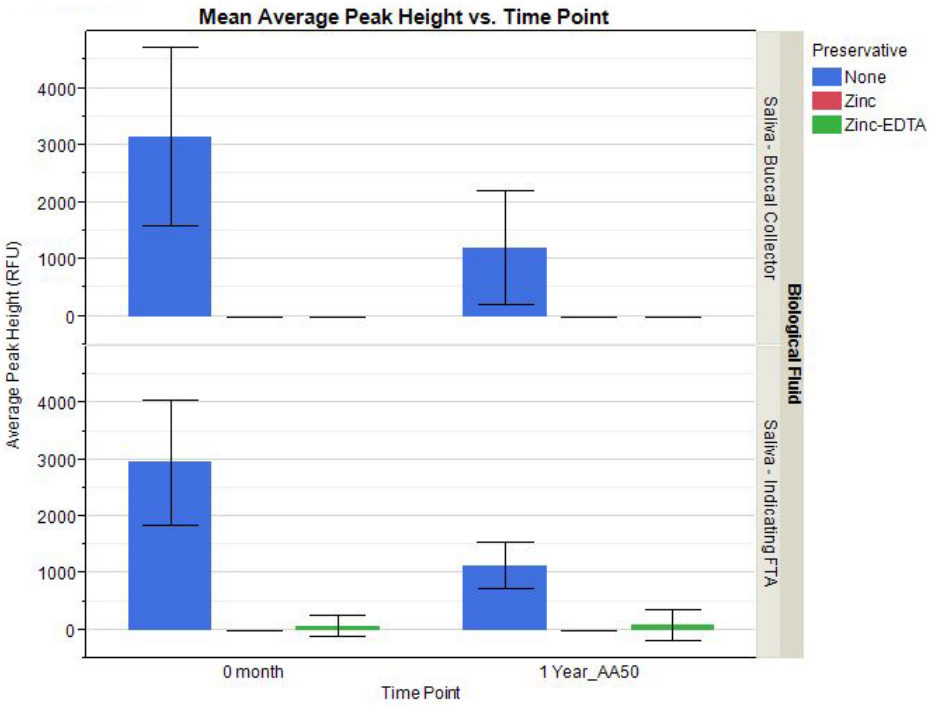

Figure 57: Mean average peak heights generated for all saliva on indicating FTA and saliva on Buccal DNA Collector samples treated with Zinc and Zinc-EDTA across all Phase III time points. The untreated control samples are represented as "None." Each error bar is constructed using one standard deviation from the mean.

Profile Balance Ratios

Figure 58 displays the overall profile balance results that were generated for the saliva samples that generated full DNA profiles. Because the majority of the treated saliva samples did not produce full profiles, all but one sample represented in the graph belongs to the untreated control samples. Regardless of the collection substrate, the overall profile imbalance increased over time for the untreated control samples. This result suggests degradation of the saliva over time or amplification inhibition of the DNA by the applied preservative.

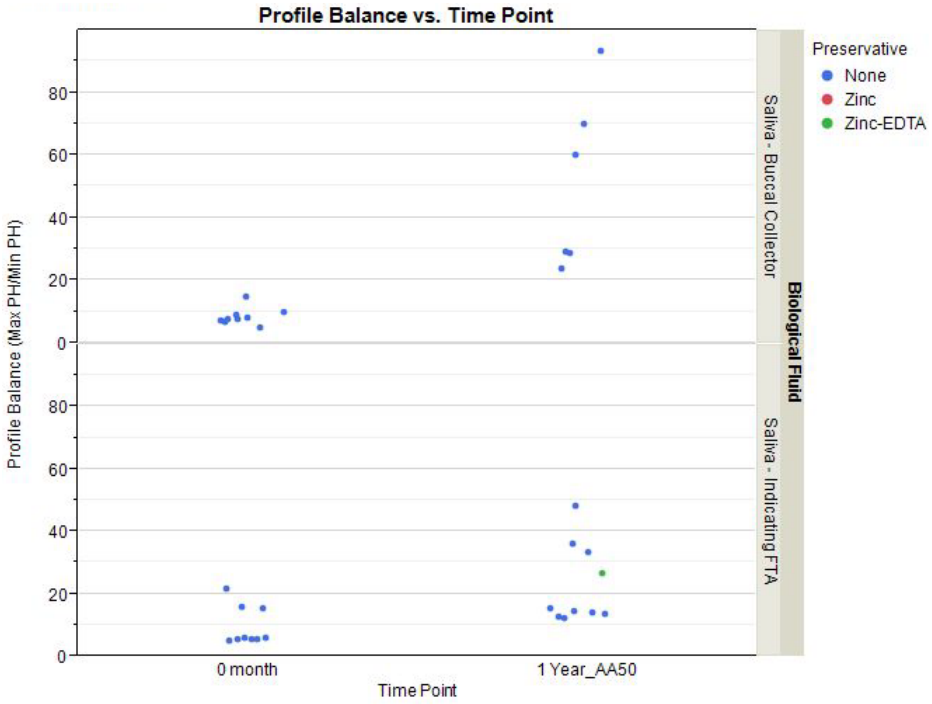

Figure 58: The overall profile balance generated for all saliva on indicating FTA and saliva on Buccal DNA Collector samples treated with Zinc and Zinc-EDTA across all Phase III time points. The untreated control samples are represented as "None."

Conclusions

Discussion of Findings

Phase I

After examining twelve COTS preservatives that covered a range of preservative types, including nuclease inhibitors, antimicrobials, chelators, fixatives, and antioxidants, the most effective preservatives were found to be Sodium Azide (antimicrobial), Parabens (antimicrobial), EDTA (chelator), Zinc (fixative), and Propyl Gallate (antioxidant). Across varying time points and biological fluids, the peak height values and percent profiles generated by these preservatives demonstrated statistically significant increases when compared to the untreated samples.

To understand why these preservatives were the most effective, first it is necessary to understand some of the processes behind DNA degradation. Although DNA degradation may be enhanced in the presence of nucleases, bacteria, and fungi, the principle processes involved in DNA degradation are hydrolysis and oxidation [53, 54]. During hydrolysis, the bonds between the sugar and base of the nucleotides are attacked, and depending on the pH conditions, the DNA may be depurinated (low pH) or cleaved at apurinic or apyramidinic sites (high pH) [54]. Depurination is loss of guanine or adenine residues at specific sites in the DNA. At these sites, the DNA is weakened and more likely to undergo strand breakage.

Oxidation is another process that can cause DNA damage, particularly at the guanine residues. Reactive oxygen species (ROS), such as superoxide ($\bullet O_2^-$) and singlet oxygen ($^1O_2$), are generated from normal oxygen metabolism that occurs in cells. Superoxide is not generally dangerous to DNA; however, it is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. Hydrogen peroxide presents a threat to DNA after it is converted to hydroxyl radicals (•OH) by the Fenton Reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$). The hydroxyl radicals then create DNA lesions by converting guanine to 8-oxoguanine [55]. Similarly, $^1O_2$ reacts with guanine to produce 8-oxo-7,8-dihydroguanine and spiroiminodihydantoin [56]. As with the depurinated DNA sites, these sites become more likely to undergo strand breakage.

Based on this information, why did Sodium Azide, Parabens, EDTA, Zinc, and Propyl Gallate demonstrate preservative effects on the stored DNA samples? Of these preservatives, the two that are most frequently used in forensic molecular biology are EDTA and Sodium Azide. EDTA is commonly used in TE^{-4} buffer to promote the preservation of extracted DNA, and its mode of action is well categorized. EDTA is a chelator that binds to divalent metal ions, such as Mg^{2+}, Ca^{2+}, and Fe^{2+}. With its four carboxyl groups, EDTA becomes negatively charged under alkaline conditions and forms coordinate (or dative) covalent bonds with divalent cations that function as cofactors for nucleases. Nuclease inhibition via chelation is an important function of EDTA, but the chelating properties of EDTA also have an antioxidant effect. With the Fenton Reaction in mind, it can be hypothesized that EDTA chelates the free iron (Fe^{2+}) necessary to produce hydroxyl radicals, preventing oxidative damage to the guanine residues. Sodium Azide is also used in molecular biology as a preservative in reagents and buffers. For instance, Qiagen includes Sodium Azide in their Buffer ATE and Buffer AVE. As previously stated, Sodium Azide is a bacteriostatic that inhibits the growth of Gram-negative bacteria by inhibiting cytochrome oxidase [19]. This is achieved because Sodium Azide is a known quencher of singlet oxygen ($^1O_2$), which has been shown to induce oxidative DNA damage [56, 57]. As such, Sodium Azide is an antioxidant. Although the bacteriostatic properties of Sodium Azide are of interest to the forensic community, the antioxidant properties of Sodium Azide are perhaps more effective for DNA preservation.

Propyl Gallate is a phenolic antioxidant. Phenolic antioxidants function by interrupting the free radical chain reaction. This can be accomplished through two mechanisms: hydrogen atom transfer (HAT) and/or electron transfer [58]. The chain reaction and disruption occur as follows: a free radical ($R^\bullet$) is generated (reaction 1), O_2 is added (reaction 2), and lipid molecules (RH) are converted into lipid hydroperoxide (ROOH) (reaction 3), resulting in oxidation.

Reaction 1: $RH \rightarrow R^\bullet$

Reaction 2: $R^\bullet + O_2 \rightarrow RO_2^\bullet$

Reaction 3: $RO_2^\bullet + RH \rightarrow ROOH + R^\bullet$

During HAT, the antioxidant (ArOH) disrupts the chain reaction by taking the place of the lipid molecule (reaction 4), whereas during electron transfer, the radical cation is formed and then deprotonated (reaction 5). Propyl Gallate has been shown to be more likely to function by HAT [58, 59].

Reaction 4: $RO_2^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet}$

Reaction 5: $R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet+} \rightarrow ArO^{\bullet} + RH$

Parabens were classified as antimicrobials that are effective against fungi and Gram-positive bacteria. By targeting the proton motive force involved in active transport, oxidative phosphorylation, and ATP synthesis, parabens function as membrane active agents that are active at the cytoplasmic membrane level of the bacterial cell [60]. Propyl paraben also alters the integrity of bacterial membranes [61]. Along with their antimicrobial properties, Parabens are also phenolic antioxidants, which function as described above.

Zinc chloride has been used as a fixative since the 1990s, when it gained popularity as a substitute for highly toxic mercury chloride. The mechanism of action for both zinc chloride and mercury chloride is not fully understood or well documented; however, it is known that mercury chloride reacts with amines, amides, amino acids and sulphydryl groups [62]. Mercury chloride is also known to cross-link cysteine, some proteins, and some thiol groups [63, 64, 65]. Similarly, zinc chloride reacts with amino, carboxyl and sulphydryl groups, forming reversible reaction products. Zinc also has antioxidant properties [66, 67]. There are three proposed mechanisms for the antioxidant activities of zinc: long-term exposure to zinc can be linked to the induction of another substance that serves as the ultimate antioxidant, acute exposure to zinc may protect protein sulfhydryl groups, and acute exposure may also reduce the formation of ·OH from H_2O_2 and $\bullet O_2^-$.

All of the preservatives that demonstrated a statistically significant beneficial effect on the samples possessed antioxidant properties; however, at most time points Ascorbic Acid, the other antioxidant examined in this study, did not differ significantly from the untreated samples. In fact, at the 2.5 year RT equivalent time point, the Ascorbic Acid blood samples demonstrated a statistically significant decrease in peak height values when compared to the control samples. It is unknown why the Ascorbic Acid treated samples did not perform as well as those that were treated with the other antioxidants; however, the pH of the preservative may be a factor. As noted above, overly acidic or alkaline conditions can adversely affect the quality of the DNA. Although the pH of most of the preservatives was not measured, it is known that Ascorbic Acid produces a mildly acidic solution when mixed in water. Furthermore, Bronopol was shown to be the worst performing preservative across all data analysis metrics, and it likely had the lowest pH of all of the preservatives as it was prepared by dissolution in McIlvaine Buffer, pH 3.2.

Overall, the Phase I results indicated that it may be beneficial to further examine chelators and antioxidants for the preservation of forensic DNA samples.

Forensic Index

In this study, the Forensic Index was found to be a useful method for the assessment of profile quality. This index assesses the quality of a DNA profile by providing a single quantitative value that takes three factors into consideration: overall peak height, profile balance within each locus in a profile, and the profile balance across all loci of a profile. When comparing the FI values from the treated samples to those from the untreated control samples, statistically significant increases

in FI were observed from the Sodium Azide treated, Parabens treated, EDTA treated, Zinc treated, and Propyl Gallate treated samples. It was also observed that the Bronopol treated samples generated the lowest quality profiles across all time points and biological fluids. In general, the results from the Forensic Index rankings and the previous data analyses were in concordance. Ultimately, if the FI had been the only method used to analyze the data in this study, the conclusions made regarding the most effective preservatives would have been same as those made by examining the percent profiles, peak height values, and profile balances. Because the FI assigned a single value to each sample, it simplified the processes used to assess the overall profile quality and to determine the most effective preservatives.

Phase II

Statistically significant increases in profile quality (peak height and percent profile) were observed from all of the preservative combinations, except for Nisin-Lysozyme. Zinc-EDTA, Sodium Azide-EDTA, Parabens-EDTA, Propyl Gallate-EDTA, Nisin-EDTA, Lysozyme-EDTA, Zinc-EDTA-Sodium Azide, and Nisin-Lysozyme-EDTA were shown to generate statistically significant differences in percent profile and peak height values when compared to the untreated blood, saliva, and vaginal fluid samples. This demonstrated that combining preservatives can be effective; however, further studies will have to be conducted to determine if the combined preservatives were more effective that the individual preservatives.

In Phase II, a comparison of the PowerPlex Fusion kit and PowerPlex 16 kit was also performed. On average, the PowerPlex Fusion samples generated higher average peak height values than the PowerPlex 16 samples. This is of particular interest because when amplifying with PowerPlex Fusion, 1.0 ng of DNA was targeted, whereas 1.5 ng of DNA was targeted with PowerPlex 16. Additionally, the PowerPlex Fusion amplification was performed with one fewer PCR cycle (29 cycles) than the PowerPlex 16 amplification (30 cycles). These results demonstrated the sensitivity of the PowerPlex Fusion System. The PowerPlex Fusion kit also produced more full profiles than the PowerPlex 16 kit; however, the PowerPlex 16 kit generally produced more balanced profiles. Overall, the data gathered from this study indicated that the PowerPlex Fusion kit is a robust and sensitive system. With the advent of new amplification kits such as PowerPlex Fusion, it will be possible to obtain more information from lower quality DNA samples, consequently diminishing the need for preservatives such as those examined in this study.

Phase III

Direct amplification with Promega's PowerPlex Fusion kit was successful with the Zinc treated and Zinc-EDTA treated blood on FTA samples. While no statistically significant differences were observed between the peak height values and profiles balance ratios from the untreated control samples and the Zinc treated blood samples, statistically significant decreases in peak heights values and statistically significant increases in profile balance ratios were observed when the Zinc-EDTA treated blood on FTA samples were compared to both the untreated control samples and the Zinc treated samples. As all Phase III samples were prepared and stored simultaneously and the Zinc-EDTA preservative combination was shown to be effective in Phase II, it is unlikely that the decreases in profile quality were due to sample degradation. Amplification inhibition most

likely caused the decreased peak height values and increased profile imbalance. This is unsurprising as EDTA is a known amplification inhibitor. When enough EDTA is present during PCR, it can bind the free magnesium that functions as a co-factor for the polymerase in the reaction mix. If enough free magnesium is chelated by the EDTA, the activity of the enzyme will be reduced, and the amplification will be less effective. In Phase II, inhibition was not an issue with the Zinc-EDTA treated blood samples because those samples were extracted, which produced a purified DNA extract that was free of excess EDTA. In future studies, a range of EDTA concentrations could be investigated to determine a concentration that effectively preserves the DNA but no longer inhibits the amplification reaction.

Direct amplification was not successful with saliva samples on FTA and Buccal DNA Collectors that were treated Zinc and Zinc-EDTA. While the untreated control saliva samples produced high partial to full profiles, the treated saliva samples generally failed to generate profiles. Because the untreated control saliva samples produced profiles, it is most likely that the direct amplification of the treated samples failed due amplification inhibition. Unlike the blood samples, the saliva samples likely did not contain enough DNA to overcome the inhibitory effects of the preservatives. As stated above, in future studies, titrations of preservative concentrations could be investigated to determine a concentration that effectively preserves the DNA but no longer inhibits the amplification reaction.

Implications for policy and practice

This proposal outlined a method by which biological evidence collected on swabs may be effectively preserved for extensive periods of time. Evidence at crime scenes is frequently collected on sterile cotton tipped swabs and stored for future analysis. Many times evidence is stored for months to years at a time awaiting state or federal funding, and in that time precious sample may be lost to degradative insults such as microbes, nucleases, or general environmental conditions. Commercial off the shelf (COTS) preservatives used for decades in the food and cosmetics industries may have direct applications for forensic practices to preserve biological evidence. These COTS preservatives are very inexpensive and safe, and could easily be applied to the cotton swab by the forensic investigator at the crime scene. If done in this manner, the forensic investigator could be confident that the sample being collected will result in favorable results, regardless of when the evidence is processed. This method represents a novel mechanism for preservation of sample as currently there are no measures being taken to prevent sample degradation. This method does not require expensive instruments or specialized skills, and can easily be adopted by any state crime lab regardless of funding level. The application of preservatives now could aid in the processing of cold cases in the future by preventing the degradation of DNA evidence kept in long-term storage. It is of great importance to obtain full DNA profiles in order to convict suspects.

Study limitations

Although this study provided a large quantity of data, it was also subject to several limitations. First, temperature was the only factor that was examined. Samples were stored at room temperature or 50°C/60°C to artificially age the samples. Under these conditions, the degradation observed in the blood and semen samples was limited. It would be beneficial to examine additional environmental factors that may impact sample integrity during storage. For example, different temperatures or humidity conditions may encourage the blood and semen samples to degrade, allowing for a better assessment of the COTS preservatives' effects on those sample types. Another possible limitation presented by the accelerated aging temperatures was that the effects of the temperatures on any bacteria present were unknown. The accelerated aging samples were subjected to storage at 50°C or 60°C for as many as 262 days. Most bacterial growth occurs between 5°C-60°C; however, the optimal growth temperature is 20°C-45°C for many food borne bacteria and 37°C for many bacteria found in the human microbiome. At the temperatures used in the study, some of the bacteria of interest may have been killed. Another limitation to be considered is that all of the blood used in the study was purchased from a commercial vendor and contained potassium-EDTA preservatives that prevent coagulation. Such additives would not be present in blood samples collected from a crime scene and may have impacted the degradation of the blood samples and the effectiveness of the preservatives on those samples. It should also be noted that the biological fluids were spotted on the swabs in a clean environment, and all the samples were stored in clean conditions. In general, any microbes present on the samples would have been naturally occurring microbial flora found in the human microbiome; however, no efforts were made to identify what, if any, microbes were present on the swabs. Some of the preservatives may have had different effects if they were in the presence of different types of microbes, such as microbes not typically found in the human microbiome that may be collected when swabbing a sample at a crime scene. Additionally, a "ski slope" effect was often observed in the profiles, particularly in profiles produced by the Zinc-EDTA treated blood on FTA samples examined in Phase III. In these profiles, the smaller loci amplified preferentially over the larger loci. When this occurs, it is generally caused by degraded template DNA or the presence of inhibitors during amplifications; however, this study did not attempt to confirm if the preferential amplification was due to DNA degradation or amplification inhibition by the preservatives.

Implications for further research

Despite its limitations, this study provides a solid basis for further research. Future studies could examine additional environmental conditions that would be conducive to bacterial growth. To encourage bacterial growth, 37°C temperature conditions and varying humidity levels could be studied. It would also be beneficial to test the efficacy of the preservatives by examining them in conjunction with plated colonies of known microbes frequently associated with forensic samples. As previously noted, this study did not attempt to determine if preferential amplification of the smaller loci was a result of sample degradation or amplification inhibition by the preservatives. In future studies, gel electrophoresis could be used to assess the quality of the DNA prior to amplification. In Phase III of this study, profiles were not successfully produced following direct amplification of saliva samples on FTA paper and Buccal DNA Collectors. It was strongly

believed that this was due to inhibition by the preservatives. To address inhibition issues, further research could be conducted to examine various preservative concentrations and determine the ideal concentration that maintains the preservative effect without inhibiting the samples. Furthermore, the data generated in this study can be used to augment and refine the preservation methods used in forensic science. Additional COTS preservatives that function via the same mechanisms of action as the effective preservatives should be identified and examined for their efficacy when used to preserve biological evidence. Finally, a true long-term room temperature storage study can be performed, and the results from this study can be compared to the accelerated aging results generated in Phase I. By pursuing these avenues for further research, it will be possible to strengthen and expand upon the data already generated.

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Dissemination of Research Findings

The results of this NIJ funded grant have been disseminated to the forensic community at the following:

1. On Tuesday June 19th 2012, Heather Cunningham participated in a concurrent panel entitled" "Contamination and Degradation: A Quick and Dirty Dilemma in Viable Evidence Retention" at the NIJ Conference 2012. The presentation focused on the results generated to date for this NIJ funded (2010-DN-BX-K193) research project the "Effective Long-term Preservation of Biological Evidence".

2. Heather Cunningham was invited by John Butler from the National Institute of Standards and Technology (NIST) to present the interim results of this NIJ funded (2010-DN-BX-K193) research project the "Effective Long-term Preservation of Biological Evidence" to the members of his laboratory. Ms. Cunningham presented the findings on September 10th, 2012.

3. On Tuesday October 16th 2012, Heather Cunningham presented a poster at the 23rd International Symposium on Human Identification in Nashville, TN detailing the interim results of this NIJ funded (2010-DN-BX-K193) research project the "Effective Long-term Preservation of Biological Evidence".

4. On Wednesday October 9th, 2013 Jamia Fillinger presented a poster at the 24th International Symposium on Human Identification in Atlanta, Georgia detailing results generated to date of this NIJ funded (2010-DN-BX-K193) research project the "Effective Long-term Preservation of Biological Evidence".

APPENDIX A: FORENSIC INDEX DATA

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Actin	0 month RT	126738	0.93	2.50	2.54	0.92	-0.03	2.95	9.53
			100610	0.93	2.56	1.72	0.95	0.20	2.51	9.21
			56521	0.93	2.45	0.33	0.92	-0.23	0.95	8.09
		2 months RT	107073	0.93	2.51	1.92	0.89	0.03	2.46	9.18
			101109	0.93	2.54	1.74	0.94	0.13	2.46	9.18
			122009	0.89	2.54	2.39	0.39	0.11	2.45	9.17
		6 months RT	78109	0.94	2.49	1.01	1.00	-0.06	1.73	8.65
			83174	0.92	2.49	1.17	0.81	-0.06	1.69	8.63
			76707	0.91	2.51	0.97	0.54	0.02	1.34	8.37
		8 months RT	115927	0.93	2.54	2.20	0.93	0.13	2.83	9.45
			105247	0.95	2.52	1.87	1.12	0.04	2.66	9.32
			88363	0.89	2.53	1.33	0.31	0.10	1.49	8.48
		10 months RT	78361	0.95	2.53	1.02	1.16	0.10	2.05	8.88
			71174	0.93	2.52	0.79	0.91	0.07	1.59	8.55
			77225	0.92	2.52	0.98	0.69	0.07	1.54	8.51
		1 year_AA50	84626	0.92	2.48	1.22	0.71	-0.09	1.60	8.56
			65431	0.91	2.43	0.61	0.58	-0.28	0.80	7.99
			34653	0.94	2.38	-0.35	0.99	-0.49	0.20	7.55
		1 year_AA60	72359	0.87	2.05	0.83	0.03	-1.79	-0.93	6.73
			59072	0.84	2.04	0.41	-0.34	-1.82	-1.66	6.21
			87038	0.74	1.98	1.29	-1.75	-2.04	-2.50	5.61
		2.5 year_AA50	86839	0.93	2.40	1.29	0.89	-0.43	1.52	8.50
			105153	0.84	2.23	1.86	-0.39	-1.09	0.16	7.52
			65568	0.88	2.25	0.62	0.24	-1.00	-0.18	7.28
		5 year_AA50	41560	0.85	2.15	-0.14	-0.23	-1.40	-1.61	6.24
			38054	0.85	2.03	-0.25	-0.22	-1.84	-2.11	5.89
			32224	0.88	1.92	-0.43	0.23	-2.30	-2.25	5.79
		5 year_AA60	76	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.16
			12090	0.70	1.16	-1.06	-2.20	-5.24	-7.81	1.78
			7727	0.58	0.88	-1.20	-3.91	-6.34	-10.57	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Ascorbic Acid	0 month RT	102884	0.96	2.55	1.79	1.34	0.18	2.94	9.52
			96803	0.94	2.55	1.60	1.04	0.16	2.46	9.18
			92969	0.91	2.53	1.48	0.59	0.10	1.88	8.76
		2 months RT	110790	0.94	2.53	2.04	0.97	0.09	2.70	9.35
			119821	0.90	2.51	2.32	0.42	0.03	2.34	9.09
			93954	0.94	2.53	1.51	1.07	0.07	2.34	9.09
		6 months RT	75632	0.92	2.46	0.93	0.78	-0.16	1.37	8.39
			57726	0.92	2.52	0.37	0.80	0.06	1.13	8.22
			54314	0.92	2.49	0.26	0.77	-0.05	0.92	8.07
		8 months RT	116334	0.93	2.50	2.21	0.95	-0.01	2.73	9.37
			113707	0.92	2.53	2.13	0.68	0.08	2.48	9.19
			91504	0.93	2.54	1.43	0.83	0.11	2.08	8.90
		10 months RT	64633	0.91	2.49	0.59	0.66	-0.07	1.05	8.16
			54174	0.94	2.48	0.26	0.98	-0.12	1.05	8.16
			57164	0.92	2.50	0.35	0.73	-0.01	0.98	8.11
		1 year_AA50	78281	0.93	2.46	1.02	0.89	-0.18	1.53	8.51
			57116	0.93	2.43	0.35	0.89	-0.30	0.87	8.03
			53618	0.94	2.42	0.24	1.01	-0.32	0.87	8.03
		1 year_AA60	127283	0.91	2.30	2.56	0.68	-0.79	2.03	8.87
			49042	0.92	2.33	0.10	0.79	-0.70	0.20	7.55
			69704	0.86	2.23	0.75	0.00	-1.09	-0.39	7.12
		10 year_AA60	25574	0.76	1.63	-0.64	-1.40	-3.41	-5.00	3.80
			20450	0.75	1.41	-0.80	-1.56	-4.26	-6.07	3.03
			14764	0.74	1.37	-0.98	-1.71	-4.42	-6.51	2.71
		2.5 year_AA50	98298	0.90	2.27	1.65	0.48	-0.92	0.97	8.10
			80998	0.90	2.36	1.10	0.51	-0.57	0.87	8.03
			90217	0.87	2.34	1.39	0.06	-0.67	0.59	7.83
		5 year_AA50	55923	0.89	2.02	0.31	0.40	-1.89	-1.09	6.62
			43159	0.85	2.12	-0.09	-0.16	-1.51	-1.61	6.25
			39654	0.86	1.97	-0.20	-0.06	-2.08	-2.13	5.87
		5 year_AA60	33223	0.82	1.63	-0.40	-0.67	-3.43	-4.12	4.44
			18400	0.77	1.30	-0.87	-1.31	-4.70	-6.29	2.87
			12382	0.68	1.40	-1.06	-2.57	-4.30	-7.28	2.16
		10 year_AA60	25574	0.76	1.63	-0.64	-1.40	-3.41	-5.00	3.80
			20450	0.75	1.41	-0.80	-1.56	-4.26	-6.07	3.03
			14764	0.74	1.37	-0.98	-1.71	-4.42	-6.51	2.71

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	ATA	0 month RT	109011	0.95	2.54	1.98	1.19	0.12	2.89	9.49
			110064	0.92	2.55	2.02	0.69	0.17	2.48	9.19
			92265	0.93	2.55	1.46	0.90	0.17	2.22	9.01
		2 months RT	88403	0.95	2.53	1.34	1.12	0.10	2.27	9.04
			92354	0.94	2.52	1.46	1.03	0.05	2.24	9.02
			88200	0.94	2.54	1.33	1.06	0.13	2.24	9.02
		6 months RT	82730	0.92	2.48	1.16	0.78	-0.09	1.62	8.57
			64702	0.93	2.52	0.59	0.91	0.04	1.40	8.42
			57088	0.93	2.49	0.35	0.91	-0.05	1.12	8.21
		8 months RT	97150	0.94	2.52	1.61	1.03	0.07	2.38	9.12
			106792	0.92	2.52	1.91	0.79	0.04	2.37	9.11
			95043	0.89	2.52	1.54	0.36	0.05	1.66	8.60
		10 months RT	74546	0.93	2.55	0.90	0.92	0.16	1.78	8.69
			71531	0.92	2.50	0.81	0.76	-0.04	1.36	8.38
			53325	0.89	2.49	0.23	0.29	-0.05	0.42	7.71
		1 year_AA50	68295	0.92	2.41	0.70	0.71	-0.36	0.93	8.07
			59591	0.91	2.45	0.43	0.66	-0.21	0.80	7.98
			68498	0.90	2.40	0.71	0.54	-0.40	0.74	7.94
		1 year_AA60	106025	0.90	2.31	1.89	0.54	-0.78	1.35	8.38
			110904	0.89	2.29	2.04	0.40	-0.85	1.29	8.33
			84960	0.91	2.12	1.23	0.68	-1.51	0.27	7.60
		2.5 year_AA50	75314	0.92	2.28	0.92	0.76	-0.88	0.68	7.89
			94634	0.88	2.23	1.53	0.25	-1.10	0.50	7.76
			104017	0.85	2.29	1.83	-0.25	-0.84	0.49	7.76
		5 year_AA50	39850	0.90	2.04	-0.19	0.41	-1.80	-1.41	6.39
			37854	0.86	1.94	-0.25	-0.08	-2.20	-2.31	5.74
			23231	0.81	2.08	-0.71	-0.74	-1.65	-2.81	5.38
		5 year_AA60	10804	0.67	1.18	-1.10	-2.64	-5.15	-8.17	1.52
			10185	0.62	1.04	-1.12	-3.29	-5.71	-9.34	0.67
			14759	0.61	1.03	-0.98	-3.53	-5.73	-9.46	0.59

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Bronopol	0 month RT	117679	0.90	2.52	2.26	0.41	0.05	2.29	9.06
			80640	0.95	2.52	1.09	1.09	0.06	2.00	8.85
			55862	0.93	2.49	0.31	0.88	-0.07	1.04	8.15
		2 months RT	111544	0.93	2.45	2.06	0.84	-0.22	2.31	9.07
			120803	0.87	2.46	2.35	0.12	-0.20	1.87	8.76
			68032	0.92	2.42	0.70	0.77	-0.34	0.99	8.12
		6 months RT	60354	0.94	2.47	0.45	0.99	-0.13	1.21	8.28
			54017	0.92	2.42	0.25	0.75	-0.33	0.63	7.86
			55703	0.91	2.30	0.31	0.66	-0.79	0.15	7.52
		8 months RT	71942	0.93	2.40	0.82	0.86	-0.40	1.13	8.22
			52577	0.91	2.30	0.21	0.61	-0.81	0.01	7.41
			36478	0.91	2.41	-0.30	0.57	-0.37	-0.04	7.38
		10 months RT	52103	0.93	2.44	0.19	0.92	-0.26	0.80	7.98
			41152	0.95	2.34	-0.15	1.15	-0.66	0.38	7.68
			55967	0.87	2.22	0.32	0.13	-1.10	-0.63	6.95
		1 year_AA50	32308	0.93	2.14	-0.43	0.82	-1.43	-0.88	6.77
			21656	0.87	2.15	-0.76	0.04	-1.39	-1.87	6.06
			33209	0.82	1.97	-0.40	-0.62	-2.09	-2.85	5.35
		1 year_AA60	82984	0.91	1.99	1.17	0.57	-2.00	-0.32	7.17
			57195	0.81	1.67	0.35	-0.81	-3.25	-3.48	4.90
			57147	0.78	1.71	0.35	-1.15	-3.10	-3.66	4.76
		2.5 year_AA50	47419	0.77	1.67	0.05	-1.29	-3.27	-4.20	4.38
			46873	0.83	1.68	0.03	-0.46	-3.23	-3.38	4.97
			40383	0.84	1.51	-0.17	-0.38	-3.87	-4.06	4.48
		5 year_AA50	42612	0.73	1.67	-0.10	-1.84	-3.24	-4.83	3.92
			33545	0.73	1.36	-0.39	-1.78	-4.46	-6.13	2.99
			25013	0.63	1.22	-0.66	-3.24	-5.00	-8.25	1.46
		5 year_AA60	3134	0.45	0.56	-1.35	-5.69	-7.58	-13.54	0.05
			14643	0.67	1.12	-0.98	-2.60	-5.39	-8.26	1.45
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Chitosan	0 month RT	64067	0.95	2.58	0.57	1.15	0.29	1.85	8.74
			79229	0.92	2.56	1.05	0.76	0.20	1.78	8.69
			59601	0.94	2.56	0.43	1.05	0.20	1.55	8.52
		2 months RT	125977	0.93	2.50	2.52	0.91	-0.01	2.93	9.52
			97564	0.93	2.53	1.62	0.82	0.10	2.21	9.00
			94744	0.91	2.54	1.54	0.58	0.15	1.96	8.82
		6 months RT	105373	0.94	2.49	1.87	1.08	-0.08	2.51	9.21
			63722	0.93	2.55	0.56	0.86	0.17	1.44	8.44
			36635	0.95	2.52	-0.29	1.09	0.07	0.87	8.03
		8 months RT	96631	0.92	2.55	1.59	0.72	0.17	2.16	8.96
			51371	0.94	2.55	0.17	0.98	0.18	1.24	8.30
			104714	0.92	2.53	1.85	0.72	0.10	2.30	9.07
		10 months RT	71901	0.93	2.55	0.82	0.94	0.16	1.72	8.64
			49542	0.93	2.57	0.11	0.83	0.26	1.14	8.23
			41237	0.93	2.56	-0.15	0.89	0.21	0.93	8.07
		1 year_AA50	92043	0.92	2.51	1.45	0.71	0.01	1.88	8.76
			61607	0.93	2.54	0.49	0.91	0.15	1.42	8.43
			61960	0.93	2.52	0.50	0.89	0.05	1.32	8.35
		1 year_AA60	160736	0.94	2.44	3.61	1.09	-0.26	3.77	10.12
			126998	0.88	2.35	2.55	0.19	-0.59	1.74	8.66
			59650	0.88	2.23	0.43	0.14	-1.06	-0.49	7.05
		2.5 year_AA50	122381	0.90	2.41	2.40	0.48	-0.38	2.08	8.91
			102450	0.89	2.44	1.78	0.29	-0.25	1.51	8.50
			103224	0.87	2.39	1.80	0.13	-0.45	1.20	8.27
		5 year_AA50	52761	0.88	2.19	0.21	0.26	-1.22	-0.69	6.90
			39807	0.91	2.16	-0.19	0.62	-1.34	-0.79	6.83
			28232	0.90	2.21	-0.56	0.42	-1.17	-1.12	6.60
		5 year_AA60	39101	0.87	1.52	-0.21	0.06	-3.83	-3.64	4.78
			22682	0.76	1.65	-0.73	-1.46	-3.35	-5.08	3.74
			25084	0.66	1.32	-0.66	-2.73	-4.63	-7.42	2.06
		10 year_AA60	19723	0.76	1.36	-0.82	-1.40	-4.44	-6.10	3.01
			15170	0.70	1.28	-0.97	-2.24	-4.77	-7.32	2.13
			24448	0.62	1.17	-0.68	-3.29	-5.21	-8.50	1.28

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	EDTA	0 month RT	97315	0.93	2.56	1.62	0.87	0.19	2.34	9.09
			81383	0.91	2.56	1.11	0.64	0.22	1.73	8.65
			102147	0.83	2.55	1.77	-0.43	0.17	1.19	8.26
		2 months RT	102841	0.94	2.53	1.79	1.06	0.10	2.59	9.27
			103692	0.90	2.50	1.82	0.50	-0.04	1.94	8.80
			90116	0.91	2.51	1.39	0.56	0.02	1.70	8.63
		6 months RT	101392	0.92	2.50	1.74	0.80	-0.01	2.19	8.99
			71407	0.93	2.53	0.80	0.85	0.09	1.56	8.53
			50206	0.91	2.48	0.13	0.61	-0.12	0.58	7.83
		8 months RT	88950	0.94	2.52	1.35	1.05	0.06	2.18	8.98
			89203	0.94	2.51	1.36	1.03	0.01	2.12	8.93
			97120	0.89	2.51	1.61	0.34	0.00	1.65	8.59
		10 months RT	54684	0.94	2.49	0.28	0.99	-0.08	1.10	8.20
			45308	0.93	2.52	-0.02	0.83	0.04	0.82	7.99
			51097	0.91	2.44	0.16	0.62	-0.26	0.49	7.76
		1 year_AA50	64924	0.94	2.48	0.60	0.96	-0.10	1.32	8.36
			72461	0.91	2.48	0.83	0.62	-0.10	1.19	8.27
			55708	0.90	2.50	0.31	0.46	-0.04	0.66	7.88
		1 year_AA60	105345	0.93	2.46	1.87	0.91	-0.16	2.27	9.04
			97961	0.93	2.47	1.64	0.90	-0.14	2.08	8.91
			98136	0.92	2.42	1.64	0.74	-0.32	1.77	8.68
		2.5 year_AA50	149453	0.91	2.43	3.26	0.61	-0.30	2.99	9.56
			126835	0.93	2.47	2.54	0.90	-0.13	2.84	9.45
			103781	0.91	2.44	1.82	0.56	-0.24	1.82	8.71
		5 year_AA50	79393	0.91	2.37	1.05	0.68	-0.524	1.04	8.15
			55376	0.95	2.41	0.30	1.15	-0.39	0.99	8.12
			27495	0.92	2.32	-0.58	0.70	-0.73	-0.48	7.06
		5 year_AA60	60084	0.82	2.26	0.45	-0.56	-0.94	-1.04	6.66
			33291	0.84	2.22	-0.40	-0.31	-1.13	-1.67	6.20
			35411	0.90	1.94	-0.33	0.46	-2.21	-1.85	6.07
		10 year_AA60	43171	0.88	2.14	-0.09	0.15	-1.42	-1.23	6.52
			49545	0.84	2.17	0.11	-0.30	-1.33	-1.41	6.39
			38630	0.87	2.07	-0.23	0.12	-1.70	-1.63	6.23

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Lysozyme	0 month RT	141684	0.93	2.54	3.01	0.84	0.12	3.40	9.85
			111625	0.94	2.54	2.07	0.97	0.15	2.76	9.40
			126397	0.91	2.50	2.53	0.58	-0.01	2.63	9.30
		2 months RT	132955	0.93	2.47	2.74	0.93	-0.13	3.03	9.59
			109605	0.95	2.55	2.00	1.15	0.15	2.90	9.49
			113992	0.93	2.52	2.14	0.84	0.03	2.60	9.28
		6 months RT	82444	0.95	2.49	1.15	1.16	-0.05	2.02	8.86
			81636	0.95	2.50	1.12	1.10	-0.03	1.96	8.82
			78368	0.92	2.51	1.02	0.71	0.00	1.52	8.50
		8 months RT	133761	0.94	2.50	2.76	1.01	-0.02	3.22	9.73
			110051	0.94	2.53	2.02	0.98	0.10	2.69	9.35
			86889	0.90	2.52	1.29	0.54	0.07	1.64	8.59
		10 months RT	72165	0.94	2.52	0.83	1.05	0.05	1.74	8.66
			73262	0.93	2.53	0.86	0.84	0.11	1.61	8.57
			86635	0.90	2.53	1.28	0.44	0.08	1.56	8.53
		1 year_AA50	76483	0.94	2.48	0.96	1.02	-0.11	1.68	8.61
			74142	0.94	2.48	0.89	1.03	-0.12	1.62	8.57
			68222	0.94	2.49	0.70	0.96	-0.06	1.45	8.45
		1 year_AA60	131721	0.91	2.44	2.70	0.65	-0.25	2.62	9.29
			70957	0.93	2.40	0.79	0.92	-0.42	1.14	8.23
			66127	0.92	2.39	0.64	0.78	-0.44	0.87	8.03
		2.5 year_AA50	105714	0.93	2.37	1.88	0.94	-0.54	1.96	8.82
			93352	0.88	2.34	1.49	0.22	-0.64	0.85	8.02
			82250	0.86	2.28	1.14	-0.09	-0.90	0.02	7.42
		5 year_AA50	52561	0.89	2.07	0.21	0.39	-1.71	-1.02	6.67
			41631	0.87	2.15	-0.14	0.08	-1.40	-1.32	6.45
			9026	0.59	1.37	-1.16	-3.79	-4.40	-8.65	1.17
		5 year_AA60	121861	0.82	1.98	2.39	-0.59	-2.05	-0.49	7.05
			29565	0.83	2.03	-0.51	-0.51	-1.85	-2.61	5.53
			14438	0.69	1.52	-0.99	-2.38	-3.85	-6.63	2.62
		10 year_AA60	15329	0.69	1.36	-0.96	-2.42	-4.46	-7.21	2.21
			14136	0.63	1.29	-1.00	-3.17	-4.74	-8.22	1.48

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Nisin	0 month RT	148181	0.93	2.55	3.22	0.94	0.16	3.70	10.07
			115676	0.92	2.54	2.19	0.70	0.15	2.62	9.29
			88202	0.93	2.54	1.33	0.94	0.13	2.12	8.93
		2 months RT	111880	0.94	2.52	2.07	0.99	0.06	2.71	9.36
			104616	0.93	2.51	1.85	0.84	0.02	2.34	9.09
			104476	0.92	2.51	1.84	0.77	0.02	2.28	9.05
		6 months RT	56746	0.88	2.52	0.34	0.15	0.06	0.48	7.75
			48257	0.87	2.20	0.07	0.13	-1.19	-0.91	6.75
			100806	0.94	2.52	1.73	1.08	0.06	2.52	9.22
		8 months RT	98909	0.92	2.52	1.67	0.73	0.03	2.11	8.92
			66552	0.95	2.52	0.65	1.14	0.05	1.68	8.61
			90397	0.91	2.54	1.40	0.67	0.14	1.92	8.79
		10 months RT	84151	0.93	2.54	1.20	0.83	0.14	1.91	8.79
			68811	0.93	2.52	0.72	0.90	0.06	1.52	8.50
			89654	0.94	2.50	1.38	1.00	-0.03	2.06	8.89
		1 year_AA50	85098	0.91	2.48	1.23	0.55	-0.10	1.45	8.45
			64988	0.94	2.49	0.60	1.00	-0.06	1.40	8.41
			136905	0.91	2.36	2.86	0.64	-0.57	2.44	9.17
		1 year_AA60	102762	0.91	2.34	1.79	0.65	-0.63	1.52	8.50
			70178	0.91	2.30	0.76	0.67	-0.80	0.53	7.79
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		2.5 year_AA50	124378	0.91	2.41	2.47	0.66	-0.36	2.33	9.09
			115546	0.89	2.42	2.19	0.40	-0.32	1.89	8.76
			105284	0.92	2.39	1.87	0.72	-0.44	1.83	8.72
		5 year_AA50	57716	0.90	2.30	0.37	0.45	-0.81	-0.01	7.40
			56956	0.90	2.24	0.35	0.42	-1.05	-0.27	7.21
			10012	0.64	1.39	-1.13	-3.12	-4.35	-7.93	1.69
		5 year_AA60	22444	0.82	1.70	-0.74	-0.60	-3.13	-4.06	4.48
			32413	0.78	1.75	-0.43	-1.18	-2.94	-4.18	4.39
			16521	0.76	1.68	-0.92	-1.38	-3.22	-5.04	3.77
		10 year_AA60	45319	0.82	1.75	-0.02	-0.64	-2.95	-3.34	5.00
			32208	0.85	1.62	-0.43	-0.14	-3.46	-3.67	4.76
			26023	0.83	1.66	-0.63	-0.52	-3.29	-4.03	4.50

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	None	0 month RT	85775	0.94	2.56	1.25	1.01	0.21	2.20	8.99
			89248	0.92	2.52	1.36	0.75	0.06	1.89	8.77
			57283	0.90	2.44	0.36	0.53	-0.26	0.57	7.81
		2 months RT	121642	0.96	2.52	2.38	1.29	0.05	3.25	9.74
			94762	0.93	2.54	1.54	0.88	0.12	2.22	9.01
			83806	0.92	2.52	1.19	0.80	0.03	1.78	8.69
		6 months RT	85175	0.94	2.52	1.23	1.01	0.07	2.06	8.89
			81086	0.93	2.52	1.11	0.89	0.06	1.82	8.72
			89571	0.91	2.51	1.37	0.59	0.02	1.72	8.65
		8 months RT	109787	0.93	2.54	2.01	0.82	0.12	2.56	9.25
			103632	0.92	2.54	1.81	0.76	0.11	2.33	9.08
			98515	0.92	2.54	1.65	0.79	0.11	2.22	9.01
		10 months RT	71970	0.94	2.55	0.82	1.06	0.17	1.85	8.74
			75528	0.92	2.53	0.93	0.79	0.11	1.62	8.57
			71070	0.92	2.53	0.79	0.77	0.08	1.47	8.46
		1 year_AA50	97818	0.92	2.51	1.63	0.76	0.03	2.10	8.92
			77156	0.95	2.53	0.98	1.17	0.07	2.01	8.85
			56625	0.93	2.50	0.34	0.86	-0.02	1.09	8.19
		1 year_AA60	147971	0.93	2.52	3.21	0.87	0.04	3.51	9.93
			147119	0.92	2.50	3.18	0.73	-0.02	3.30	9.79
			79474	0.91	2.44	1.05	0.68	-0.26	1.28	8.33
		2.5 year_AA50	127379	0.93	2.42	2.56	0.85	-0.34	2.62	9.29
			126529	0.92	2.41	2.53	0.71	-0.36	2.44	9.17
			111706	0.91	2.45	2.07	0.58	-0.24	2.04	8.88
		5 year_AA50	57393	0.94	2.37	0.36	1.05	-0.52	0.83	8.00
			66585	0.89	2.33	0.65	0.29	-0.68	0.19	7.54
			57652	0.91	2.32	0.37	0.58	-0.74	0.18	7.53
		5 year_AA60	76090	0.90	2.26	0.95	0.48	-0.95	0.37	7.68
			49373	0.92	2.32	0.11	0.77	-0.71	0.18	7.53
			52673	0.91	2.15	0.21	0.58	-1.39	-0.55	7.01
		10 year_AA60	26307	0.91	2.13	-0.62	0.58	-1.47	-1.30	6.47
			37688	0.90	2.04	-0.26	0.47	-1.80	-1.41	6.39
			20152	0.86	2.14	-0.81	-0.13	-1.41	-2.09	5.90

Fluid	Pres.	Time Point	TPH	MLB	SH	tph	mlb	sh	pc	FI
Blood	Parabens	0 month RT	122580	0.95	2.51	2.41	1.19	0.02	3.15	9.67
			117058	0.93	2.54	2.24	0.88	0.14	2.81	9.43
			107379	0.94	2.56	1.93	0.96	0.22	2.71	9.36
			83872	0.93	2.57	1.19	0.94	0.23	2.10	8.92
		2 months RT	118275	0.93	2.50	2.28	0.85	-0.01	2.68	9.34
			99435	0.95	2.54	1.68	1.16	0.13	2.63	9.30
			105120	0.93	2.52	1.86	0.85	0.06	2.41	9.14
			102226	0.93	2.53	1.77	0.86	0.09	2.37	9.12
		6 months RT	92832	0.92	2.51	1.48	0.79	0.00	1.98	8.83
			89287	0.92	2.53	1.36	0.78	0.08	1.95	8.81
			60687	0.92	2.54	0.46	0.81	0.14	1.29	8.33
			41472	0.94	2.49	-0.14	1.06	-0.06	0.85	8.02
		8 months RT	108845	0.94	2.54	1.98	0.99	0.13	2.71	9.36
			111637	0.93	2.53	2.07	0.84	0.11	2.61	9.28
			104292	0.93	2.53	1.84	0.86	0.10	2.44	9.16
			106013	0.91	2.52	1.89	0.63	0.06	2.21	9.00
		10 months RT	126201	0.94	2.49	2.52	1.00	-0.05	2.99	9.56
			92031	0.93	2.55	1.45	0.82	0.15	2.12	8.94
			81089	0.94	2.54	1.11	1.05	0.13	2.04	8.88
			87723	0.92	2.53	1.31	0.81	0.09	1.94	8.81
		1 year_AA50	92669	0.94	2.52	1.47	1.07	0.06	2.29	9.06
			93445	0.93	2.53	1.49	0.93	0.10	2.22	9.00
			85031	0.93	2.53	1.23	0.83	0.10	1.90	8.77
			69693	0.93	2.54	0.75	0.85	0.13	1.55	8.52
		1 year_AA60	141542	0.93	2.50	3.01	0.91	-0.02	3.33	9.81
			128635	0.94	2.48	2.60	1.06	-0.09	3.08	9.62
			128152	0.94	2.49	2.59	1.03	-0.05	3.08	9.62
			94356	0.91	2.44	1.52	0.58	-0.27	1.57	8.53
		2.5 year_AA50	136919	0.93	2.43	2.86	0.92	-0.29	2.98	9.55
			139587	0.92	2.47	2.95	0.71	-0.15	2.97	9.55
			144511	0.91	2.46	3.10	0.59	-0.19	2.95	9.53
			140281	0.89	2.46	2.97	0.39	-0.17	2.66	9.32
		5 year_AA50	88983	0.93	2.39	1.35	0.88	-0.47	1.53	8.51
			59777	0.92	2.37	0.44	0.81	-0.52	0.66	7.88
			58109	0.90	2.38	0.38	0.42	-0.50	0.26	7.59
			56166	0.92	2.30	0.32	0.70	-0.82	0.19	7.54
		5 year_AA60	91060	0.93	2.31	1.42	0.82	-0.75	1.26	8.32
			79210	0.88	2.27	1.05	0.23	-0.92	0.24	7.58
			17871	0.85	2.00	-0.88	-0.23	-1.97	-2.76	5.42
			33882	0.83	1.90	-0.38	-0.53	-2.35	-2.97	5.26
		10 year_AA60	36081	0.85	2.05	-0.31	-0.23	-1.76	-2.09	5.90
			22322	0.85	1.72	-0.74	-0.26	-3.07	-3.68	4.75
			20575	0.82	1.80	-0.80	-0.67	-2.76	-3.84	4.64
			28797	0.78	1.50	-0.54	-1.10	-3.92	-5.09	3.73

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Propyl Gallate	0 month RT	117697	0.94	2.55	2.26	0.99	0.19	2.98	9.55
			111449	0.94	2.53	2.06	0.96	0.11	2.72	9.37
			77053	0.92	2.54	0.98	0.78	0.13	1.67	8.61
		2 months RT	134680	0.92	2.53	2.79	0.72	0.11	3.09	9.63
			94252	0.94	2.55	1.52	1.04	0.18	2.42	9.15
			108088	0.91	2.51	1.95	0.67	0.02	2.27	9.04
		6 months RT	91983	0.93	2.51	1.45	0.87	0.03	2.05	8.88
			89255	0.91	2.53	1.36	0.65	0.11	1.85	8.74
			73578	0.92	2.53	0.87	0.71	0.08	1.48	8.47
		8 months RT	119610	0.92	2.51	2.32	0.78	0.01	2.66	9.32
			58580	0.93	2.56	0.40	0.90	0.21	1.38	8.40
			116290	0.87	2.52	2.21	0.10	0.05	1.96	8.82
		10 months RT	117712	0.94	2.54	2.26	1.00	0.14	2.95	9.53
			92273	0.95	2.53	1.46	1.12	0.10	2.37	9.11
			73480	0.92	2.55	0.87	0.77	0.17	1.62	8.57
		1 year_AA50	91752	0.95	2.53	1.44	1.09	0.10	2.32	9.08
			89513	0.94	2.54	1.37	0.96	0.13	2.17	8.97
			84615	0.92	2.53	1.22	0.72	0.10	1.79	8.70
		1 year_AA60	175259	0.92	2.47	4.07	0.75	-0.13	3.95	10.25
			142067	0.93	2.50	3.02	0.86	-0.03	3.28	9.77
			109109	0.93	2.46	1.99	0.86	-0.20	2.28	9.05
		2.5 year_AA50	120691	146692	0.93	2.48	3.17	0.94	-0.12	3.40
			107335	145316	0.91	2.46	3.13	0.55	-0.19	2.93
			104578	133818	0.93	2.45	2.76	0.88	-0.23	2.91
		5 year_AA50	88860	62757	0.93	2.39	0.53	0.85	-0.44	0.84
			93550	71702	0.90	2.35	0.81	0.48	-0.61	0.57
			73770	48146	0.90	2.12	0.07	0.44	-1.49	-0.90
		5 year_AA60	54216	55093	0.92	2.23	0.29	0.71	-1.08	-0.08
			54130	47570	0.92	2.14	0.05	0.75	-1.43	-0.55
			46125	45106	0.86	2.19	-0.03	-0.05	-1.25	-1.21
		10 year_AA60	57739	56964	0.89	2.28	0.35	0.40	-0.89	-0.14
			39273	29700	0.87	2.13	-0.51	0.00	-1.48	-1.78
			38950	36353	0.88	1.94	-0.30	0.18	-2.21	-2.10

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Sodium Azide	0 month RT	124384	0.94	2.53	2.47	0.97	0.08	3.04	9.60
			94075	0.93	2.55	1.51	0.91	0.18	2.28	9.05
			51515	0.94	2.52	0.18	1.04	0.06	1.21	8.27
		2 months RT	118618	0.94	2.49	2.29	0.96	-0.05	2.76	9.40
			98260	0.93	2.54	1.65	0.93	0.11	2.35	9.10
			93540	0.91	2.53	1.50	0.65	0.07	1.93	8.79
		6 months RT	69125	0.93	2.55	0.73	0.94	0.16	1.65	8.60
			62616	0.92	2.51	0.52	0.79	0.02	1.21	8.28
			64904	0.91	2.51	0.60	0.67	0.03	1.16	8.24
		8 months RT	98320	0.94	2.54	1.65	1.09	0.14	2.53	9.23
			98489	0.93	2.54	1.65	0.84	0.11	2.27	9.04
			74147	0.87	2.52	0.89	0.12	0.07	0.91	8.06
		10 months RT	75980	0.93	2.53	0.95	0.83	0.09	1.66	8.60
			59255	0.93	2.54	0.42	0.95	0.12	1.37	8.39
			72110	0.91	2.53	0.82	0.63	0.09	1.37	8.39
		1 year_AA50	98997	0.93	2.53	1.67	0.94	0.09	2.37	9.11
			77166	0.94	2.52	0.98	1.07	0.04	1.88	8.76
			77335	0.92	2.54	0.99	0.81	0.13	1.71	8.64
		1 year_AA60	143566	0.92	2.38	3.07	0.79	-0.48	2.85	9.46
			110880	0.89	2.50	2.04	0.36	-0.04	1.99	8.84
			84667	0.90	2.22	1.22	0.42	-1.11	0.39	7.69
		2.5 year_AA50	133074	0.94	2.46	2.74	1.00	-0.18	3.05	9.61
			133774	0.92	2.45	2.76	0.73	-0.22	2.78	9.41
			103630	0.92	2.43	1.81	0.71	-0.31	1.89	8.77
		5 year_AA50	73364	0.90	2.35	0.86	0.51	-0.60	0.65	7.87
			54971	0.92	2.36	0.28	0.80	-0.57	0.48	7.75
			27700	0.87	1.85	-0.57	0.08	-2.54	-2.73	5.44
		5 year_AA60	133059	0.88	1.93	2.74	0.24	-2.25	0.42	7.71
			47326	0.77	2.05	0.04	-1.32	-1.77	-2.86	5.35
			29053	0.78	1.56	-0.53	-1.22	-3.69	-5.00	3.80
		10 year_AA60	52287	0.89	2.37	0.20	0.35	-0.55	0.00	7.41
			36502	0.87	1.83	-0.30	0.03	-2.61	-2.61	5.52
			28420	0.69	1.51	-0.55	-2.38	-3.88	-6.31	2.86

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Blood	Zinc	0 month RT	104925	0.94	2.53	1.86	1.08	0.10	2.66	9.33
			112394	0.92	2.54	2.09	0.74	0.12	2.54	9.24
			102325	0.91	2.57	1.77	0.56	0.24	2.22	9.01
		2 months RT	124963	0.91	2.53	2.49	0.58	0.09	2.68	9.34
			97751	0.94	2.52	1.63	1.05	0.05	2.40	9.13
			87901	0.95	2.54	1.32	1.11	0.11	2.26	9.03
		6 months RT	74286	0.94	2.52	0.89	0.96	0.05	1.71	8.64
			74001	0.93	2.53	0.88	0.90	0.10	1.69	8.62
			75969	0.91	2.51	0.94	0.57	0.03	1.35	8.38
		8 months RT	122755	0.92	2.54	2.42	0.80	0.12	2.87	9.48
			103643	0.93	2.52	1.82	0.86	0.05	2.37	9.11
			80024	0.93	2.53	1.07	0.88	0.11	1.83	8.72
		10 months RT	90044	0.95	2.55	1.39	1.14	0.18	2.40	9.14
			97862	0.93	2.52	1.63	0.94	0.07	2.31	9.07
			78017	0.89	2.53	1.01	0.36	0.11	1.27	8.32
		1 year_AA50	86853	0.95	2.52	1.29	1.19	0.05	2.25	9.03
			82954	0.93	2.51	1.16	0.93	0.03	1.87	8.75
			76764	0.90	2.53	0.97	0.52	0.10	1.38	8.40
		1 year_AA60	124733	0.92	2.44	2.48	0.80	-0.25	2.58	9.26
			111675	0.94	2.46	2.07	1.00	-0.17	2.51	9.22
			103764	0.94	2.45	1.82	0.97	-0.21	2.23	9.02
		2.5 year_AA50	140294	0.93	2.46	2.97	0.87	-0.18	3.12	9.65
			119038	0.91	2.43	2.30	0.67	-0.28	2.28	9.05
			97242	0.90	2.40	1.61	0.46	-0.40	1.41	8.42
		5 year_AA50	60771	0.91	2.35	0.47	0.68	-0.60	0.49	7.76
			47951	0.92	2.37	0.06	0.78	-0.54	0.32	7.63
			54791	0.92	2.31	0.28	0.69	-0.77	0.18	7.54
		5 year_AA60	58268	0.85	2.24	0.39	-0.22	-1.05	-0.86	6.79
			26801	0.88	2.27	-0.60	0.16	-0.93	-1.20	6.54
			43808	0.88	2.12	-0.07	0.19	-1.49	-1.24	6.51
		10 year_AA60	114733	0.89	2.19	2.16	0.29	-1.25	0.91	8.06
			25326	0.92	2.17	-0.65	0.76	-1.31	-1.00	6.68
			40673	0.88	2.01	-0.17	0.19	-1.92	-1.72	6.17

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Actin	0 month RT	35700	0.84	2.38	-0.32	-0.35	-0.47	-1.04	6.66
			26537	0.90	2.20	-0.61	0.48	-1.19	-1.13	6.59
			28008	0.86	2.33	-0.56	-0.09	-0.67	-1.17	6.56
		2 months RT	43941	0.91	2.33	-0.06	0.68	-0.68	-0.03	7.39
			63582	0.85	2.34	0.56	-0.19	-0.63	-0.30	7.19
			43591	0.85	2.26	-0.07	-0.27	-0.94	-1.18	6.55
		6 months RT	68982	0.91	2.32	0.72	0.67	-0.73	0.57	7.81
			14652	0.92	2.37	-0.98	0.74	-0.55	-0.60	6.98
			29932	0.82	2.16	-0.50	-0.61	-1.36	-2.25	5.78
		8 months RT	21598	0.93	2.23	-0.77	0.87	-1.09	-0.80	6.83
			44989	0.84	2.24	-0.03	-0.31	-1.02	-1.26	6.49
			36065	0.82	2.31	-0.31	-0.55	-0.76	-1.48	6.34
		10 months RT	30602	0.85	2.26	-0.48	-0.19	-0.95	-1.46	6.36
			24182	0.89	2.14	-0.68	0.31	-1.44	-1.59	6.26
			22478	0.89	2.12	-0.74	0.29	-1.51	-1.72	6.16
		1 year_AA50	33970	0.90	2.16	-0.38	0.42	-1.35	-1.15	6.57
			54509	0.83	2.25	0.27	-0.50	-1.00	-1.19	6.55
			44205	0.86	2.17	-0.05	-0.02	-1.29	-1.25	6.51
		1 year_AA60	94574	0.89	2.25	1.53	0.33	-0.98	0.67	7.89
			91755	0.88	2.18	1.44	0.19	-1.25	0.22	7.56
			54813	0.90	2.09	0.28	0.51	-1.63	-0.78	6.85
		2.5 year_AA50	44113	0.82	1.75	-0.06	-0.64	-2.93	-3.35	4.99
			38284	0.73	1.82	-0.24	-1.83	-2.65	-4.39	4.24
			24697	0.80	1.65	-0.67	-0.92	-3.33	-4.50	4.16
		5 year_AA50	20591	0.87	2.07	-0.80	0.12	-1.70	-2.10	5.89
			15283	0.78	1.35	-0.96	-1.21	-4.49	-6.07	3.03
			9953	0.57	1.81	-1.13	-3.99	-2.69	-7.24	2.19
		5 year_AA60	21258	0.72	1.77	-0.78	-1.99	-2.88	-5.20	3.66
			17881	0.73	1.45	-0.88	-1.88	-4.10	-6.30	2.86
			7452	0.66	1.45	-1.21	-2.81	-4.13	-7.49	2.00

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Ascorbic Acid	0 month RT	26467	0.85	2.42	-0.61	-0.14	-0.34	-0.95	6.72
			31675	0.88	2.25	-0.45	0.24	-1.00	-1.05	6.65
			28712	0.87	2.13	-0.54	0.12	-1.47	-1.67	6.20
		2 months RT	32411	0.88	2.42	-0.43	0.16	-0.32	-0.49	7.05
			30112	0.88	2.18	-0.50	0.26	-1.25	-1.31	6.46
			47253	0.84	2.16	0.04	-0.36	-1.35	-1.56	6.28
		6 months RT	76744	0.91	2.35	0.97	0.60	-0.62	0.81	7.99
			29715	0.87	2.31	-0.51	0.06	-0.75	-1.04	6.65
			30835	0.89	2.19	-0.47	0.32	-1.25	-1.23	6.52
		8 months RT	44309	0.90	2.37	-0.05	0.47	-0.54	-0.08	7.34
			29714	0.90	2.23	-0.51	0.46	-1.06	-0.96	6.72
			33541	0.82	2.27	-0.39	-0.65	-0.93	-1.79	6.11
		10 months RT	24934	0.90	2.22	-0.66	0.52	-1.12	-1.07	6.63
			14510	0.77	2.24	-0.99	-1.31	-1.02	-3.01	5.24
			18118	0.80	1.98	-0.87	-0.94	-2.04	-3.50	4.88
		1 year_AA50	135485	0.90	2.31	2.82	0.52	-0.77	2.11	8.93
			57600	0.89	2.24	0.37	0.30	-1.05	-0.37	7.14
			25672	0.87	2.12	-0.64	0.06	-1.50	-1.85	6.07
		1 year_AA60	109077	0.89	2.20	1.99	0.39	-1.18	0.93	8.07
			91925	0.92	2.21	1.45	0.68	-1.17	0.77	7.96
			84773	0.93	2.17	1.22	0.90	-1.31	0.67	7.89
		2.5 year_AA50	35133	0.80	1.76	-0.34	-0.88	-2.91	-3.79	4.67
			42239	0.77	1.70	-0.12	-1.30	-3.13	-4.21	4.37
			39997	0.76	1.69	-0.19	-1.42	-3.18	-4.44	4.20
		5 year_AA50	17400	0.75	1.35	-0.90	-1.50	-4.52	-6.33	2.84
			14364	0.64	1.37	-0.99	-3.11	-4.43	-7.88	1.73
		5 year_AA60	18016	0.74	1.38	-0.88	-1.72	-4.37	-6.40	2.80
			8599	0.68	1.26	-1.17	-2.52	-4.84	-7.83	1.76
			10979	0.59	1.43	-1.10	-3.75	-4.19	-8.36	1.38

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	ATA	0 month RT	70489	0.94	2.35	0.77	1.09	-0.61	1.12	8.21
			47754	0.85	2.44	0.06	-0.22	-0.26	-0.41	7.11
			21343	0.80	2.09	-0.77	-0.87	-1.63	-2.98	5.26
		2 months RT	35617	0.87	2.41	-0.32	0.05	-0.36	-0.55	7.01
			24726	0.89	2.15	-0.67	0.38	-1.40	-1.47	6.35
			31674	0.82	2.33	-0.45	-0.66	-0.69	-1.63	6.23
		6 months RT	75664	0.89	2.31	0.94	0.32	-0.78	0.37	7.67
			47121	0.91	2.24	0.04	0.61	-1.04	-0.33	7.16
			16691	0.84	2.06	-0.92	-0.35	-1.75	-2.70	5.46
		8 months RT	39600	0.89	2.28	-0.20	0.28	-0.87	-0.70	6.90
			30949	0.88	2.26	-0.47	0.25	-0.95	-1.02	6.67
			16186	0.85	1.90	-0.94	-0.25	-2.35	-3.17	5.12
		10 months RT	30302	0.84	2.20	-0.49	-0.30	-1.21	-1.81	6.10
			14802	0.85	2.08	-0.98	-0.20	-1.64	-2.50	5.60
			13041	0.79	1.86	-1.03	-1.00	-2.52	-4.12	4.43
		1 year_AA50	21691	0.88	2.01	-0.76	0.15	-1.92	-2.24	5.79
			17566	0.82	2.02	-0.89	-0.62	-1.89	-3.06	5.20
			12315	0.71	1.65	-1.06	-2.04	-3.32	-5.88	3.16
		1 year_AA60	63751	0.88	2.07	0.56	0.16	-1.72	-0.96	6.71
			60682	0.84	2.03	0.46	-0.40	-1.86	-1.71	6.17
			26078	0.80	1.77	-0.62	-0.88	-2.87	-4.00	4.52
		2.5 year_AA50	34312	0.78	1.57	-0.37	-1.15	-3.63	-4.74	3.99
			22440	0.68	1.47	-0.74	-2.58	-4.05	-6.81	2.50
			5660	0.66	1.28	-1.27	-2.86	-4.77	-8.17	1.52
		5 year_AA50	19590	0.76	1.38	-0.83	-1.46	-4.40	-6.13	2.99
			4334	0.61	1.17	-1.31	-3.50	-5.21	-9.23	0.75
			6295	0.62	1.10	-1.25	-3.38	-5.48	-9.30	0.70
		5 year_AA60	7585	0.67	1.15	-1.21	-2.70	-5.27	-8.42	1.34
			8409	0.65	1.18	-1.18	-2.94	-5.14	-8.52	1.26
			6077	0.68	1.00	-1.25	-2.56	-5.87	-8.89	1.00

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Bronopol	0 month RT	99141	0.88	2.38	1.67	0.15	-0.49	1.07	8.18
			33219	0.89	2.34	-0.40	0.31	-0.66	-0.64	6.94
			32855	0.90	2.18	-0.41	0.49	-1.26	-1.03	6.67
		2 months RT	31530	0.77	2.13	-0.45	-1.27	-1.46	-2.93	5.29
			15847	0.79	1.78	-0.95	-1.06	-2.84	-4.41	4.23
			13170	0.76	1.64	-1.03	-1.46	-3.38	-5.36	3.54
		6 months RT	32992	0.82	1.67	-0.41	-0.56	-3.26	-3.87	4.62
			35617	0.76	1.83	-0.32	-1.47	-2.63	-4.09	4.46
			15997	0.72	1.31	-0.94	-1.95	-4.65	-6.92	2.41
		8 months RT	23245	0.82	1.76	-0.71	-0.55	-2.89	-3.77	4.69
			10845	0.64	1.55	-1.10	-3.03	-3.73	-7.25	2.18
			5298	0.58	0.88	-1.28	-3.84	-6.35	-10.57	0.05
		10 months RT	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			2888	0.57	0.57	-1.35	-4.03	-7.53	-11.90	0.05
			5017	0.55	0.94	-1.29	-4.26	-6.11	-10.77	0.05
		1 year_AA50	13434	0.69	1.12	-1.02	-2.44	-5.40	-8.15	1.53
			446	0.40	0.30	-1.43	-6.33	-8.57	-15.13	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		1 year_AA60	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		2.5 year_AA50	16225	0.73	1.44	-0.93	-1.87	-4.15	-6.38	2.80
			287	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.17
			98	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.16
		5 year_AA50	2217	0.61	1.88	-1.37	-3.46	-2.43	-6.69	2.58
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		5 year_AA60	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			659	0.47	0.37	-1.42	-5.42	-8.32	-14.02	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Chitosan	0 month RT	43255	0.90	2.28	-0.08	0.45	-0.89	-0.45	7.08
			27915	0.87	2.37	-0.57	0.09	-0.53	-0.86	6.78
			29372	0.82	2.32	-0.52	-0.64	-0.74	-1.72	6.16
		2 months RT	37428	0.88	2.47	-0.27	0.26	-0.14	-0.10	7.33
			33497	0.92	2.22	-0.39	0.73	-1.12	-0.65	6.94
			30304	0.87	2.31	-0.49	0.12	-0.75	-0.98	6.70
		6 months RT	86528	0.89	2.34	1.28	0.33	-0.66	0.77	7.96
			67652	0.93	2.31	0.68	0.87	-0.77	0.69	7.90
			18733	0.89	2.26	-0.86	0.37	-0.96	-1.23	6.52
		8 months RT	59322	0.88	2.23	0.42	0.15	-1.07	-0.49	7.05
			35292	0.88	2.30	-0.33	0.18	-0.79	-0.83	6.81
			29675	0.90	2.23	-0.51	0.46	-1.08	-0.97	6.71
		10 months RT	26153	0.85	2.19	-0.62	-0.25	-1.24	-1.89	6.04
			15408	0.85	2.12	-0.96	-0.19	-1.51	-2.36	5.70
			19305	0.84	2.09	-0.84	-0.36	-1.64	-2.53	5.58
		1 year_AA50	23547	0.87	2.31	-0.70	0.08	-0.78	-1.22	6.53
			38852	0.87	2.19	-0.22	0.06	-1.24	-1.26	6.50
			30394	0.85	2.22	-0.49	-0.26	-1.12	-1.68	6.19
		1 year_AA60	87352	0.85	2.18	1.30	-0.18	-1.27	-0.27	7.21
			50816	0.89	2.07	0.15	0.38	-1.69	-1.05	6.65
			42204	0.91	1.98	-0.12	0.67	-2.07	-1.35	6.43
		2.5 year_AA50	36332	0.80	2.04	-0.30	-0.86	-1.83	-2.75	5.42
			35875	0.77	1.95	-0.32	-1.31	-2.16	-3.50	4.88
			169	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.16
		5 year_AA50	40401	0.83	1.73	-0.17	-0.48	-3.02	-3.38	4.97
			24971	0.76	1.74	-0.66	-1.37	-2.98	-4.59	4.09
			9853	0.64	1.60	-1.13	-3.07	-3.53	-7.12	2.27
		5 year_AA60	22505	0.76	1.60	-0.74	-1.50	-3.54	-5.29	3.59
			15164	0.73	1.37	-0.97	-1.83	-4.44	-6.63	2.63
			16060	0.68	1.36	-0.94	-2.53	-4.48	-7.32	2.13

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	EDTA	0 month RT	42143	0.88	2.43	-0.12	0.22	-0.29	-0.14	7.30
			40256	0.90	2.35	-0.18	0.45	-0.60	-0.27	7.21
			36667	0.86	2.25	-0.29	-0.04	-1.01	-1.21	6.53
		2 months RT	54876	0.92	2.37	0.28	0.79	-0.54	0.50	7.76
			60288	0.90	2.39	0.45	0.42	-0.44	0.37	7.67
			28776	0.88	2.11	-0.54	0.20	-1.53	-1.66	6.21
		6 months RT	94488	0.91	2.33	1.53	0.65	-0.70	1.23	8.29
			94114	0.88	2.37	1.52	0.15	-0.54	0.90	8.05
			65876	0.90	2.25	0.63	0.53	-1.00	0.11	7.48
		8 months RT	55252	0.89	2.20	0.29	0.33	-1.20	-0.55	7.01
			39791	0.89	2.15	-0.19	0.38	-1.38	-1.06	6.64
			15761	0.85	2.05	-0.95	-0.16	-1.79	-2.58	5.55
		10 months RT	25055	0.85	2.03	-0.66	-0.27	-1.85	-2.49	5.61
			14522	0.78	1.77	-0.99	-1.20	-2.87	-4.60	4.09
			15660	0.73	1.62	-0.95	-1.82	-3.46	-5.71	3.29
		1 year_AA50	60614	0.92	2.45	0.46	0.72	-0.20	0.89	8.04
			61535	0.91	2.43	0.49	0.60	-0.29	0.72	7.92
			33699	0.86	2.34	-0.38	-0.06	-0.66	-0.98	6.70
		1 year_AA60	123836	0.92	2.47	2.45	0.71	-0.15	2.56	9.25
			73183	0.92	2.47	0.86	0.69	-0.15	1.23	8.29
			61348	0.91	2.40	0.48	0.57	-0.40	0.58	7.82
		2.5 year_AA50	108118	0.89	2.39	1.96	0.34	-0.44	1.54	8.51
			84774	0.88	2.42	1.22	0.26	-0.32	0.96	8.09
			52985	0.87	2.29	0.22	0.13	-0.82	-0.45	7.08
		5 year_AA50	40243	0.94	2.25	-0.18	1.00	-1.00	-0.10	7.33
			41332	0.87	2.49	-0.14	0.07	-0.08	-0.13	7.31
			40138	0.92	2.27	-0.18	0.78	-0.92	-0.24	7.24
		5 year_AA60	40700	0.90	2.30	-0.16	0.49	-0.81	-0.41	7.11
			32930	0.81	2.42	-0.41	-0.71	-0.32	-1.31	6.46
			16934	0.89	2.21	-0.91	0.36	-1.16	-1.47	6.35
		10 year_AA60	49914	0.92	2.32	0.13	0.80	-0.71	0.21	7.56
			35197	0.89	2.36	-0.34	0.27	-0.59	-0.56	7.00
			29845	0.92	2.18	-0.51	0.78	-1.28	-0.84	6.80

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Lysozyme	0 month RT	50367	0.89	2.44	0.14	0.38	-0.25	0.26	7.59
			33422	0.91	2.30	-0.39	0.63	-0.79	-0.44	7.09
			43977	0.84	2.29	-0.06	-0.36	-0.85	-1.18	6.56
		2 months RT	60683	0.88	2.43	0.46	0.25	-0.30	0.34	7.65
			27170	0.89	2.34	-0.59	0.29	-0.67	-0.82	6.82
			36666	0.84	2.24	-0.29	-0.39	-1.02	-1.55	6.29
		6 months RT	80619	0.88	2.34	1.09	0.19	-0.63	0.50	7.77
			25722	0.91	2.17	-0.64	0.67	-1.29	-1.06	6.64
			34485	0.85	2.30	-0.36	-0.18	-0.79	-1.19	6.55
		8 months RT	44673	0.87	2.33	-0.04	0.11	-0.70	-0.57	6.99
			39965	0.85	2.28	-0.19	-0.17	-0.88	-1.13	6.59
			33708	0.88	2.14	-0.38	0.20	-1.43	-1.43	6.37
		10 months RT	21352	0.89	2.39	-0.77	0.28	-0.46	-0.80	6.83
			24962	0.88	2.06	-0.66	0.25	-1.73	-1.90	6.04
			19076	0.87	2.12	-0.84	0.05	-1.51	-2.03	5.94
		1 year_AA50	58485	0.90	2.14	0.39	0.42	-1.43	-0.58	6.99
			38217	0.82	2.20	-0.24	-0.65	-1.18	-1.91	6.03
			26123	0.84	1.97	-0.62	-0.28	-2.09	-2.70	5.46
		1 year_AA60	100982	0.90	2.26	1.73	0.53	-0.96	1.05	8.16
			70777	0.91	2.17	0.78	0.67	-1.32	0.07	7.46
			73299	0.88	2.06	0.86	0.20	-1.73	-0.69	6.91
		2.5 year_AA50	65838	0.83	1.95	0.63	-0.51	-2.16	-1.96	5.99
			49356	0.81	1.92	0.11	-0.79	-2.30	-2.78	5.40
			44580	0.81	1.78	-0.04	-0.77	-2.81	-3.35	4.99
		5 year_AA50	28961	0.76	1.57	-0.53	-1.39	-3.65	-5.12	3.71
			24193	0.77	1.41	-0.68	-1.26	-4.28	-5.70	3.29
			12821	0.65	1.36	-1.04	-2.86	-4.46	-7.70	1.85
		5 year_AA60	41191	0.83	1.72	-0.15	-0.54	-3.05	-3.44	4.92
			18103	0.67	1.63	-0.88	-2.62	-3.40	-6.36	2.82
			8637	0.71	1.15	-1.17	-2.14	-5.29	-7.88	1.73
		10 year_AA60	12294	0.60	1.28	-1.06	-3.59	-4.79	-8.72	1.12
			7798	0.66	1.05	-1.20	-2.83	-5.67	-8.91	0.98

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Nisin	0 month RT	74477	0.91	2.42	0.90	0.62	-0.35	1.02	8.14
			49383	0.87	2.18	0.11	0.09	-1.26	-0.98	6.70
			27773	0.89	2.11	-0.57	0.28	-1.53	-1.60	6.25
		2 months RT	59589	0.86	2.45	0.43	-0.04	-0.24	0.10	7.48
			46723	0.92	2.33	0.02	0.69	-0.70	0.04	7.44
			42543	0.90	2.21	-0.11	0.50	-1.15	-0.66	6.93
		6 months RT	94677	0.89	2.35	1.53	0.32	-0.62	1.00	8.13
			26030	0.91	2.45	-0.63	0.64	-0.22	-0.10	7.34
			25066	0.87	2.12	-0.66	0.08	-1.49	-1.84	6.08
		8 months RT	51816	0.83	2.32	0.19	-0.49	-0.72	-0.99	6.69
			17386	0.88	2.05	-0.90	0.16	-1.79	-2.23	5.80
			32445	0.91	2.28	-0.42	0.58	-0.88	-0.60	6.97
		10 months RT	20548	0.89	2.30	-0.80	0.32	-0.80	-1.08	6.63
			35271	0.83	2.28	-0.34	-0.43	-0.87	-1.49	6.33
			24664	0.91	2.08	-0.67	0.57	-1.68	-1.55	6.29
		1 year_AA50	25588	0.86	2.12	-0.64	-0.01	-1.51	-1.92	6.02
			28246	0.87	2.01	-0.56	0.00	-1.94	-2.24	5.79
			29081	0.86	1.97	-0.53	-0.08	-2.11	-2.45	5.64
		1 year_AA60	78433	0.90	2.11	1.02	0.47	-1.56	-0.14	7.30
			74488	0.86	2.21	0.90	-0.03	-1.14	-0.34	7.16
			73966	0.87	2.04	0.88	0.09	-1.80	-0.84	6.80
		2.5 year_AA50	39160	0.83	1.76	-0.21	-0.52	-2.92	-3.36	4.98
			34357	0.82	1.79	-0.36	-0.63	-2.78	-3.46	4.91
			22379	0.71	1.63	-0.74	-2.09	-3.42	-5.76	3.25
		5 year_AA50	15372	0.79	2.04	-0.96	-1.00	-1.82	-3.43	4.93
			28015	0.73	1.44	-0.56	-1.80	-4.16	-6.02	3.06
			14249	0.63	1.46	-1.00	-3.24	-4.06	-7.66	1.88
		5 year_AA60	15158	0.77	1.75	-0.97	-1.29	-2.94	-4.74	3.99
			19566	0.72	1.42	-0.83	-2.04	-4.22	-6.52	2.70
			9846	0.65	1.38	-1.13	-2.98	-4.37	-7.81	1.77

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	None	0 month RT	41301	0.93	2.36	-0.15	0.93	-0.57	0.26	7.59
			37034	0.88	2.43	-0.28	0.19	-0.31	-0.33	7.17
			8192	0.72	1.62	-1.19	-2.04	-3.47	-6.12	2.99
		2 months RT	70878	0.90	2.43	0.78	0.44	-0.29	0.80	7.98
			40617	0.88	2.25	-0.17	0.24	-0.99	-0.81	6.82
			24085	0.82	2.25	-0.69	-0.54	-0.99	-2.00	5.96
		6 months RT	92762	0.89	2.29	1.47	0.37	-0.83	0.81	7.99
			57443	0.92	2.38	0.36	0.73	-0.47	0.56	7.81
			48978	0.92	2.29	0.10	0.75	-0.86	0.01	7.41
		8 months RT	35252	0.88	2.27	-0.34	0.16	-0.92	-0.97	6.71
			23458	0.88	2.23	-0.71	0.27	-1.08	-1.31	6.46
			23767	0.86	2.26	-0.70	-0.06	-0.97	-1.52	6.31
		10 months RT	33539	0.90	2.40	-0.39	0.41	-0.43	-0.32	7.18
			33543	0.88	2.30	-0.39	0.23	-0.82	-0.84	6.80
			22024	0.86	2.21	-0.75	0.00	-1.17	-1.70	6.18
		1 year_AA50	68099	0.88	2.28	0.70	0.14	-0.90	-0.11	7.32
			31596	0.91	2.23	-0.45	0.62	-1.06	-0.74	6.87
			27418	0.86	1.99	-0.58	-0.01	-2.02	-2.34	5.72
		1 year_AA60	107638	0.91	2.22	1.94	0.59	-1.13	1.13	8.22
			102279	0.90	2.21	1.77	0.44	-1.14	0.83	8.00
			40418	0.89	2.11	-0.17	0.28	-1.55	-1.30	6.47
		2.5 year_AA50	41775	0.83	1.79	-0.13	-0.52	-2.80	-3.17	5.12
			46460	0.79	1.80	0.02	-1.01	-2.73	-3.46	4.91
			30486	0.78	1.69	-0.49	-1.22	-3.17	-4.48	4.18
		5 year_AA50	10523	0.72	1.34	-1.11	-1.93	-4.55	-6.95	2.40
			14225	0.69	1.33	-1.00	-2.42	-4.57	-7.35	2.11
			8352	0.57	1.39	-1.18	-4.08	-4.33	-8.88	1.01
		5 year_AA60	17068	0.73	1.53	-0.91	-1.83	-3.80	-5.99	3.08
			9680	0.63	1.40	-1.14	-3.20	-4.31	-7.97	1.66
			10253	0.67	1.18	-1.12	-2.70	-5.14	-8.24	1.47
		10 year_AA60	5903	0.57	1.02	-1.26	-4.01	-5.79	-10.22	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Parabens	0 month RT	64215	0.85	2.45	0.58	-0.17	-0.22	0.11	7.48
			40267	0.89	2.19	-0.18	0.37	-1.25	-0.94	6.73
		2 months RT	66503	0.84	2.43	0.65	-0.38	-0.30	-0.12	7.32
			43654	0.91	2.28	-0.07	0.62	-0.88	-0.27	7.21
		6 months RT	55094	0.93	2.45	0.29	0.82	-0.21	0.84	8.01
			28165	0.90	2.25	-0.56	0.44	-1.01	-0.96	6.72
		8 months RT	52299	0.87	2.44	0.20	0.11	-0.28	0.02	7.42
			47010	0.89	2.25	0.03	0.37	-0.98	-0.51	7.04
		10 months RT	39855	0.92	2.39	-0.19	0.70	-0.46	0.10	7.47
			21678	0.86	2.19	-0.76	-0.12	-1.22	-1.86	6.07
		1 year_AA50	47574	0.89	2.33	0.05	0.36	-0.70	-0.26	7.22
			43938	0.86	2.31	-0.06	-0.03	-0.77	-0.79	6.84
		1 year_AA60	163098	0.92	2.42	3.68	0.71	-0.35	3.40	9.85
			66432	0.90	2.32	0.64	0.49	-0.73	0.33	7.64
		2.5 year_AA50	55106	0.89	2.18	0.29	0.37	-1.29	-0.59	6.98
			60254	0.87	2.11	0.45	0.06	-1.53	-0.97	6.71
		5 year_AA50	33815	0.87	1.88	-0.38	0.05	-2.45	-2.51	5.59
			34581	0.85	1.86	-0.36	-0.23	-2.52	-2.83	5.37
		5 year_AA60	28597	0.84	1.90	-0.55	-0.33	-2.36	-2.93	5.29
			20640	0.78	1.87	-0.80	-1.16	-2.48	-4.04	4.49
		10 year_AA60	9882	0.62	1.39	-1.13	-3.30	-4.35	-8.10	1.57
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Propyl Gallate	0 month RT	55328	0.87	2.34	0.30	0.04	-0.66	-0.32	7.17
			29250	0.86	2.33	-0.52	-0.04	-0.68	-1.09	6.62
			37973	0.89	2.16	-0.25	0.28	-1.33	-1.16	6.57
		2 months RT	62592	0.88	2.47	0.52	0.15	-0.16	0.44	7.72
			33075	0.87	2.44	-0.40	0.06	-0.27	-0.52	7.03
			40430	0.91	2.20	-0.17	0.60	-1.19	-0.65	6.93
		6 months RT	37632	0.87	2.44	-0.26	0.05	-0.25	-0.40	7.12
			31591	0.88	2.29	-0.45	0.18	-0.83	-0.96	6.71
			37165	0.88	2.17	-0.28	0.26	-1.29	-1.17	6.57
		8 months RT	54765	0.87	2.38	0.28	0.13	-0.50	-0.11	7.33
			59185	0.89	2.23	0.42	0.34	-1.07	-0.31	7.18
			38406	0.90	2.24	-0.24	0.51	-1.04	-0.66	6.93
		10 months RT	31806	0.88	2.44	-0.44	0.16	-0.24	-0.43	7.10
			19099	0.88	2.24	-0.84	0.17	-1.02	-1.47	6.34
			27055	0.87	2.21	-0.59	0.05	-1.14	-1.49	6.33
		1 year_AA50	48709	0.88	2.25	0.09	0.22	-0.98	-0.62	6.96
			48412	0.84	2.29	0.08	-0.31	-0.86	-1.02	6.67
			31135	0.87	2.16	-0.47	0.09	-1.33	-1.52	6.31
		1 year_AA60	115764	0.93	2.29	2.20	0.89	-0.85	1.88	8.76
			80791	0.89	2.23	1.10	0.36	-1.08	0.26	7.59
			65120	0.86	2.25	0.60	-0.08	-0.99	-0.49	7.05
		2.5 year_AA50	53786	0.86	1.94	0.25	-0.11	-2.20	-1.93	6.02
			51362	0.84	1.98	0.17	-0.33	-2.03	-2.04	5.93
			38589	0.80	1.83	-0.23	-0.82	-2.62	-3.38	4.97
		5 year_AA50	25604	0.86	1.53	-0.64	-0.06	-3.82	-4.09	4.46
			21053	0.80	1.75	-0.78	-0.85	-2.95	-4.17	4.40
			19177	0.75	1.49	-0.84	-1.58	-3.97	-5.85	3.19
		5 year_AA60	28658	0.74	1.64	-0.54	-1.64	-3.38	-5.13	3.71
			15154	0.80	1.51	-0.97	-0.85	-3.90	-5.19	3.66
			12258	0.70	1.47	-1.06	-2.24	-4.04	-6.73	2.55
		10 year_AA60	8091	0.66	1.19	-1.19	-2.72	-5.12	-8.30	1.42
			6678	0.52	1.01	-1.23	-4.66	-5.81	-10.84	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Sodium Azide	0 month RT	46600	0.86	2.28	0.02	-0.07	-0.89	-0.86	6.78
			33981	0.86	2.28	-0.38	-0.02	-0.88	-1.14	6.59
			13640	0.83	1.99	-1.02	-0.41	-2.01	-3.08	5.19
		2 months RT	50433	0.88	2.39	0.14	0.15	-0.46	-0.17	7.29
			47271	0.85	2.33	0.04	-0.22	-0.67	-0.80	6.83
			28458	0.83	2.15	-0.55	-0.43	-1.39	-2.14	5.86
		6 months RT	61687	0.90	2.34	0.50	0.53	-0.65	0.32	7.64
			26673	0.90	2.25	-0.61	0.44	-0.99	-0.98	6.70
			50072	0.88	2.06	0.13	0.15	-1.73	-1.34	6.44
		8 months RT	36346	0.89	2.39	-0.30	0.40	-0.45	-0.28	7.20
			29932	0.86	2.41	-0.50	-0.07	-0.38	-0.83	6.81
			17531	0.81	2.04	-0.89	-0.68	-1.80	-3.04	5.21
		10 months RT	26550	0.86	2.50	-0.61	-0.03	-0.02	-0.55	7.01
			24737	0.90	2.16	-0.67	0.41	-1.33	-1.38	6.41
			15313	0.88	2.12	-0.96	0.20	-1.49	-1.97	5.99
		1 year_AA50	32343	0.92	2.26	-0.43	0.70	-0.97	-0.57	6.99
			29517	0.85	2.20	-0.52	-0.14	-1.21	-1.67	6.20
			26066	0.85	2.18	-0.62	-0.19	-1.27	-1.86	6.06
		1 year_AA60	115666	0.93	2.40	2.19	0.85	-0.42	2.24	9.02
			105804	0.92	2.37	1.88	0.76	-0.53	1.79	8.70
			61820	0.90	2.15	0.50	0.47	-1.40	-0.42	7.10
		2.5 year_AA50	100702	0.92	2.30	1.72	0.73	-0.80	1.39	8.41
			63631	0.84	2.22	0.56	-0.39	-1.10	-0.93	6.74
			32672	0.80	2.04	-0.42	-0.88	-1.81	-2.85	5.35
		5 year_AA50	42703	0.88	2.15	-0.10	0.20	-1.39	-1.16	6.57
			41376	0.89	2.12	-0.14	0.30	-1.51	-1.21	6.53
			23562	0.87	2.00	-0.70	0.11	-1.96	-2.27	5.77
		5 year_AA60	24555	0.86	2.13	-0.67	-0.10	-1.47	-2.00	5.96
			25973	0.83	2.11	-0.63	-0.43	-1.53	-2.33	5.73
			18721	0.84	1.87	-0.86	-0.35	-2.47	-3.31	5.02
		10 year_AA60	20390	0.85	2.01	-0.80	-0.16	-1.94	-2.60	5.53
			21441	0.87	1.85	-0.77	0.13	-2.57	-2.87	5.34
			18345	0.83	1.96	-0.87	-0.54	-2.12	-3.18	5.11

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Saliva	Zinc	0 month RT	32726	0.85	2.37	-0.42	-0.20	-0.55	-1.04	6.66
			33180	0.88	2.17	-0.40	0.16	-1.32	-1.39	6.40
			29364	0.82	2.39	-0.52	-0.58	-0.47	-1.41	6.39
		2 months RT	55203	0.91	2.43	0.29	0.65	-0.29	0.60	7.84
			44287	0.91	2.41	-0.05	0.65	-0.37	0.25	7.58
			45884	0.88	2.30	0.00	0.22	-0.81	-0.54	7.02
		6 months RT	40829	0.88	2.43	-0.16	0.18	-0.30	-0.23	7.24
			31366	0.92	2.24	-0.46	0.78	-1.05	-0.59	6.98
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		8 months RT	50380	0.85	2.47	0.14	-0.14	-0.15	-0.16	7.29
			31722	0.87	2.40	-0.45	0.02	-0.40	-0.71	6.89
			34373	0.89	2.24	-0.36	0.39	-1.04	-0.88	6.77
		10 months RT	33830	0.91	2.41	-0.38	0.64	-0.37	-0.04	7.38
			24714	0.90	2.33	-0.67	0.50	-0.67	-0.68	6.91
			25136	0.93	2.18	-0.65	0.91	-1.28	-0.84	6.80
		1 year_AA50	50759	0.88	2.38	0.15	0.20	-0.49	-0.14	7.31
			33922	0.89	2.31	-0.38	0.29	-0.78	-0.74	6.87
			26139	0.87	2.18	-0.62	0.13	-1.27	-1.55	6.29
		1 year_AA60	61276	0.91	2.39	0.48	0.68	-0.47	0.62	7.85
			52470	0.90	2.40	0.21	0.46	-0.42	0.23	7.57
			51857	0.91	2.28	0.19	0.62	-0.88	-0.06	7.36
		2.5 year_AA50	83068	0.89	2.26	1.17	0.30	-0.96	0.37	7.67
			49460	0.89	2.14	0.11	0.30	-1.45	-0.94	6.73
			64697	0.83	2.19	0.59	-0.50	-1.22	-1.12	6.60
		5 year_AA50	46016	0.88	2.07	0.00	0.20	-1.71	-1.37	6.42
			31610	0.87	1.80	-0.45	0.09	-2.77	-2.82	5.37
			22950	0.83	1.80	-0.72	-0.47	-2.75	-3.57	4.83
		5 year_AA60	23071	0.80	2.11	-0.72	-0.92	-1.53	-2.89	5.32
			26576	0.83	1.91	-0.61	-0.52	-2.31	-3.12	5.15
			15983	0.85	1.84	-0.94	-0.24	-2.60	-3.40	4.96
		10 year_AA60	24779	0.76	1.93	-0.67	-1.46	-2.25	-4.02	4.51
			22504	0.84	1.63	-0.74	-0.39	-3.43	-4.13	4.43
			10337	0.73	1.50	-1.12	-1.86	-3.92	-6.31	2.86

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Actin	0 month RT	112306	0.94	2.53	2.09	0.97	0.10	2.74	9.38
			98896	0.93	2.54	1.67	0.84	0.12	2.28	9.05
			94807	0.92	2.53	1.54	0.78	0.11	2.12	8.93
		2 months RT	120631	0.94	2.52	2.35	1.00	0.04	2.94	9.52
			81782	0.93	2.54	1.13	0.88	0.14	1.91	8.78
			75539	0.90	2.52	0.93	0.42	0.06	1.23	8.29
		6 months RT	102789	0.93	2.54	1.79	0.85	0.12	2.40	9.13
			57511	0.87	2.56	0.36	0.06	0.20	0.54	7.79
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		8 months RT	99570	0.94	2.52	1.69	0.98	0.05	2.38	9.12
			110482	0.91	2.51	2.03	0.60	0.00	2.25	9.03
			83675	0.94	2.54	1.19	0.97	0.14	2.04	8.88
		10 months RT	87639	0.93	2.54	1.31	0.84	0.14	2.02	8.86
			82106	0.92	2.53	1.14	0.77	0.08	1.75	8.67
			65790	0.91	2.51	0.62	0.61	0.03	1.13	8.22
		1 year_AA50	87368	0.93	2.46	1.30	0.84	-0.17	1.72	8.65
			47683	0.89	2.49	0.06	0.30	-0.06	0.27	7.60
			48631	0.92	2.37	0.08	0.72	-0.54	0.27	7.60
		1 year_AA60	123049	0.92	2.48	2.43	0.70	-0.12	2.56	9.25
			91332	0.91	2.47	1.43	0.64	-0.14	1.66	8.60
			82548	0.92	2.41	1.15	0.73	-0.36	1.32	8.35
		2.5 year_AA50	118477	0.89	2.40	2.28	0.32	-0.40	1.82	8.72
			108982	0.90	2.34	1.98	0.51	-0.65	1.53	8.51
			46426	0.88	2.30	0.02	0.19	-0.79	-0.53	7.02
		5 year_AA50	44095	0.94	2.23	-0.06	1.07	-1.07	0.01	7.41
			39054	0.94	2.13	-0.22	0.97	-1.48	-0.61	6.97
			26326	0.87	2.35	-0.62	0.05	-0.62	-1.03	6.66
		5 year_AA60	85768	0.88	2.05	1.25	0.21	-1.79	-0.41	7.11
			53755	0.90	2.02	0.25	0.44	-1.91	-1.13	6.59
			32361	0.80	2.04	-0.43	-0.82	-1.80	-2.80	5.39
		10 year_AA60	40110	0.86	1.97	-0.18	-0.03	-2.07	-2.08	5.90
			44678	0.83	1.99	-0.04	-0.49	-1.99	-2.34	5.72
			41101	0.86	1.83	-0.15	-0.10	-2.65	-2.65	5.49

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Ascorbic Acid	0 month RT	103911	0.94	2.55	1.82	1.09	0.17	2.71	9.36
			61958	0.93	2.54	0.50	0.90	0.13	1.40	8.42
		2 months RT	137199	0.97	2.54	2.87	1.36	0.14	3.80	10.15
			123685	0.92	2.51	2.45	0.82	0.00	2.80	9.42
		6 months RT	106985	0.93	2.52	1.92	0.88	0.03	2.46	9.18
			92624	0.93	2.52	1.47	0.82	0.04	2.03	8.87
		8 months RT	85606	0.91	2.53	1.25	0.61	0.07	1.69	8.62
			78208	0.91	2.51	1.02	0.57	0.00	1.38	8.40
		10 months RT	101736	0.93	2.54	1.76	0.87	0.12	2.39	9.13
			78754	0.92	2.53	1.03	0.76	0.11	1.68	8.62
		1 year_AA50	86252	0.94	2.46	1.27	0.97	-0.19	1.80	8.70
			68233	0.93	2.46	0.70	0.85	-0.20	1.22	8.28
		1 year_AA60	78431	0.93	2.32	1.02	0.88	-0.71	1.04	8.15
			60833	0.94	2.25	0.47	0.95	-1.02	0.37	7.67
		2.5 year_AA50	99996	0.94	2.32	1.70	1.06	-0.73	1.75	8.67
			104616	0.92	2.35	1.85	0.79	-0.60	1.72	8.65
		5 year_AA50	55299	0.87	2.13	0.29	0.11	-1.47	-1.00	6.68
			26893	0.91	2.06	-0.60	0.55	-1.75	-1.57	6.28
		5 year_AA60	70426	0.91	2.28	0.77	0.62	-0.90	0.40	7.70
			72211	0.92	1.91	0.83	0.78	-2.31	-0.69	6.91
		10 year_AA60	102318	0.94	2.52	1.77	0.99	0.07	2.48	9.19
			66761	0.90	2.02	0.66	0.50	-1.89	-0.71	6.89

Fluid	Pres.	Time Point	TPH	MLB	SH	tph	mlb	sh	pc	FI
Semen	ATA	0 month RT	111640	0.95	2.56	2.07	1.19	0.22	3.05	9.60
			79951	0.94	2.54	1.07	0.96	0.12	1.92	8.79
			79945	0.92	2.55	1.07	0.76	0.15	1.75	8.67
			90694	0.89	2.52	1.41	0.39	0.07	1.60	8.56
		2 months RT	106635	0.95	2.56	1.91	1.22	0.19	2.92	9.51
			102712	0.94	2.53	1.79	1.01	0.09	2.53	9.23
			104286	0.92	2.53	1.84	0.81	0.09	2.37	9.11
			98221	0.90	2.52	1.64	0.45	0.04	1.82	8.72
		6 months RT	102591	0.92	2.52	1.78	0.76	0.04	2.24	9.02
			105763	0.91	2.52	1.88	0.68	0.04	2.24	9.02
			95536	0.93	2.51	1.56	0.93	0.03	2.21	9.00
			82621	0.91	2.52	1.15	0.55	0.06	1.54	8.51
		8 months RT	116962	0.95	2.51	2.23	1.14	0.00	2.94	9.52
			97484	0.94	2.51	1.62	0.98	0.02	2.29	9.06
			96854	0.93	2.51	1.60	0.91	0.01	2.20	8.99
			65430	0.91	2.51	0.61	0.62	0.01	1.11	8.21
		10 months RT	92056	0.93	2.51	1.45	0.89	0.03	2.08	8.90
			87607	0.92	2.51	1.31	0.77	0.03	1.85	8.74
			91144	0.91	2.50	1.42	0.55	-0.02	1.68	8.62
			56495	0.88	2.48	0.33	0.24	-0.10	0.41	7.70
		1 year_AA50	87921	0.93	2.46	1.32	0.87	-0.20	1.74	8.66
			93128	0.92	2.45	1.48	0.69	-0.21	1.70	8.63
			79469	0.95	2.43	1.05	1.11	-0.31	1.65	8.60
			54532	0.90	2.43	0.27	0.50	-0.31	0.42	7.71
		1 year_AA60	85127	0.86	2.50	1.23	-0.04	-0.03	0.95	8.09
			58114	0.91	2.36	0.38	0.61	-0.57	0.38	7.68
			60641	0.93	2.18	0.46	0.84	-1.27	0.02	7.42
			68167	0.88	2.22	0.70	0.19	-1.13	-0.27	7.21
		2.5 year_AA50	131380	0.95	2.41	2.69	1.09	-0.36	2.94	9.52
			114931	0.92	2.40	2.17	0.78	-0.41	2.16	8.96
			100857	0.89	2.42	1.73	0.40	-0.32	1.51	8.50
			93259	0.92	2.29	1.49	0.73	-0.85	1.14	8.23
		5 year_AA50	40061	0.86	2.41	-0.18	-0.07	-0.39	-0.57	6.99
			42654	0.90	2.25	-0.10	0.46	-1.02	-0.58	6.99
			35615	0.92	2.21	-0.32	0.69	-1.14	-0.65	6.94
			24301	0.91	2.20	-0.68	0.67	-1.18	-1.00	6.68
		5 year_AA60	84671	0.86	2.27	1.22	-0.03	-0.93	0.12	7.49
			61711	0.87	2.13	0.50	0.09	-1.47	-0.85	6.79
			55275	0.83	2.19	0.29	-0.46	-1.22	-1.32	6.45
			31822	0.84	2.22	-0.44	-0.40	-1.11	-1.76	6.13
		10 year_AA60	48788	0.90	2.09	0.09	0.51	-1.62	-0.92	6.74
			41387	0.89	2.13	-0.14	0.36	-1.47	-1.12	6.60
			44060	0.84	1.92	-0.06	-0.30	-2.30	-2.45	5.64

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Bronopol	0 month RT	67365	0.92	2.50	0.67	0.71	-0.04	1.20	8.27
			86764	0.89	2.46	1.28	0.29	-0.17	1.18	8.26
			72726	0.93	2.37	0.84	0.91	-0.55	1.07	8.18
		2 months RT	112199	0.94	2.50	2.08	1.04	-0.02	2.70	9.35
			87262	0.93	2.48	1.30	0.87	-0.09	1.82	8.72
			85675	0.86	2.43	1.25	-0.09	-0.31	0.65	7.87
		6 months RT	46243	0.90	2.34	0.01	0.49	-0.65	-0.12	7.32
			51614	0.85	2.35	0.18	-0.20	-0.62	-0.61	6.96
			29430	0.86	2.21	-0.52	-0.01	-1.14	-1.48	6.34
		8 months RT	34614	0.91	2.24	-0.36	0.58	-1.04	-0.69	6.91
			25859	0.94	2.16	-0.63	1.00	-1.36	-0.80	6.83
			18610	0.87	2.19	-0.86	0.08	-1.23	-1.76	6.14
		10 months RT	58377	0.93	2.24	0.39	0.82	-1.04	0.16	7.52
			40988	0.89	2.15	-0.16	0.38	-1.37	-1.02	6.67
			26485	0.83	2.13	-0.61	-0.48	-1.48	-2.32	5.73
		1 year_AA50	63039	0.94	2.43	0.54	1.04	-0.31	1.16	8.24
			59774	0.91	2.31	0.44	0.59	-0.78	0.20	7.55
			32491	0.88	1.93	-0.42	0.18	-2.23	-2.23	5.80
		1 year_AA60	51215	0.74	1.45	0.17	-1.73	-4.10	-5.29	3.59
			34981	0.73	1.43	-0.34	-1.84	-4.21	-5.91	3.14
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		2.5 year_AA50	88381	0.90	2.27	1.34	0.49	-0.94	0.71	7.92
			52167	0.83	2.26	0.20	-0.42	-0.97	-1.13	6.59
			56521	0.87	2.06	0.33	0.10	-1.76	-1.25	6.51
		5 year_AA50	25531	0.84	1.72	-0.64	-0.28	-3.08	-3.62	4.79
			23131	0.80	1.76	-0.72	-0.84	-2.91	-4.07	4.47
			2789	0.59	1.03	-1.36	-3.73	-5.74	-9.97	0.22
		5 year_AA60	9593	0.61	2.07	-1.14	-3.44	-1.71	-5.82	3.21
			24370	0.67	0.87	-0.68	-2.67	-6.39	-8.99	0.93
			21316	0.58	0.82	-0.77	-3.90	-6.57	-10.42	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Chitosan	0 month RT	88820	0.93	2.49	1.35	0.87	-0.05	1.90	8.78
			55531	0.93	2.45	0.30	0.90	-0.21	0.92	8.07
			40991	0.90	2.50	-0.16	0.41	-0.04	0.24	7.58
		2 months RT	126930	0.93	2.50	2.55	0.90	-0.04	2.93	9.52
			89436	0.92	2.48	1.37	0.82	-0.08	1.83	8.73
			64961	0.91	2.40	0.60	0.61	-0.43	0.69	7.90
		6 months RT	103381	0.94	2.53	1.81	1.02	0.11	2.57	9.26
			105179	0.93	2.52	1.86	0.88	0.05	2.43	9.16
			68749	0.88	2.54	0.72	0.16	0.14	0.87	8.04
		8 months RT	109581	0.94	2.52	2.00	1.08	0.06	2.74	9.38
			93886	0.93	2.55	1.51	0.85	0.19	2.23	9.01
			85380	0.89	2.54	1.24	0.34	0.13	1.47	8.47
		10 months RT	132316	0.95	2.53	2.72	1.15	0.07	3.41	9.86
			99545	0.94	2.54	1.69	1.00	0.14	2.49	9.20
			34351	0.86	2.37	-0.36	-0.09	-0.53	-0.86	6.78
		1 year_AA50	73859	0.92	2.46	0.88	0.80	-0.18	1.33	8.36
			63781	0.91	2.47	0.56	0.67	-0.14	0.97	8.11
			35110	0.86	2.38	-0.34	-0.02	-0.50	-0.76	6.86
		1 year_AA60	93472	0.91	2.34	1.50	0.64	-0.63	1.26	8.31
			75489	0.93	2.37	0.93	0.88	-0.52	1.14	8.22
			51167	0.82	2.16	0.16	-0.56	-1.37	-1.66	6.21
		2.5 year_AA50	82664	0.95	2.30	1.16	1.09	-0.81	1.26	8.31
			74654	0.90	2.24	0.90	0.42	-1.05	0.19	7.54
			60559	0.82	2.32	0.46	-0.57	-0.72	-0.83	6.80
		5 year_AA50	38986	0.91	2.14	-0.22	0.64	-1.41	-0.86	6.79
			34394	0.89	2.25	-0.36	0.29	-0.99	-0.93	6.73
			39331	0.86	1.98	-0.21	-0.03	-2.06	-2.09	5.90
		5 year_AA60	54283	0.85	2.02	0.26	-0.15	-1.91	-1.68	6.20
			38277	0.88	2.00	-0.24	0.15	-1.98	-1.87	6.06
			38889	0.80	2.01	-0.22	-0.84	-1.93	-2.77	5.41
		10 year_AA60	36093	0.85	1.93	-0.31	-0.24	-2.24	-2.54	5.57
			37131	0.81	1.98	-0.28	-0.74	-2.07	-2.84	5.36
			37300	0.86	1.74	-0.27	-0.02	-2.98	-2.97	5.26

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	EDTA	0 month RT	90071	0.93	2.55	1.39	0.91	0.17	2.17	8.97
			88216	0.92	2.55	1.33	0.81	0.15	2.02	8.86
			35543	0.91	2.58	-0.33	0.58	0.27	0.53	7.79
		2 months RT	103648	0.92	2.47	1.82	0.72	-0.15	2.04	8.88
			85372	0.92	2.48	1.24	0.74	-0.08	1.66	8.60
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		6 months RT	93067	0.92	2.48	1.48	0.70	-0.10	1.80	8.70
			83515	0.87	2.48	1.18	0.01	-0.09	0.89	8.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		8 months RT	79165	0.95	2.44	1.05	1.18	-0.27	1.75	8.67
			71050	0.92	2.44	0.79	0.71	-0.26	1.09	8.19
			40726	0.92	2.37	-0.16	0.80	-0.54	0.14	7.51
		10 months RT	71452	0.95	2.42	0.80	1.15	-0.33	1.47	8.46
			66676	0.92	2.39	0.65	0.78	-0.45	0.87	8.04
			28933	0.84	2.32	-0.53	-0.33	-0.73	-1.43	6.37
		1 year_AA50	78628	0.94	2.51	1.03	1.05	0.01	1.86	8.75
			84218	0.93	2.49	1.20	0.86	-0.05	1.78	8.69
			43896	0.90	2.45	-0.06	0.45	-0.20	0.19	7.54
		1 year_AA60	152669	0.93	2.48	3.36	0.95	-0.09	3.59	9.99
			92864	0.91	2.47	1.48	0.67	-0.15	1.72	8.65
			111623	0.91	2.30	2.07	0.65	-0.79	1.60	8.56
		2.5 year_AA50	122235	0.93	2.48	2.40	0.83	-0.11	2.67	9.33
			126848	0.93	2.43	2.54	0.84	-0.31	2.62	9.30
			98288	0.92	2.56	1.65	0.78	0.19	2.28	9.05
		5 year_AA50	47262	0.96	2.38	0.04	1.31	-0.50	0.84	8.01
			53028	0.91	2.45	0.22	0.65	-0.23	0.59	7.83
			43475	0.93	2.32	-0.08	0.82	-0.74	0.05	7.44
		5 year_AA60	188772	0.89	2.44	4.49	0.27	-0.26	3.72	10.09
			89229	0.94	2.39	1.36	0.96	-0.44	1.64	8.59
			94421	0.90	2.23	1.53	0.47	-1.08	0.72	7.93
		10 year_AA60	80327	0.92	2.27	1.08	0.77	-0.93	0.78	7.97
			63002	0.92	2.26	0.54	0.80	-0.96	0.33	7.64
			31533	0.87	2.20	-0.45	0.01	-1.20	-1.46	6.35

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Lysozyme	0 month RT	105288	0.92	2.54	1.87	0.69	0.13	2.32	9.08
			94448	0.92	2.56	1.53	0.69	0.22	2.12	8.94
			73106	0.89	2.53	0.85	0.28	0.10	1.06	8.17
			41183	0.93	2.50	-0.15	0.84	-0.04	0.65	7.88
		2 months RT	129495	0.93	2.54	2.63	0.94	0.13	3.18	9.70
			98399	0.91	2.53	1.65	0.67	0.08	2.07	8.90
			88884	0.92	2.53	1.35	0.71	0.09	1.88	8.76
			97483	0.89	2.51	1.62	0.32	0.02	1.67	8.61
		6 months RT	108963	0.94	2.52	1.98	1.04	0.05	2.68	9.34
			110211	0.92	2.53	2.02	0.75	0.07	2.46	9.18
			89806	0.94	2.53	1.38	1.08	0.09	2.26	9.03
			88532	0.90	2.53	1.34	0.52	0.07	1.67	8.61
		8 months RT	104479	0.94	2.54	1.84	1.08	0.12	2.67	9.33
			95165	0.93	2.52	1.55	0.93	0.07	2.23	9.01
			109364	0.89	2.49	1.99	0.33	-0.05	1.91	8.78
			11435	0.94	2.50	-1.08	1.01	-0.02	0.06	7.45
		10 months RT	98023	0.94	2.55	1.64	0.99	0.17	2.46	9.18
			102654	0.92	2.54	1.78	0.76	0.14	2.33	9.08
			94262	0.94	2.52	1.52	1.04	0.06	2.31	9.07
			39714	0.87	2.49	-0.20	0.11	-0.08	-0.13	7.31
		1 year_AA50	93310	0.95	2.48	1.49	1.10	-0.11	2.18	8.98
			83286	0.92	2.48	1.17	0.72	-0.10	1.56	8.53
			84876	0.91	2.46	1.22	0.62	-0.19	1.43	8.43
			70164	0.89	2.49	0.76	0.38	-0.06	0.94	8.08
		1 year_AA60	71200	0.94	2.28	0.79	1.00	-0.86	0.82	8.00
			69005	0.90	2.33	0.73	0.52	-0.68	0.47	7.75
			64813	0.91	2.23	0.59	0.55	-1.07	0.03	7.43
			45386	0.91	2.21	-0.02	0.56	-1.16	-0.54	7.01
		2.5 year_AA50	111224	0.89	2.35	2.05	0.37	-0.61	1.49	8.48
			80163	0.89	2.31	1.08	0.34	-0.78	0.50	7.77
			48972	0.87	2.37	0.10	0.07	-0.52	-0.33	7.17
			18774	0.90	2.18	-0.85	0.46	-1.27	-1.42	6.38
		5 year_AA50	18856	0.91	2.21	-0.85	0.55	-1.13	-1.21	6.53
			31381	0.90	2.09	-0.46	0.51	-1.62	-1.38	6.41
			31013	0.90	2.01	-0.47	0.48	-1.94	-1.71	6.17
			32617	0.87	1.93	-0.42	0.09	-2.25	-2.32	5.73
		5 year_AA60	45813	0.88	1.35	0.00	0.25	-4.51	-3.90	4.59
			31235	0.76	1.84	-0.46	-1.48	-2.61	-4.20	4.38
			38998	0.74	1.54	-0.22	-1.71	-3.76	-5.27	3.61
			19420	0.74	1.30	-0.83	-1.75	-4.69	-6.68	2.59
		10 year_AA60	38377	0.86	1.73	-0.24	-0.05	-3.01	-3.01	5.24
			17340	0.77	1.72	-0.90	-1.29	-3.07	-4.80	3.95
			22398	0.72	1.14	-0.74	-2.03	-5.31	-7.44	2.04
			3163	0.58	0.70	-1.35	-3.87	-7.03	-11.29	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Nisin	0 month RT	104656	0.96	2.52	1.85	1.24	0.05	2.76	9.39
			86479	0.94	2.54	1.28	1.02	0.12	2.15	8.95
			74918	0.90	2.53	0.91	0.44	0.10	1.27	8.32
		2 months RT	116963	0.96	2.51	2.23	1.28	0.02	3.09	9.63
			119324	0.90	2.51	2.31	0.44	0.00	2.33	9.08
			98027	0.91	2.55	1.64	0.67	0.15	2.14	8.94
		6 months RT	89148	0.90	2.54	1.36	0.43	0.12	1.65	8.59
			44853	0.90	2.42	-0.03	0.53	-0.34	0.17	7.53
			1448	0.48	0.94	-1.40	-5.21	-6.08	-11.75	0.05
		8 months RT	50740	0.93	2.47	0.15	0.95	-0.14	0.92	8.06
			54560	0.92	2.47	0.27	0.81	-0.14	0.87	8.03
			112083	0.71	2.54	2.08	-2.11	0.13	-0.20	7.26
		10 months RT	102574	0.94	2.52	1.78	0.99	0.06	2.48	9.19
			97703	0.92	2.53	1.63	0.70	0.09	2.09	8.91
			46204	0.84	2.48	0.01	-0.28	-0.08	-0.34	7.16
		1 year_AA50	79942	0.93	2.46	1.07	0.85	-0.20	1.51	8.50
			54063	0.93	2.39	0.26	0.94	-0.44	0.71	7.92
			48878	0.85	2.48	0.09	-0.18	-0.11	-0.20	7.26
		1 year_AA60	141780	0.92	2.46	3.01	0.74	-0.17	3.04	9.60
			74194	0.93	2.39	0.89	0.86	-0.44	1.15	8.23
			78579	0.91	2.39	1.03	0.63	-0.47	1.02	8.14
		2.5 year_AA50	115087	0.91	2.40	2.17	0.64	-0.43	2.01	8.86
			80065	0.92	2.34	1.07	0.81	-0.64	1.07	8.17
			71850	0.84	2.42	0.82	-0.37	-0.32	0.02	7.42
		5 year_AA50	29691	0.89	2.38	-0.51	0.28	-0.49	-0.60	6.97
			37236	0.90	2.15	-0.27	0.42	-1.40	-1.11	6.61
			51218	0.88	2.12	0.17	0.14	-1.52	-1.12	6.60
		5 year_AA60	80239	0.91	2.20	1.08	0.67	-1.21	0.43	7.71
			49185	0.89	2.13	0.10	0.36	-1.46	-0.91	6.75
			10528	0.77	2.16	-1.11	-1.31	-1.35	-3.41	4.94
		10 year_AA60	44128	0.91	2.30	-0.06	0.64	-0.81	-0.17	7.28
			42248	0.85	1.84	2.17	0.64	-0.43	2.01	8.86

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	None	0 month RT	101988	0.93	2.54	1.76	0.84	0.12	2.37	9.11
			78249	0.89	2.50	1.02	0.30	-0.01	1.12	8.21
			39719	0.76	2.51	-0.20	-1.39	0.00	-1.50	6.33
		2 months RT	95756	0.94	2.54	1.57	1.04	0.11	2.39	9.13
			94062	0.89	2.52	1.51	0.41	0.05	1.68	8.62
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		6 months RT	101518	0.95	2.54	1.75	1.10	0.12	2.60	9.28
			98848	0.95	2.53	1.66	1.17	0.10	2.59	9.27
			102473	0.92	2.52	1.78	0.70	0.04	2.17	8.97
		8 months RT	107492	0.93	2.52	1.94	0.84	0.06	2.46	9.18
			96038	0.94	2.52	1.58	1.00	0.04	2.29	9.06
			92575	0.92	2.52	1.47	0.80	0.03	2.01	8.85
		10 months RT	96898	0.96	2.52	1.60	1.28	0.03	2.58	9.27
			82324	0.94	2.52	1.14	0.99	0.03	1.93	8.79
			65302	0.93	2.50	0.61	0.89	-0.03	1.33	8.36
		1 year_AA50	70435	0.95	2.47	0.77	1.15	-0.16	1.60	8.56
			84702	0.88	2.47	1.22	0.25	-0.13	1.13	8.22
			69014	0.91	2.48	0.73	0.64	-0.11	1.11	8.21
		1 year_AA60	103612	0.94	2.48	1.81	1.01	-0.11	2.36	9.11
			76666	0.93	2.32	0.97	0.95	-0.72	1.05	8.16
			43527	0.82	2.42	-0.08	-0.59	-0.35	-0.95	6.72
		2.5 year_AA50	113636	0.91	2.39	2.13	0.55	-0.47	1.85	8.74
			110922	0.89	2.37	2.04	0.40	-0.53	1.58	8.54
			72513	0.89	2.37	0.84	0.37	-0.54	0.55	7.80
		5 year_AA50	32562	0.92	2.29	-0.42	0.76	-0.83	-0.38	7.13
			25495	0.86	2.31	-0.64	-0.13	-0.76	-1.35	6.43
			36563	0.86	2.19	-0.29	-0.08	-1.24	-1.45	6.36
		5 year_AA60	73131	0.90	2.25	0.86	0.49	-0.99	0.27	7.60
			34843	0.89	2.36	-0.35	0.37	-0.58	-0.46	7.07
			53905	0.86	2.02	0.25	-0.13	-1.88	-1.65	6.21
		10 year_AA60	31439	0.88	2.42	-0.46	0.27	-0.35	-0.44	7.09
			52016	0.90	2.07	0.19	0.43	-1.70	-0.99	6.69
			44571	0.90	1.94	-0.04	0.43	-2.22	-1.65	6.22

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Parabens	0 month RT	116123	0.95	2.55	2.21	1.21	0.15	3.12	9.65
			107219	0.93	2.55	1.93	0.88	0.16	2.58	9.26
			88013	0.94	2.52	1.32	1.06	0.04	2.14	8.95
		2 months RT	132644	0.94	2.49	2.73	0.95	-0.08	3.09	9.63
			95067	0.93	2.54	1.55	0.82	0.14	2.19	8.98
			102577	0.89	2.51	1.78	0.39	0.02	1.86	8.74
		6 months RT	106052	0.91	2.55	1.89	0.58	0.18	2.28	9.05
			86396	0.91	2.51	1.27	0.62	0.02	1.66	8.60
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		8 months RT	124621	0.95	2.49	2.47	1.12	-0.08	3.03	9.59
			101637	0.93	2.53	1.75	0.89	0.09	2.39	9.12
			104401	0.90	2.53	1.84	0.53	0.09	2.11	8.92
		10 months RT	110804	0.94	2.51	2.04	1.03	0.03	2.70	9.35
			83858	0.92	2.54	1.19	0.81	0.13	1.88	8.76
			58740	0.89	2.49	0.40	0.35	-0.05	0.63	7.86
		1 year_AA50	80149	0.95	2.49	1.08	1.13	-0.05	1.93	8.80
			78913	0.90	2.51	1.04	0.54	0.03	1.40	8.41
			65173	0.93	2.49	0.61	0.93	-0.05	1.35	8.37
		1 year_AA60	168662	0.94	2.51	3.86	1.06	0.02	4.21	10.44
			75515	0.91	2.45	0.93	0.63	-0.21	1.18	8.25
			62935	0.91	2.42	0.53	0.62	-0.34	0.72	7.93
		2.5 year_AA50	120227	0.92	2.45	2.34	0.72	-0.20	2.43	9.16
			111306	0.91	2.40	2.06	0.61	-0.42	1.89	8.77
			46757	0.85	2.39	0.03	-0.14	-0.44	-0.52	7.03
		5 year_AA50	40455	0.91	2.35	-0.17	0.61	-0.63	-0.13	7.31
			40673	0.89	2.30	-0.17	0.33	-0.82	-0.58	6.99
			24608	0.86	2.37	-0.67	-0.04	-0.53	-1.08	6.63
		5 year_AA60	61939	0.91	2.32	0.50	0.63	-0.73	0.34	7.65
			45840	0.88	2.48	0.00	0.18	-0.12	0.06	7.45
			85411	0.91	2.01	1.24	0.67	-1.92	-0.09	7.34
		10 year_AA60	51223	0.89	2.16	0.17	0.40	-1.35	-0.72	6.89
			42352	0.88	2.27	-0.11	0.15	-0.94	-0.81	6.82
			31450	0.85	1.88	-0.46	-0.19	-2.46	-2.82	5.37

Fluid	Pres.	Time Point	TPH	MLB	SH	tph	mlb	sh	pc	FI
Semen	Propyl Gallate	0 month RT	109408	0.94	2.54	2.00	0.96	0.13	2.68	9.34
			99970	0.94	2.56	1.70	0.99	0.21	2.54	9.24
			101421	0.92	2.55	1.75	0.74	0.17	2.30	9.07
		2 months RT	119789	0.93	2.50	2.32	0.83	-0.01	2.70	9.35
			112216	0.93	2.53	2.08	0.85	0.09	2.61	9.29
			86492	0.87	2.52	1.28	0.10	0.04	1.19	8.26
		6 months RT	113584	0.95	2.52	2.13	1.19	0.04	2.93	9.52
			131409	0.91	2.53	2.69	0.64	0.09	2.91	9.50
			123344	0.93	2.48	2.43	0.91	-0.09	2.80	9.42
		8 months RT	96757	0.95	2.52	1.60	1.17	0.06	2.49	9.20
			108527	0.91	2.49	1.97	0.63	-0.06	2.17	8.97
			87393	0.93	2.52	1.30	0.82	0.06	1.91	8.78
		10 months RT	101560	0.96	2.55	1.75	1.26	0.15	2.79	9.42
			123693	0.89	2.50	2.45	0.34	-0.04	2.30	9.07
			53363	0.90	2.50	0.23	0.47	-0.03	0.62	7.85
		1 year_AA50	94044	0.94	2.50	1.51	1.00	-0.04	2.18	8.97
			90023	0.94	2.50	1.39	0.96	-0.04	2.02	8.86
			61675	0.89	2.49	0.50	0.33	-0.06	0.67	7.89
		1 year_AA60	100444	0.91	2.29	1.71	0.65	-0.86	1.25	8.31
			69703	0.89	2.45	0.75	0.36	-0.22	0.77	7.96
			50811	0.93	2.40	0.15	0.93	-0.42	0.63	7.86
		2.5 year_AA50	120559	0.93	2.41	2.35	0.82	-0.36	2.38	9.12
			113927	0.93	2.42	2.14	0.91	-0.33	2.34	9.09
			107518	0.90	2.47	1.94	0.43	-0.15	1.87	8.75
		5 year_AA50	36773	0.93	2.29	-0.29	0.83	-0.83	-0.20	7.26
			36426	0.88	2.42	-0.30	0.15	-0.35	-0.42	7.10
			38946	0.90	2.30	-0.22	0.48	-0.82	-0.47	7.07
		5 year_AA60	47543	0.92	1.93	0.05	0.71	-2.22	-1.32	6.46
			42632	0.82	2.15	-0.10	-0.67	-1.40	-2.01	5.96
			61304	0.94	2.29	0.48	0.95	-0.84	0.55	7.80
		10 year_AA60	29980	0.82	2.22	-0.50	-0.55	-1.13	-1.98	5.98
			37105	0.87	1.98	-0.28	0.11	-2.07	-2.02	5.95
			120559	0.93	2.41	2.35	0.82	-0.36	2.38	9.12

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Sodium Azide	0 month RT	90591	0.93	2.56	1.40	0.91	0.22	2.23	9.02
			70777	0.94	2.52	0.78	1.08	0.06	1.74	8.66
			78430	0.90	2.52	1.02	0.48	0.05	1.35	8.38
		2 months RT	109423	0.93	2.52	2.00	0.91	0.06	2.57	9.26
			86965	0.94	2.56	1.29	1.01	0.22	2.23	9.02
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		6 months RT	98946	0.93	2.55	1.67	0.91	0.19	2.42	9.15
			93110	0.94	2.55	1.48	0.97	0.15	2.30	9.06
			85265	0.88	2.54	1.24	0.25	0.15	1.39	8.41
		8 months RT	102951	0.90	2.54	1.79	0.47	0.13	2.05	8.88
			39965	0.95	2.41	-0.19	1.14	-0.38	0.59	7.83
			29040	0.95	2.40	-0.53	1.18	-0.42	0.31	7.63
		10 months RT	95707	0.92	2.55	1.57	0.76	0.15	2.17	8.97
			90998	0.93	2.54	1.42	0.83	0.15	2.10	8.92
			10341	0.82	2.42	-1.12	-0.57	-0.35	-1.79	6.11
		1 year_AA50	89132	0.97	2.52	1.36	1.46	0.04	2.56	9.25
			97934	0.94	2.51	1.64	0.95	0.01	2.27	9.04
			47881	0.88	2.48	0.06	0.19	-0.09	0.15	7.51
		1 year_AA60	89807	0.92	2.41	1.38	0.78	-0.36	1.55	8.52
			89803	0.85	2.46	1.38	-0.24	-0.18	0.74	7.94
			79671	0.89	2.36	1.06	0.28	-0.59	0.60	7.84
		2.5 year_AA50	107374	0.95	2.41	1.93	1.15	-0.36	2.37	9.11
			102094	0.95	2.44	1.77	1.11	-0.25	2.29	9.06
			69600	0.86	2.39	0.74	-0.04	-0.47	0.15	7.51
		5 year_AA50	44055	0.89	2.30	-0.06	0.38	-0.79	-0.41	7.11
			18892	0.90	2.34	-0.85	0.46	-0.65	-0.86	6.79
			38868	0.83	2.24	-0.22	-0.43	-1.04	-1.55	6.29
		5 year_AA60	91564	0.90	2.05	1.44	0.49	-1.80	0.01	7.41
			72138	0.86	2.31	0.82	-0.08	-0.78	-0.12	7.32
			55918	0.88	2.18	0.31	0.22	-1.27	-0.69	6.91
		10 year_AA60	50748	0.91	2.01	0.15	0.68	-1.92	-0.99	6.69
			45409	0.84	1.92	-0.02	-0.33	-2.27	-2.41	5.67
			11321	0.84	2.02	-1.09	-0.37	-1.91	-3.01	5.24

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Semen	Zinc	0 month RT	93884	0.95	2.56	1.51	1.16	0.22	2.57	9.25
			96052	0.91	2.53	1.58	0.64	0.10	2.01	8.86
			81180	0.93	2.53	1.11	0.85	0.10	1.82	8.72
		2 months RT	106160	0.94	2.54	1.89	0.99	0.12	2.63	9.30
			111683	0.93	2.50	2.07	0.90	-0.01	2.55	9.25
			90031	0.90	2.50	1.39	0.42	-0.02	1.52	8.50
		6 months RT	124573	0.92	2.51	2.47	0.80	0.02	2.82	9.44
			107000	0.93	2.52	1.92	0.94	0.06	2.54	9.23
			86614	0.91	2.54	1.28	0.60	0.15	1.77	8.68
		8 months RT	114043	0.94	2.53	2.14	1.00	0.11	2.82	9.44
			113103	0.93	2.52	2.11	0.88	0.06	2.65	9.31
			104548	0.94	2.52	1.84	1.04	0.06	2.57	9.26
		10 months RT	93968	0.94	2.55	1.51	1.08	0.19	2.45	9.17
			95724	0.94	2.54	1.57	0.95	0.11	2.31	9.07
			29383	0.87	2.55	-0.52	0.08	0.16	-0.21	7.26
		1 year_AA50	87980	0.96	2.48	1.32	1.29	-0.08	2.25	9.03
			81941	0.95	2.49	1.13	1.15	-0.07	1.98	8.83
			69839	0.90	2.48	0.75	0.43	-0.11	0.94	8.08
		1 year_AA60	92187	0.95	2.44	1.45	1.11	-0.28	2.01	8.85
			77050	0.92	2.45	0.98	0.68	-0.23	1.25	8.31
			57802	0.90	2.41	0.37	0.50	-0.36	0.46	7.74
		2.5 year_AA50	123079	0.93	2.40	2.43	0.87	-0.43	2.45	9.17
			124350	0.91	2.44	2.47	0.55	-0.24	2.34	9.09
			109917	0.90	2.44	2.01	0.51	-0.27	1.90	8.77
		5 year_AA50	45595	0.91	2.34	-0.01	0.60	-0.63	-0.01	7.40
			22235	0.91	2.38	-0.75	0.59	-0.50	-0.50	7.04
			39666	0.87	2.26	-0.20	0.13	-0.97	-0.92	6.74
		5 year_AA60	94052	0.91	2.32	1.51	0.57	-0.71	1.15	8.23
			70798	0.94	2.32	0.78	1.04	-0.73	0.98	8.11
			35337	0.79	2.21	-0.33	-0.99	-1.15	-2.28	5.76
		10 year_AA60	57503	0.92	2.22	0.36	0.76	-1.12	0.00	7.41
			63241	0.91	2.22	0.54	0.61	-1.13	0.00	7.40
			54595	0.88	2.30	0.27	0.19	-0.80	-0.32	7.17

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Actin	0 month RT	71628	0.88	2.48	0.81	0.14	-0.12	0.69	7.90
			57282	0.88	2.47	0.36	0.21	-0.15	0.35	7.66
			43985	0.87	2.55	-0.06	0.05	0.17	0.15	7.51
		2 months RT	103260	0.90	2.52	1.80	0.46	0.06	1.98	8.83
			70992	0.91	2.49	0.79	0.55	-0.06	1.13	8.22
			63792	0.89	2.51	0.56	0.29	0.00	0.74	7.94
		6 months RT	79190	0.91	2.49	1.05	0.59	-0.08	1.35	8.38
			38209	0.89	2.57	-0.24	0.36	0.25	0.38	7.68
			54951	0.87	2.50	0.28	0.01	-0.03	0.22	7.56
		8 months RT	80879	0.91	2.49	1.10	0.55	-0.07	1.37	8.39
			88652	0.89	2.49	1.34	0.33	-0.07	1.36	8.39
			77358	0.89	2.47	0.99	0.38	-0.13	1.06	8.17
		10 months RT	52234	0.92	2.45	0.20	0.70	-0.22	0.64	7.87
			39563	0.88	2.54	-0.20	0.25	0.12	0.19	7.54
			38808	0.86	2.41	-0.22	-0.04	-0.37	-0.56	7.00
		1 year_AA50	30939	0.90	2.19	-0.47	0.47	-1.23	-1.06	6.64
			19397	0.80	1.98	-0.83	-0.86	-2.05	-3.40	4.96
			7775	0.77	1.31	-1.20	-1.30	-4.66	-6.51	2.71
		1 year_AA60	73653	0.86	2.31	0.87	-0.08	-0.76	-0.06	7.36
			59227	0.88	2.18	0.42	0.18	-1.26	-0.64	6.94
			50330	0.86	2.16	0.14	-0.06	-1.37	-1.20	6.54
		2.5 year_AA50	20101	0.83	1.76	-0.81	-0.52	-2.91	-3.83	4.64
			29448	0.81	1.65	-0.52	-0.80	-3.32	-4.24	4.35
			14412	0.59	1.62	-0.99	-3.68	-3.43	-7.51	1.99
		5 year_AA50	74820	0.96	2.53	0.91	1.27	0.08	2.04	8.88
			1501	0.49	1.01	-1.40	-5.12	-5.82	-11.42	0.05
			3131	0.44	0.98	-1.35	-5.77	-5.93	-12.10	0.05
		5 year_AA60	21408	0.67	1.59	-0.77	-2.60	-3.55	-6.39	2.80
			15539	0.63	1.25	-0.96	-3.21	-4.88	-8.36	1.38
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		10 year_AA60	19081	0.71	1.45	-0.84	-2.12	-4.11	-6.50	2.72

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Ascorbic Acid	0 month RT	45450	0.90	2.47	-0.02	0.41	-0.13	0.27	7.60
			46403	0.86	2.53	0.01	-0.02	0.11	0.09	7.47
			36828	0.87	2.49	-0.29	0.06	-0.08	-0.25	7.22
			33896	0.85	2.57	-0.38	-0.19	0.23	-0.28	7.21
		2 months RT	78999	0.90	2.49	1.04	0.51	-0.08	1.27	8.32
			65376	0.89	2.47	0.61	0.41	-0.14	0.77	7.96
			64349	0.89	2.46	0.58	0.28	-0.19	0.57	7.82
			51678	0.90	2.50	0.18	0.41	-0.03	0.52	7.78
		6 months RT	84577	0.90	2.47	1.22	0.54	-0.16	1.38	8.40
			88364	0.90	2.47	1.33	0.41	-0.13	1.37	8.40
			61464	0.88	2.50	0.49	0.24	-0.04	0.60	7.84
			44756	0.88	2.55	-0.04	0.21	0.16	0.32	7.63
		8 months RT	84996	0.90	2.49	1.23	0.47	-0.08	1.39	8.40
			76155	0.89	2.50	0.95	0.40	-0.03	1.14	8.23
			79216	0.90	2.46	1.05	0.43	-0.17	1.12	8.21
			78316	0.85	2.49	1.02	-0.24	-0.06	0.55	7.80
		10 months RT	48400	0.89	2.46	0.08	0.35	-0.19	0.23	7.57
			38611	0.88	2.55	-0.23	0.21	0.17	0.17	7.53
			36968	0.87	2.36	-0.28	0.08	-0.56	-0.67	6.92
			44076	0.84	2.41	-0.06	-0.39	-0.37	-0.76	6.86
		1 year_AA50	44735	0.86	2.20	-0.04	-0.05	-1.20	-1.19	6.55
			31039	0.82	2.21	-0.47	-0.58	-1.14	-1.99	5.97
			35924	0.82	2.15	-0.31	-0.57	-1.40	-2.09	5.90
			16616	0.79	2.02	-0.92	-1.09	-1.89	-3.54	4.85
		1 year_AA60	70286	0.90	2.24	0.77	0.49	-1.05	0.14	7.50
			59334	0.88	2.20	0.42	0.16	-1.19	-0.59	6.98
			31509	0.84	2.02	-0.45	-0.34	-1.89	-2.44	5.65
			13118	0.79	1.88	-1.03	-1.00	-2.43	-4.04	4.49
		2.5 year_AA50	14477	0.76	1.75	-0.99	-1.38	-2.96	-4.86	3.90
			27200	0.75	1.57	-0.59	-1.56	-3.65	-5.33	3.56
			20441	0.75	1.55	-0.80	-1.62	-3.73	-5.64	3.34
			15181	0.76	1.54	-0.97	-1.46	-3.75	-5.64	3.34
		5 year_AA50	4070	0.63	1.60	-1.32	-3.14	-3.53	-7.35	2.11
			3462	0.59	1.50	-1.34	-3.79	-3.93	-8.36	1.38
			2507	0.49	0.88	-1.37	-5.07	-6.31	-11.80	0.05
			764	0.42	0.92	-1.42	-6.05	-6.17	-12.65	0.05
		5 year_AA60	53037	0.81	2.10	0.22	-0.78	-1.58	-2.01	5.95
			18315	0.72	1.55	-0.87	-1.96	-3.71	-6.01	3.07
			19425	0.73	1.46	-0.83	-1.81	-4.07	-6.16	2.97
			13329	0.72	1.46	-1.03	-1.98	-4.07	-6.48	2.74

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	ATA	0 month RT	43671	0.89	2.46	-0.07	0.28	-0.18	0.05	7.44
			39816	0.92	2.32	-0.19	0.70	-0.74	-0.16	7.29
		2 months RT	63470	0.89	2.48	0.55	0.36	-0.09	0.72	7.93
			58972	0.88	2.49	0.41	0.26	-0.08	0.52	7.78
		6 months RT	87685	0.89	2.49	1.31	0.31	-0.06	1.32	8.36
			77061	0.88	2.47	0.98	0.26	-0.15	0.92	8.07
		8 months RT	66327	0.89	2.47	0.64	0.33	-0.14	0.71	7.92
			27783	0.87	2.33	-0.57	0.08	-0.70	-1.04	6.66
		10 months RT	53067	0.89	2.38	0.22	0.34	-0.49	0.06	7.45
			36585	0.83	2.37	-0.29	-0.41	-0.53	-1.12	6.59
		1 year_AA50	18632	0.76	1.73	-0.86	-1.48	-3.00	-4.89	3.88
			20054	0.76	1.69	-0.81	-1.38	-3.19	-4.92	3.86
		1 year_AA60	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			6901	0.53	1.02	-1.23	-4.55	-5.78	-10.69	0.05
		2.5 year_AA50	9568	0.76	1.62	-1.14	-1.39	-3.46	-5.45	3.48
			15609	0.74	1.48	-0.95	-1.63	-4.01	-6.03	3.06
		5 year_AA50	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			609	0.55	0.71	-1.43	-4.26	-7.00	-11.69	0.05
		5 year_AA60	421	0.40	0.00	-1.43	-6.33	0.00	-7.26	2.17
			2035	0.48	0.74	-1.38	-5.30	-6.87	-12.54	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Bronopol	0 month RT	75327	0.90	2.50	0.92	0.50	-0.03	1.22	8.29
			79628	0.86	2.51	1.06	-0.14	0.03	0.77	7.96
			25254	0.89	2.38	-0.65	0.37	-0.51	-0.65	6.94
		2 months RT	45525	0.85	2.29	-0.01	-0.14	-0.83	-0.91	6.75
			27082	0.84	2.02	-0.59	-0.40	-1.88	-2.59	5.54
			33863	0.83	1.93	-0.38	-0.52	-2.24	-2.88	5.33
		6 months RT	40424	0.83	2.10	-0.17	-0.43	-1.58	-2.00	5.96
			5829	0.63	1.39	-1.26	-3.19	-4.34	-8.10	1.57
			4258	0.58	1.13	-1.31	-3.91	-5.37	-9.77	0.36
		8 months RT	24076	0.84	1.79	-0.69	-0.34	-2.80	-3.47	4.90
			14461	0.75	1.49	-0.99	-1.57	-3.97	-5.97	3.10
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		10 months RT	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		1 year_AA50	255	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.17
			228	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.17
			214	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.17
		1 year_AA60	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		2.5 year_AA50	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		5 year_AA50	4746	0.85	2.18	-1.30	-0.21	-1.27	-2.43	5.66
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		5 year_AA60	0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Chitosan	0 month RT	43520	0.86	2.43	-0.08	-0.07	-0.31	-0.41	7.11
			21952	0.90	2.37	-0.75	0.50	-0.51	-0.61	6.96
			18880	0.86	2.41	-0.85	-0.10	-0.39	-1.16	6.57
		2 months RT	56986	0.88	2.47	0.35	0.23	-0.14	0.38	7.68
			46569	0.87	2.43	0.02	0.00	-0.31	-0.27	7.21
			41011	0.81	2.42	-0.15	-0.75	-0.33	-1.15	6.58
		6 months RT	22362	0.86	2.47	-0.74	-0.04	-0.15	-0.79	6.84
			14056	0.83	2.47	-1.00	-0.44	-0.15	-1.39	6.40
			23402	0.82	2.42	-0.71	-0.66	-0.32	-1.52	6.31
		8 months RT	87951	0.90	2.46	1.32	0.41	-0.17	1.33	8.36
			50204	0.88	2.48	0.13	0.23	-0.11	0.23	7.57
			53427	0.85	2.53	0.24	-0.18	0.08	0.09	7.47
		10 months RT	31106	0.88	2.39	-0.47	0.22	-0.45	-0.59	6.98
			24348	0.85	2.36	-0.68	-0.25	-0.56	-1.32	6.46
			8954	0.76	2.11	-1.16	-1.40	-1.53	-3.71	4.73
		1 year_AA50	18955	0.80	2.04	-0.85	-0.89	-1.81	-3.21	5.09
			21146	0.81	1.89	-0.78	-0.71	-2.39	-3.52	4.87
			2541	0.48	1.13	-1.36	-5.25	-5.37	-11.11	0.05
		1 year_AA60	14527	0.76	2.05	-0.99	-1.37	-1.78	-3.77	4.69
			11893	0.72	2.09	-1.07	-1.93	-1.60	-4.21	4.37
			5528	0.71	1.81	-1.27	-2.17	-2.71	-5.62	3.35
		2.5 year_AA50	14302	0.76	1.86	-0.99	-1.47	-2.51	-4.53	4.14
			16584	0.67	1.42	-0.92	-2.63	-4.22	-7.16	2.25
			11783	0.64	1.42	-1.07	-3.07	-4.24	-7.73	1.84
		5 year_AA50	3394	0.53	1.76	-1.34	-4.58	-2.89	-8.16	1.52
			477	0.43	0.68	-1.43	-5.94	-7.10	-13.42	0.05
			1447	0.46	0.83	-1.40	-5.57	-6.52	-12.50	0.05
		5 year_AA60	9398	0.68	1.40	-1.15	-2.47	-4.31	-7.27	2.16
			12580	0.64	1.44	-1.05	-2.99	-4.17	-7.57	1.95
			9944	0.62	1.25	-1.13	-3.28	-4.89	-8.57	1.23

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	EDTA	0 month RT	70898	0.89	2.52	0.79	0.39	0.04	1.06	8.17
			72647	0.89	2.49	0.84	0.37	-0.05	1.00	8.13
			1410	0.51	2.04	-1.40	-4.83	-1.82	-7.47	2.02
		2 months RT	97896	0.90	2.50	1.63	0.42	-0.03	1.72	8.64
			89958	0.89	2.53	1.38	0.32	0.08	1.52	8.50
			82992	0.87	2.49	1.17	0.08	-0.05	1.00	8.12
		6 months RT	99923	0.92	2.51	1.70	0.74	0.02	2.13	8.94
			88117	0.90	2.49	1.33	0.46	-0.05	1.49	8.48
			63030	0.89	2.42	0.54	0.29	-0.33	0.42	7.70
		8 months RT	43860	0.89	2.40	-0.07	0.33	-0.42	-0.12	7.32
			59628	0.86	2.39	0.43	-0.09	-0.44	-0.14	7.31
			38133	0.87	2.32	-0.25	0.03	-0.74	-0.85	6.79
		10 months RT	30142	0.85	2.31	-0.50	-0.23	-0.77	-1.33	6.44
			27516	0.90	2.04	-0.58	0.45	-1.83	-1.73	6.16
			25376	0.80	2.28	-0.65	-0.91	-0.90	-2.24	5.79
		1 year_AA50	123816	0.89	2.50	2.45	0.30	-0.04	2.27	9.04
			72173	0.87	2.55	0.83	0.03	0.19	0.88	8.04
			53538	0.87	2.49	0.24	0.05	-0.05	0.20	7.55
		1 year_AA60	103428	0.90	2.48	1.81	0.41	-0.09	1.81	8.71
			83795	0.92	2.47	1.19	0.71	-0.16	1.52	8.50
			91821	0.88	2.47	1.44	0.27	-0.14	1.32	8.35
		2.5 year_AA50	74329	0.92	2.49	0.89	0.75	-0.06	1.40	8.42
			59784	0.91	2.45	0.44	0.62	-0.21	0.76	7.95
			52398	0.89	2.41	0.20	0.36	-0.38	0.16	7.52
		5 year_AA50	41987	0.89	2.43	-0.12	0.33	-0.29	-0.05	7.37
			30196	0.87	2.44	-0.49	0.03	-0.26	-0.62	6.96
			13587	0.88	2.14	-1.02	0.16	-1.43	-1.99	5.97
		5 year_AA60	83708	0.85	2.50	1.19	-0.14	-0.03	0.81	7.99
			53069	0.85	2.42	0.22	-0.17	-0.32	-0.28	7.21
			40513	0.82	2.06	-0.17	-0.62	-1.72	-2.32	5.74
		10 year_AA60	67851	0.89	2.43	0.69	0.34	-0.29	0.63	7.86
			60101	0.86	2.42	0.45	-0.01	-0.36	0.03	7.43
			46492	0.84	2.29	0.02	-0.35	-0.83	-1.08	6.63

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Lysozyme	0 month RT	73682	0.87	2.50	0.87	0.04	-0.01	0.75	7.94
			24781	0.89	2.54	-0.67	0.34	0.12	-0.12	7.32
		2 months RT	73693	0.89	2.51	0.87	0.40	0.02	1.12	8.21
			63928	0.87	2.51	0.57	0.03	0.02	0.51	7.78
		6 months RT	79990	0.88	2.50	1.07	0.24	-0.02	1.09	8.19
			53712	0.86	2.51	0.24	-0.13	0.03	0.11	7.48
		8 months RT	81176	0.90	2.47	1.11	0.43	-0.13	1.21	8.28
			68027	0.90	2.49	0.69	0.50	-0.07	0.99	8.12
		10 months RT	54480	0.90	2.48	0.27	0.50	-0.11	0.60	7.84
			52309	0.87	2.47	0.20	0.13	-0.13	0.16	7.52
		1 year_AA50	24616	0.84	2.09	-0.67	-0.38	-1.60	-2.39	5.68
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		1 year_AA60	86211	0.84	2.32	1.27	-0.31	-0.73	0.08	7.46
			15218	0.77	1.67	-0.97	-1.25	-3.26	-4.98	3.81
		2.5 year_AA50	19888	0.77	1.60	-0.82	-1.35	-3.52	-5.20	3.65
			19986	0.65	1.29	-0.82	-2.93	-4.73	-7.83	1.76
		5 year_AA50	3596	0.50	1.17	-1.33	-5.03	-5.21	-10.72	0.05
			7751	0.54	1.09	-1.20	-4.49	-5.51	-10.36	0.05
		5 year_AA60	20961	0.84	1.12	-0.79	-0.33	-5.40	-5.92	3.14
			14353	0.76	1.40	-0.99	-1.46	-4.31	-6.18	2.95

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Nisin	0 month RT	53319	0.88	2.46	0.23	0.22	-0.17	0.25	7.58
			55990	0.87	2.40	0.32	0.08	-0.42	-0.05	7.37
			34305	0.88	2.51	-0.37	0.15	0.01	-0.15	7.30
		2 months RT	73492	0.88	2.49	0.87	0.26	-0.06	0.91	8.06
			70586	0.85	2.52	0.78	-0.14	0.04	0.54	7.80
			73496	0.85	2.50	0.87	-0.24	-0.04	0.44	7.73
		6 months RT	63016	0.89	2.40	0.54	0.37	-0.42	0.41	7.70
			45841	0.88	2.45	0.00	0.19	-0.20	-0.01	7.40
			40475	0.88	2.45	-0.17	0.15	-0.23	-0.21	7.26
		8 months RT	79745	0.90	2.44	1.06	0.48	-0.25	1.10	8.20
			61260	0.90	2.42	0.48	0.42	-0.32	0.51	7.77
			56707	0.90	2.44	0.34	0.45	-0.25	0.49	7.76
		10 months RT	25333	0.89	2.36	-0.65	0.37	-0.57	-0.70	6.90
			30813	0.86	2.31	-0.48	-0.07	-0.77	-1.17	6.56
			15535	0.83	2.30	-0.96	-0.47	-0.79	-1.96	5.99
		1 year_AA50	836	0.47	0.54	-1.42	-5.37	-7.66	-13.37	0.05
			3308	0.49	0.85	-1.34	-5.15	-6.44	-11.97	0.05
			2038	0.48	0.83	-1.38	-5.27	-6.53	-12.20	0.05
		1 year_AA60	19289	0.75	1.76	-0.84	-1.51	-2.89	-4.80	3.94
			10734	0.72	1.66	-1.11	-1.92	-3.31	-5.80	3.23
			3093	0.50	1.03	-1.35	-5.03	-5.76	-11.23	0.05
		2.5 year_AA50	3147	0.45	0.71	-1.35	-5.66	-6.98	-12.96	0.05
			1309	0.44	0.89	-1.40	-5.78	-6.27	-12.48	0.05
			1209	0.44	0.21	-1.41	-5.78	-8.93	-14.92	0.05
		5 year_AA50	132	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.16
			87	0.40	0.00	-1.44	-6.33	0.00	-7.27	2.16
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		5 year_AA60	1140	0.44	0.50	-1.41	-5.84	-7.81	-13.95	0.05
			6133	0.53	1.06	-1.25	-4.55	-5.63	-10.57	0.05
			5866	0.58	0.97	-1.26	-3.90	-5.98	-10.28	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	None	0 month RT	72082	0.91	2.51	0.82	0.57	0.01	1.23	8.29
			78724	0.87	2.53	1.03	0.13	0.09	1.05	8.16
			68063	0.89	2.50	0.70	0.28	-0.02	0.82	8.00
		2 months RT	90095	0.89	2.51	1.39	0.40	0.01	1.54	8.51
			61908	0.89	2.49	0.50	0.32	-0.06	0.66	7.88
			55293	0.85	2.47	0.29	-0.18	-0.13	-0.05	7.37
		6 months RT	66565	0.89	2.48	0.65	0.36	-0.12	0.77	7.96
			60091	0.88	2.49	0.45	0.16	-0.07	0.46	7.74
			56555	0.87	2.43	0.33	0.12	-0.29	0.12	7.49
		8 months RT	83026	0.90	2.49	1.17	0.41	-0.08	1.28	8.33
			79341	0.87	2.48	1.05	0.08	-0.11	0.85	8.02
			38068	0.89	2.48	-0.25	0.38	-0.12	0.05	7.44
		10 months RT	59896	0.89	2.49	0.44	0.38	-0.05	0.68	7.90
			59090	0.85	2.44	0.41	-0.14	-0.24	-0.01	7.39
			36173	0.85	2.38	-0.31	-0.19	-0.50	-0.90	6.76
		1 year_AA50	28006	0.87	1.93	-0.56	0.07	-2.26	-2.47	5.63
			25703	0.75	1.93	-0.64	-1.53	-2.24	-4.05	4.49
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		1 year_AA60	33490	0.83	2.04	-0.39	-0.44	-1.82	-2.41	5.67
			21668	0.83	2.11	-0.76	-0.41	-1.55	-2.44	5.64
			14901	0.78	1.82	-0.98	-1.13	-2.66	-4.33	4.28
		2.5 year_AA50	35390	0.88	2.01	-0.33	0.25	-1.94	-1.81	6.10
			23936	0.81	1.65	-0.69	-0.77	-3.32	-4.35	4.27
			12201	0.66	1.40	-1.06	-2.80	-4.30	-7.51	1.99
		5 year_AA50	1723	0.47	0.91	-1.39	-5.43	-6.20	-12.06	0.05
			656	0.43	0.56	-1.42	-5.86	-7.59	-13.78	0.05
			1466	0.48	0.93	-1.40	-5.28	-6.15	-11.87	0.05
		5 year_AA60	30493	0.67	2.14	-0.49	-2.70	-1.43	-4.31	4.30
			13679	0.67	1.36	-1.01	-2.65	-4.46	-7.48	2.01
			8674	0.61	1.22	-1.17	-3.41	-5.01	-8.85	1.03

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Parabens	0 month RT	78116	0.88	2.53	1.01	0.19	0.09	1.10	8.20
			63229	0.90	2.51	0.54	0.49	0.00	0.92	8.07
			48853	0.88	2.54	0.09	0.24	0.12	0.42	7.71
		2 months RT	79652	0.91	2.51	1.06	0.57	0.03	1.45	8.45
			81024	0.87	2.54	1.10	0.00	0.13	1.03	8.15
			87644	0.85	2.54	1.31	-0.18	0.12	1.02	8.14
		6 months RT	101046	0.89	2.51	1.73	0.28	0.01	1.71	8.63
			76311	0.88	2.51	0.96	0.27	0.03	1.07	8.17
			45947	0.88	2.59	0.00	0.23	0.34	0.53	7.79
		8 months RT	73435	0.89	2.52	0.86	0.31	0.04	1.05	8.16
			64667	0.90	2.55	0.59	0.43	0.16	1.05	8.16
			7680	0.89	2.47	-1.20	0.38	-0.13	-0.74	6.87
		10 months RT	49037	0.87	2.48	0.10	0.06	-0.10	0.04	7.44
			48502	0.88	2.42	0.08	0.25	-0.36	-0.02	7.39
			35327	0.87	2.44	-0.33	0.03	-0.26	-0.49	7.05
		1 year_AA50	43271	0.86	2.22	-0.08	-0.03	-1.12	-1.13	6.59
			29031	0.80	1.89	-0.53	-0.87	-2.39	-3.47	4.91
			14882	0.73	1.75	-0.98	-1.82	-2.94	-5.25	3.62
		1 year_AA60	67585	0.89	2.32	0.68	0.33	-0.72	0.22	7.56
			47033	0.86	2.10	0.03	-0.08	-1.57	-1.49	6.33
			15301	0.83	2.07	-0.96	-0.53	-1.72	-2.88	5.33
		2.5 year_AA50	31464	0.89	1.84	-0.46	0.37	-2.59	-2.39	5.68
			22556	0.72	1.51	-0.74	-2.01	-3.90	-6.12	3.00
			14278	0.69	1.47	-1.00	-2.39	-4.02	-6.81	2.50
		5 year_AA50	7048	0.63	1.61	-1.22	-3.23	-3.50	-7.33	2.12
			1417	0.46	1.08	-1.40	-5.56	-5.54	-11.59	0.05
			1364	0.45	0.89	-1.40	-5.60	-6.28	-12.31	0.05
		5 year_AA60	15650	0.75	1.74	-0.95	-1.51	-2.98	-4.97	3.82
			12388	0.70	1.55	-1.05	-2.20	-3.74	-6.42	2.78
			20916	0.78	1.04	-0.79	-1.18	-5.69	-7.00	2.36

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Propyl Gallate	0 month RT	63935	0.92	2.52	0.57	0.74	0.05	1.23	8.29
			56337	0.88	2.48	0.33	0.23	-0.10	0.40	7.69
			45296	0.84	2.51	-0.02	-0.40	0.02	-0.38	7.13
		2 months RT	81548	0.92	2.50	1.12	0.74	-0.02	1.62	8.57
			80016	0.90	2.50	1.07	0.46	-0.01	1.31	8.35
			76463	0.85	2.54	0.96	-0.16	0.13	0.76	7.95
		6 months RT	99955	0.90	2.51	1.70	0.46	0.01	1.85	8.74
			90335	0.86	2.46	1.40	-0.13	-0.17	0.86	8.03
			79474	0.86	2.48	1.05	-0.13	-0.11	0.64	7.87
		8 months RT	101406	0.88	2.50	1.74	0.19	-0.04	1.58	8.54
			65055	0.91	2.53	0.60	0.58	0.07	1.12	8.21
			12139	0.90	2.48	-1.06	0.54	-0.09	-0.44	7.09
		10 months RT	65168	0.88	2.45	0.60	0.18	-0.21	0.48	7.75
			50408	0.85	2.47	0.14	-0.18	-0.16	-0.21	7.26
			42730	0.87	2.45	-0.10	0.01	-0.21	-0.26	7.22
		1 year_AA50	49029	0.89	2.28	0.10	0.36	-0.87	-0.37	7.14
			30653	0.87	2.23	-0.48	0.10	-1.07	-1.28	6.48
			42464	0.83	2.21	-0.11	-0.47	-1.17	-1.62	6.24
		1 year_AA60	100800	0.89	2.46	1.73	0.38	-0.17	1.62	8.58
			28967	0.81	2.20	-0.53	-0.78	-1.21	-2.30	5.75
			20928	0.81	2.10	-0.79	-0.75	-1.57	-2.81	5.38
		2.5 year_AA50	63318	0.91	2.18	0.55	0.63	-1.28	-0.12	7.32
			24155	0.88	1.87	-0.68	0.18	-2.47	-2.65	5.49
			31903	0.86	1.87	-0.44	-0.03	-2.46	-2.66	5.49
		5 year_AA50	24247	0.85	1.84	-0.68	-0.15	-2.58	-3.08	5.19
			3156	0.55	1.20	-1.35	-4.35	-5.10	-9.97	0.22
			3050	0.51	1.13	-1.35	-4.88	-5.37	-10.74	0.05
		5 year_AA60	66221	0.90	1.63	0.64	0.49	-3.41	-2.14	5.87
			26407	0.74	1.75	-0.61	-1.76	-2.94	-4.89	3.88
			7858	0.50	1.20	-1.20	-4.98	-5.09	-10.44	0.05

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Sodium Azide	0 month RT	73613	0.88	2.54	0.87	0.16	0.14	0.99	8.12
			68970	0.90	2.49	0.72	0.46	-0.05	0.99	8.12
			66433	0.88	2.51	0.64	0.17	0.02	0.72	7.92
		2 months RT	101061	0.89	2.52	1.73	0.31	0.06	1.78	8.69
			85983	0.91	2.54	1.26	0.58	0.12	1.71	8.64
			70072	0.88	2.51	0.76	0.26	0.03	0.91	8.06
		6 months RT	87366	0.89	2.53	1.30	0.40	0.09	1.53	8.51
			86530	0.89	2.52	1.28	0.30	0.05	1.38	8.40
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		8 months RT	92172	0.88	2.51	1.45	0.27	0.01	1.46	8.46
			70309	0.90	2.51	0.77	0.47	0.00	1.08	8.18
			64475	0.88	2.50	0.58	0.15	-0.01	0.61	7.85
		10 months RT	35895	0.89	2.45	-0.32	0.36	-0.21	-0.11	7.33
			31894	0.87	2.45	-0.44	0.03	-0.22	-0.53	7.02
			31906	0.87	2.40	-0.44	0.04	-0.43	-0.72	6.89
		1 year_AA50	64404	0.88	2.46	0.58	0.25	-0.17	0.57	7.81
			34109	0.88	2.36	-0.37	0.17	-0.57	-0.66	6.93
			30226	0.86	2.23	-0.49	-0.01	-1.09	-1.41	6.39
		1 year_AA60	105850	0.92	2.47	1.88	0.69	-0.16	2.07	8.90
			61106	0.84	2.48	0.48	-0.38	-0.10	-0.06	7.36
			45415	0.86	2.38	-0.02	-0.01	-0.48	-0.46	7.07
		2.5 year_AA50	41221	0.90	2.22	-0.15	0.49	-1.11	-0.67	6.92
			34453	0.91	2.21	-0.36	0.58	-1.15	-0.79	6.83
			31500	0.88	2.23	-0.45	0.27	-1.09	-1.11	6.60
		5 year_AA50	13584	0.87	2.20	-1.02	0.02	-1.18	-1.91	6.03
			9490	0.81	2.17	-1.15	-0.70	-1.32	-2.83	5.37
			9846	0.80	2.13	-1.13	-0.83	-1.45	-3.07	5.19
		5 year_AA60	45663	0.85	2.31	-0.01	-0.14	-0.78	-0.86	6.79
			38233	0.87	2.22	-0.24	0.05	-1.12	-1.18	6.56
			31006	0.87	2.22	-0.47	0.03	-1.12	-1.39	6.40
		10 year_AA60	16750	0.83	1.99	-0.92	-0.45	-1.99	-3.02	5.23
			26335	0.80	1.96	-0.62	-0.88	-2.12	-3.30	5.03

Fluid	Pres.	Time Point	*TPH*	*MLB*	SH	*tph*	*mlb*	*sh*	*pc*	*FI*
Vaginal Fluid	Zinc	0 month RT	61239	0.85	2.53	0.48	-0.15	0.08	0.33	7.64
			41718	0.87	2.57	-0.13	0.07	0.23	0.17	7.53
			27758	0.88	2.49	-0.57	0.21	-0.05	-0.32	7.17
		2 months RT	114906	0.89	2.52	2.17	0.37	0.06	2.20	8.99
			79588	0.91	2.52	1.06	0.59	0.05	1.48	8.47
			83406	0.86	2.56	1.18	-0.03	0.22	1.14	8.23
		6 months RT	94984	0.90	2.52	1.54	0.52	0.06	1.83	8.73
			74342	0.87	2.50	0.89	0.09	-0.01	0.81	7.99
			0	0.40	0.00	-1.44	-6.33	0.00	-7.28	0.05
		8 months RT	68716	0.89	2.50	0.72	0.38	-0.02	0.94	8.08
			71552	0.87	2.54	0.81	0.03	0.14	0.82	8.00
			27804	0.90	2.52	-0.57	0.52	0.05	0.07	7.46
		10 months RT	47991	0.88	2.50	0.06	0.26	-0.01	0.30	7.62
			44589	0.88	2.49	-0.04	0.16	-0.08	0.04	7.44
			27227	0.89	2.49	-0.59	0.38	-0.06	-0.17	7.28
		1 year_AA50	57891	0.88	2.44	0.38	0.27	-0.25	0.34	7.65
			32708	0.87	2.37	-0.42	0.05	-0.53	-0.79	6.84
			43267	0.85	2.32	-0.08	-0.14	-0.72	-0.87	6.78
		1 year_AA60	111544	0.89	2.49	2.06	0.34	-0.05	1.98	8.83
			87972	0.89	2.44	1.32	0.36	-0.26	1.20	8.27
			92399	0.87	2.46	1.46	0.05	-0.18	1.08	8.18
		2.5 year_AA50	62407	0.86	2.26	0.52	0.00	-0.94	-0.44	7.09
			54814	0.88	2.27	0.28	0.16	-0.91	-0.45	7.08
			38147	0.86	2.16	-0.24	-0.01	-1.35	-1.44	6.36
		5 year_AA50	17030	0.87	1.98	-0.91	0.11	-2.07	-2.54	5.57
			17465	0.84	2.07	-0.90	-0.32	-1.72	-2.62	5.51
			478	0.42	0.70	-1.43	-6.06	-7.04	-13.47	0.05
		5 year_AA60	46495	0.80	2.19	0.02	-0.85	-1.25	-1.95	6.00
			38374	0.85	2.07	-0.24	-0.22	-1.71	-1.98	5.98
			29599	0.82	2.09	-0.51	-0.56	-1.61	-2.44	5.65
		10 year_AA60	51167	0.88	2.09	0.16	0.21	-1.61	-1.14	6.58
			38505	0.84	2.01	-0.23	-0.29	-1.92	-2.23	5.79
			33000	0.84	2.03	-0.41	-0.39	-1.86	-2.42	5.66