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DNA integrity in forensic samples

Samantha L. Ramey
University of Montana, Missoula

DNA Integrity in Forensic Samples

By

Samantha Leigh Allison Ramey

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Approved By:

Scott Whittenburg, Dean of the Graduate School
Graduate School

Dr. Meradeth Snow, Chair
Anthropology

Dr. Kirsten Green Mink
Anthropology

Dr. Christopher Palmer
Chemistry

Joseph Pasternak
State of Montana Forensic Science Division

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DNA Integrity in Forensic Samples

Chairperson: Dr. Meradeth Snow

Abstract

When packaged genetic evidence samples are stored in close proximity, there is a higher chance for cross-contamination, which can lead to potential false results. The goal of this study was to test DNA storage methods and environments to determine the best way to avoid potential cross-contamination. Established protocols for storing different types of genetic evidence samples were evaluated: biological swabs and DNA cards. A known concentration of pig DNA was introduced to the evidence samples. Three different evidence drying times of the DNA-free swabs and cards were implemented before packaging: immediate packaging, an hour drying, and 24 hours drying. The samples were then placed in the evidence envelopes in one of two ways. The first was with a DNA carrying swab/card in an envelope next to a non-DNA carrying swab/card in a separate envelope. The second was with two swabs/cards in the same envelope, one carrying DNA and the other not. The three drying methods and two packaging methods were completed in triplicate. A control sample of a non-DNA carrying sample was also included for both packaging techniques. The samples were placed into room temperature storage and aligned next to each other for different intervals: 72 hours, two weeks, and two months. Once the sample exposed to DNA was removed from storage, DNA analysis was completed to determine if cross-contamination occurred on the blank sample at the same time.

DNA can be a vital piece of evidence in a court of law, therefore the integrity of the DNA is important. If cross-contamination occurs during storage, then the integrity of the evidence becomes jeopardized. Not only does cross-contamination render the genetic evidence problematic; but if left undetected, it has the potential to link an individual to a case they were not actually associated with, or render a genetic profile contaminated and unusable. Either scenario is not ideal and can be detrimental to individual's lives and the judicial system. If storage methods can cause evidence contamination, then new ways to preserve the integrity of evidence must be analyzed.

Cross-contamination is a rising problem throughout all aspects of a case. Prior studies have found cross-contamination occurring during collection and transportation due to materials or procedures (Fonneløp et al., 2016; Basset and Castella, 2018). Little prior research focused on contamination occurring during storage. This paper will impact the forensic science community by introducing the need for strict regulation and procedures for genetic evidence storage due to the potential of evidence cross-contamination.

Acknowledgments

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1.0 Introduction

DNA can be a vital piece of evidence in a court of law; therefore, the integrity of the DNA is essential. If cross-contamination occurs during storage, then the integrity of the evidence becomes jeopardized. Not only does cross-contamination render the genetic evidence problematic but if left undetected, cross-contamination has the potential to link an individual to a case they were not previously associated with. Either scenario is not ideal in a court of law.

Protocols are created to increase evidence integrity; however, they can vary and that can cause inconsistencies. For instance, the National Institute of Justice considers short-term storage to be anything less than 72 hours (Ballou et al., 2013), while the International Associate for Property & Evidence Inc. does not specify a time range for temporary storage (Latta et al., 2015). This small detail could potentially affect the DNA's quality because storage methods differ between short-term and long-term storage (Ballou et al., 2013). The storage time will influence the best method of storage based on the type of genetic evidence.

The goal of this project is to test DNA storage methods and environments to determine the best way to avoid potential cross-contamination. Known protocols for storing different types of genetic evidence samples will be evaluated. When packaged genetic evidence samples are stored in close proximity to another, there is a higher chance for cross-contamination. The hypothesis may be accepted if we can reject the null hypothesis that storage methods and environments do not affect genetic evidence cross-contamination.

1.1 Thesis outline

Chapter 2 discusses the hypothesis and expectations of this research. The chapter also goes into detail on the significance of the research to the field of forensics. Chapter 3 is a literature

review of contamination and protocols of genetic evidence in a forensic context. Chapter 4 discusses the storage processes of the samples and the methods used to analyze the results. Chapter 5 is on the results of the research. Chapter 6 goes into a discussion on the results from the different storage times. Chapter 7 is the final remarks on the research and what the results imply for the field in a forensic context and what other research would help to further research this area.

2.0 Hypothesis, Expectations, and Significance

The following research examines the potential contamination of genetic evidence during varying storage times and sample dry times. Based on prior research on contamination and observations, the study will test the three hypotheses listed below. The expected results are founded on these observations and prior research.

2.1 Hypotheses

1. If packaged genetic evidence samples are stored in close proximity to one another, then there is a higher chance for cross-contamination.
2. The longer the samples are in storage, the more contamination will occur.
3. The longer the samples dry before storage reduces the overall probability of cross-contamination occurring during storage.

2.2 Expectations

Studies show that molecules can travel significant distance over time, and even migrate through packaging. During this experiment, it is expected the 72 hour blank samples to have no cross-contamination, the 14 day blank samples to have some contamination, and the 45 day blank samples to have the most contamination. The amount of contamination will decrease with an increase in dry time. It is expected that more contamination will occur in the buccal swabs than the Whatman cards because the material of the cards will hold onto the DNA.

2.3 Significance

Cross-contamination is a big problem throughout most of the investigation process. If DNA can migrate through packaging, then the integrity of genetic evidence storage becomes jeopardized. The results from this research can provide further insight into how to improve the storage process of biological evidence in order to prevent cross-contamination.

3.0 Literature Review

3.1 Cross-contamination

The source of the primary contamination can occur prior, during, or after the investigation process. Prior contamination originates from the manufacturing of materials, such as buccal swabs; these contaminations can be reduced by using DNA-free products (Margiotta et al., 2015; Pickrahn et al., 2017). Contamination during investigation originates from mishandling of evidence, such as wearing the same gloves when handling different evidence samples, with the highest source being the crime scene (Pickrahn et al., 2017). Contamination after the investigation process originates during transport or in the laboratory (Pickrahn et al., 2017).

DNA transfers onto an object one of two ways: primary and secondary transfer. Primary transfer occurs from direct contact with an object. While secondary transfer is a result from indirect contact with an object (Cale et al., 2016; Pickrahn et al., 2017). The highest rate of contamination occurs during the initial crime scene investigation due to the high levels of activity, frequently caused by secondary DNA transfer. These secondary transfers are a different source of contamination than the primary contamination. Direct and indirect are a type of secondary transfer. A direct transfer occurs with indirect contact with an object but direct contact with the area, such as coughing; while indirect transfer occurs with intermediate contact between both the object and the area (Margiotta et al., 2015).

Prior contamination is a result of the materials used during the investigation. This can consist of investigation and laboratory equipment, such as a camera or scissors, and collection materials, such as gloves, swabs, or body bags. If equipment is improperly cleaned, then there remains a high risk of contamination known as impurities (Szkuta et al., 2015; Schwendener et al., 2016). Due to use, the equipment is a known contamination risk to the materials used to

collect the genetic evidence. Many studies have found unused gloves to have trace DNA caused by manufacturing (Margiotta et al., 2015; Szkuta et al., 2015; Basset and Castella, 2018). The use of DNA free materials helps reduce the risk of manufacture contamination, thus reducing prior contamination during an investigation.

Schwendener (2016) referred to contaminations during the investigation as "pollution." Contaminations during this process are often caused by careless handling of genetic evidence. Gloves need to be changed before handling evidence because studies show gloves transfer a significant amount of DNA between surfaces (Szkuta et al., 2015; Fonneløp et al., 2016; Basset and Castella, 2018). Any precautions preventing contamination during the investigation is vital because often the collection of trace evidence occurs after transport to the laboratory, increasing the chance of contamination (Schwendener et al., 2016).

Post-contamination occurs during the final stages of the investigation process, current research focuses predominantly on evidence during the collection process and less on evidence during transport or at the laboratory facilities. These two stages of the investigation process are just as crucial to the collection process. One study found that many jurisdictions collect trace evidence from a body in their facilities after being in a body bag (Schwendener et al., 2016). This leads to a further risk of contamination. The risk comes from the body bags. Schwendener (2016) found contamination within new body bags. During transportation, genetic evidence requires precautions to prevent contamination or DNA degradation (Clermont et al., 2014).

Laboratories routinely clean the facilities based on set protocols. Different areas within the laboratory receive different treatments based on sensitivity to DNA. For example, an area with high traffic will require cleaning more than an area with less traffic. One study tested to see the recovery rate of DNA in laboratories between areas of varying sensitivity and found less

contaminating DNA is recovered in sensitive areas (areas of evidence sampling or processing) in comparison to non-sensitive areas (evidence storage or hall; (Taylor et al., 2016). It is possible for the potential of contamination within the laboratories primarily through touch DNA (Szkuta et al., 2015). Moreover, it should be noted that the predominant DNA on an item is not necessarily from the last person who touched the item (Taylor et al., 2016). Therefore, the quality of the DNA does not necessarily indicate order of contact. Szkuta et al. (2015) study examines this risk of DNA transfer from laboratory equipment, such as gloves or scissors, and emphasizes the need for following cleaning protocols. The study cleaned their equipment with 1% hypochlorite followed by 70% ethanol. However, there does not appear to be a standard cleaning protocol for laborites to follow.

The prevalent issue of contamination in crime scene evidence needs to be addressed. The rates of contamination are increasing when they should be decreasing across all sources of contamination (Kloosterman et al., 2014; Fonneløp et al., 2016). Low-level crimes, such as burglaries, have one of the highest rates of contamination (Pickrahn et al., 2017). Based on the different studies, the high contamination rate of low-level crimes is due to careless handling of evidence. The lower the chance of finding the perpetrator the higher the chance of mishandling evidence during the initial investigation. Different studies found contaminations linking an investigator to an unassigned case (Fonneløp et al., 2016; Taylor et al., 2016). Many times the individual came into contact with the evidence but did not take custody (Taylor et al., 2016). One study showed six cases where a police officer's DNA was found, but they had not been involved in the case (Fonneløp et al., 2016). In a 2017 study research found 67.1% (n=233) of the contaminated samples originated from the crime scene investigators. This same study showed 45.8% (n=159) of the contaminated samples were the swabs (Pickrahn et al., 2017). A 2014

study found contamination at the Netherland Forensic Institute (NFI) went from 49 in 2008 to 135 in 2012; the source of contamination is both external and internal to NFI (Kloosterman et al., 2014). In a recent study in Switzerland, there were 709 contamination events between 2011 and 2015, with 78% of contamination originating from the police or in the laboratory (Basset and Castella, 2018). This increase in contamination began after the new next-generation multiplex (NGM) was implemented (Kloosterman et al., 2014; Fonneløp et al., 2016). It is more likely the new system NGM shed a more accurate light on the contamination rate (Kloosterman et al., 2014).

The rates of cross-contamination can easily be reduced. Training in handling genetic evidence should be required for anyone within the investigation process. Training should include handling genetic evidence, contamination factors, proper transportation techniques, laboratory protocols, storage methods, proper extraction methods, and cleaning techniques (Szkuta et al., 2015; Fonneløp et al., 2016; Kampmann et al., 2017; Pickrahn et al., 2017). This training applied during an investigation would reduce preventable contamination significantly. The integrity of genetic evidence is vital in a court of law. The prevention of cross-contamination is a critical factor in keeping genetic integrity. Once genetic evidence is contaminated, the results become biased if there is a known contamination source (Kloosterman et al., 2014; Basset and Castella, 2018). Without a known contamination source, distinguishing the contamination and original DNA is impossible. Measures must be taken to reduce contamination (Margiotta et al., 2015).

3.2 Protocols

Protocols provide evidence integrity. However, these protocols can vary between laboratories and across the globe. Laboratory protocols differ in packaging, storage, and

evidence retention (Ballou et al., 2013; Latta et al., 2015; Martin, 2016). Organizations such as the National Institute of Standards and Technology (NIST) and the International Associate for Property & Evidence Inc. (IAPE) provide differing protocols. Some laboratory facilities struggle with adequate storage caused by limited storage capacity, improper temporary storage, inadequate packaging materials, etc. Having a universal protocol could potentially fix these issues (Ballou et al., 2013).

For example, there is no protocol on how to dry evidence. However, most protocols suggest drying evidence before packaging (Cordray, 2010; Department of Public Safety - Texas, 2012; Ballou et al., 2013; Latta et al., 2015). This could impact the quality of the DNA. Improper drying could lead to bacterial and mold growth.

Some states have publicly accessible access to their protocol documents, such as Illinois, Ohio, and Texas. The Illinois State Police have a document dedicated to evidence packaging procedures for the forensic division, detailing different types of evidence. The document suggests for the different evidence types how to label, the desired sample size, preservation, wrapping and packing, and miscellaneous tips. For instance, for swabs of stains the department suggest there is no standard for the desired sample size and to allow the swabs to dry before packaging. The swabs should be packaged in an envelope or paper bag and to label the outside of the envelope with specimen type, date sealed, investigator's initials, case number, and sample location (Ballou et al., 2013; Latta et al., 2015). The Ohio Attorney General and Texas have a document on the Guidelines for Preservation and Retention of Biological Evidence that does not break down the types of evidence, but rather a general overall protocol for packaging biological evidence (Cordray, 2010; Department of Public Safety - Texas, 2012). The Ohio guidelines do suggest to dry evidence and use paper bags or envelopes for all biological evidence similar to

	frozen	refrigerated	temperature controlled	room temperature
	Lond-Term Storage			
liquid blood	never	best		
dry stained	best			
swabs			best (dried)	
DNA extract	best (liquid)	acceptable (liquid)	acceptable (dried)	
buccal			best	
	Short-Term Storage			
liquid blood	never	best	less than 24 hr	
dry stained			best	acceptable
swabs		best (wet)	best (dried)	
DNA extract	NONE			
buccal			best	less than 24 hr

Figure 3.2.1 The optimal storage temperature for long-term and short-term storage

Illinois procedures (Cordray, 2010; Police, 2012). IAPE has a short section in their professional standards for packaging; however, it only suggests the laboratory create a guideline (Latta et al., 2016).

Forensic biological evidence is stored prior to extraction, and the storage time depends on the capacity of a forensic laboratory or quantity of cases. There are two types of storage times for evidence samples: short-term or long-term. The length of storage affects how evidence is stored. Some methods of storage are better than others depending on the type of evidence (Figure 3.2.1; Ballou et al., 2013). NIST consider short-term storage as under 72 hours (Ballou et al., 2013) However, what is considered short-term for one laboratory may be different for another. The short-term evidence should be stored in a secure location to prevent tampering and possible contamination (Ballou et al., 2013; Latta et al., 2015).

Evidence retention protocol varies per state. Retention can vary in storage time and evidence type. With advancements in genetics, it is essential to retain genetic evidence in instances of appeals to retest the DNA samples. There is a set protocol on retesting evidence but there is no set protocol for evidence retention (Martin, 2016). Even in the states with protocols for evidence retention, there is no accountability on failure to follow protocol (Martin, 2016).

3.3 Sample Collection

The collection technique depends on the state of genetic evidence. If the genetic evidence is dry, then the sample needs to be rehydrated during collection. According to studies, water is the best rehydration method and should be done while collecting the sample (van Oorschot et al., 2003). This is done by using the double swab technique, a proven technique to recovery more DNA than one swab (van Oorschot et al., 2003; Pang and Cheung, 2007; Verdon et al., 2014). By using two swabs simultaneously, one swab rehydrates the genetic evidence while the dry swab reabsorbs any remaining moisture left behind (Pang and Cheung, 2007). During the extraction process of a swab sample, a significant amount of the DNA is not recovered, especially with cotton swabs where 20%-76% of the DNA is lost (van Oorschot et al., 2003). This significant loss of DNA during extraction is why the proper collection and packaging techniques are essential in order to ensure enough DNA is recovered for analysis.

After the genetic evidence is collected, the samples require drying time prior to packaging. The time required to dry varies on the collection method. For instance, Whatman Cards suggest no less than three hours of dry time for every 125 μl of the sample (from Whatman card instruction). If the genetic evidence is collected and packaged in the field, then this can cause potential problems during storage. Therefore, it is necessary to protect the samples

while drying and during storage. If improperly dried the probability of bacterial growth increases (Ballou et al., 2013).

The collection process of genetic evidence is important. Different factors during the collection process will affect the integrity of the DNA, and over time there is a statistically significant decline in DNA recovery (Raymond et al., 2009). The length of storage affects the appropriate protection methods for the sample (Ivanova and Kuzmina, 2013). The materials used to collect genetic evidence need to correspond to the evidence sample. Not all collection material is equal. For example, swabs have different efficiency when collecting blood versus saliva based on how the material holds and releases the biological material (Verdon et al., 2014). DNA degradation can also occur from exposure to air. When exposed to air moisture can reoccur, even if the sample was thoroughly dried (Colotte et al., 2011). Therefore, packaging should be breathable to prevent bacterial growth from contaminating the samples (Ballou et al., 2013). Therefore, plastic bags should only be used for short-term storage; however, some packaging materials for swabs are plastic tubing, which can foster bacterial growth.

3.4 Evidence Storage

Forensic evidence is stored throughout the investigation process when not in use. How the evidence is stored depends on the laboratory. Evidence can be stored in individualized lockers, but this tends to be reserved for short-term storage. Typically, storage consists of standard shelving (Ballou et al., 2013). Besides packaging, there is no real separation between cases. Some laboratories even store evidence from a single case together in a box or envelope (Cordray, 2010; Department of Public Safety - Texas, 2012). Through an interview Joseph Pasternak (State of Montana Forensic Science Division) provided insight into the handling of

evidence samples once delivered to the laboratory. Until extraction, evidence samples are stored as received. To prevent contamination of biological evidence, these samples are stored separately from control standards and reagents (February 22, 2018).

3.5 DNA Leaching

Can DNA move from its original origin? Different studies found the movement of DNA known as leaching (Haile et al., 2007; Andersen et al., 2012; Thomas et al., 2018). In these studies, the soil was undisturbed. Under proper conditions, DNA can move through the soil. The texture and structure of the soil influence DNA leaching and leaching has not been detected in frozen sediments (Hebsgaard et al., 2009; Arnold et al., 2011; Andersen et al., 2012). If DNA leaches in the soil, then it migrates radially from its source (Thomas et al., 2018). Anderson et a. (2012) found DNA leaching 10 cm below two sites; while, Thomas et al. (2018) detected viable DNA up to 16 cm away from the origin. It is clear that DNA can move through the soil, but the reasoning is still not fully understood. How DNA responds to leaching depends on the organism and the source (Haile et al., 2007).

If DNA can move from its original location, then can DNA move through a material? Fonnelop et al. (2016) tested a negative control within their study. The study tested if contamination could occur from handling evidence bags with bare hands. The results detected twelve of twenty fabric samples were contaminated. The explanation for the contamination unclear since the samples were handled next to evidence bags. However, it is possible the DNA from outside the evidence bags leached through the packaging.

How does this apply to a forensic context? This study will explore if DNA can leach through evidence packaging during storage. Based on the DNA leaching studies, it is more likely

for leaching during genetic evidence storage with wet or liquid samples. The packing material will also affect the rate of cross-contamination of genetic evidence. If DNA leaching is possible, then cross-contamination is imminent, and the protocols are insufficient.

4.0 Materials and Methods

4.1 Laboratory Prep

The Modern DNA Laboratory at the University of Montana was cleaned using a 50/50 solution of water and bleach to prevent contamination from previous research. All surfaces and instruments were wiped down and new plastic ware was used.

Extracted pig DNA labeled 116 Aa, 116 aa, 116 Bb, 116 bb, and 117 A from Emily Silverman's (2018) prior research was used to conduct this research (See Appendix). Each DNA sample was diluted with 1 mL of nuclease-free water. These new diluted samples were then used on a buccal swab or Whatman card, and it was recorded which diluted DNA sample was used for collection. The human buccal swabs were collected on the cheeks 30 seconds while rotating the swab.

4.2 Buccal Swabs

The buccal swabs samples were completed in triplicate along with a control for each drying and storage time. PurFlock Ultra DNA-Free Swabs were used to reduce potential pre-contamination. The buccal swab was labeled either A (DNA) or B (Blank), those labeled A also included the original DNA sample number. The A buccal swabs were introduced to DNA by dipping the buccal swab into the diluted concentration of pig DNA in a 1.5mL tube and left to dry for a designated time (none, one-hour, and 24 hours; Figure 4.2.1; See appendix); while the B buccal swabs remained unopen to prevent contamination before storage. Three different dry times were tested: none, one hour, and 24 hours. While drying the samples were placed in an area of the lab away from activity. After the allotted dry time the swabs were put into packaging consisting of an evidence envelope containing one A buccal swab and one B buccal swab, then sealed with ActiSeal Evidence-Pro tape (Figure 4.2.2). Each envelope was labeled 1,2,3, or 4

along with the date, dry time, and storage time. Envelopes 1-3 contained one swab with known DNA (n=18), while envelope 4 were the control envelopes (n=2 in each). The control envelopes consisted of one unopened buccal swab and one exposed to the air. The envelopes were stored in a plastic crate separated by storage time, making a total of 12 envelopes per crate.

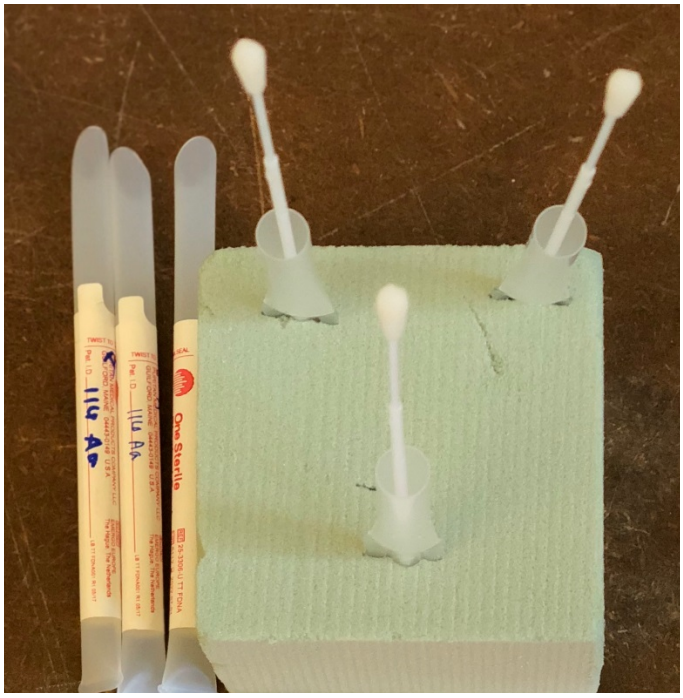

Figure 4.2.1 Buccal swabs introduced to DNA drying prior to storage.

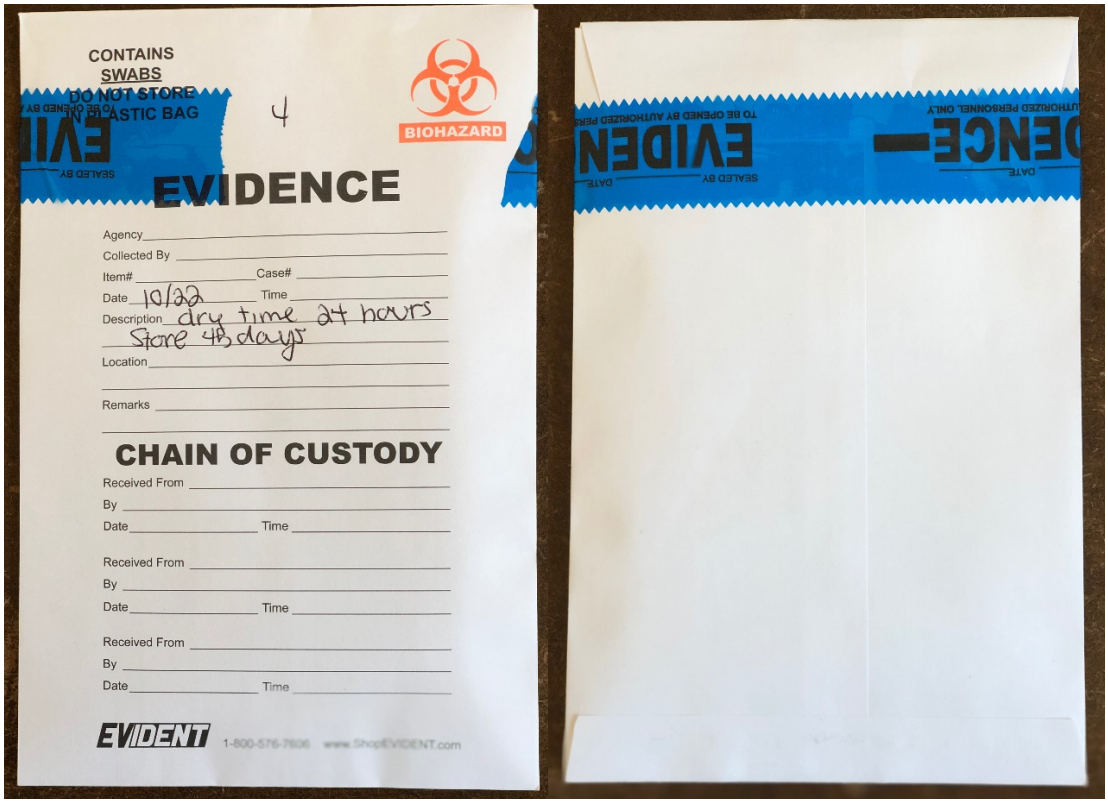

Figure 4.2.2 Buccal swabs sealed and ready for storage.

Figure 4.2.3 How the buccal swabs were stored. A container for each storage time.

4.3 Whatman Cards

The Whatman card samples were completed in triplicate along with a control for each drying and storage time. The Whatman card was labeled either A (DNA) or B (Blank), those labeled A also included the original DNA sample number. The center of the A Whatman card was introduced to 50 μl diluted pig DNA with a 10-100 μl pipette with a sterile filtered tip and then left to dry for a designated time (Figure 4.3.1), while the B Whatman cards remained unopen to prevent contamination before storage. Three different dry times were tested for the four cards: none, one hour, and 24 hours. After the allotted dry time the cards were put into packaging consisting of an evidence bag containing either an A Whatman card or a B Whatman card, then sealed with the bags seal and with ActiSeal Evidence-Pro tape (Figure 4.3.2). Unlike the buccal swabs, the Whatman cards were packaged one to an evidence bag due to the lack of individual envelopes allowing two cards to be stored together. Each evidence bag was labeled 1,2,3, or 4 along with the date, dry time, and storage time. Evidence bags 1-3 contained either one card with known DNA (A) or one blank card (B) (n=18), while bags 4 were the control envelopes (n=6). The bags were stored in a plastic crate separated by storage time, making a total of 24 evidence bags per crate.

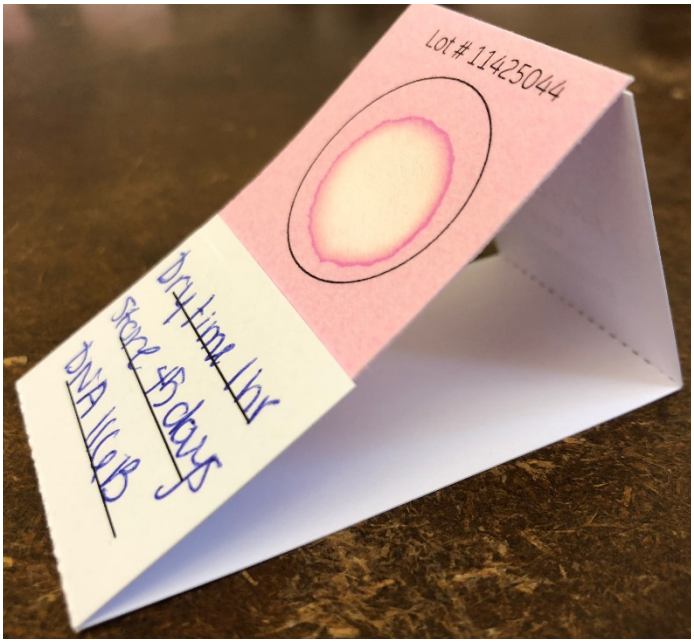

Figure 4.3.1 A Whatman card drying prior to storage.

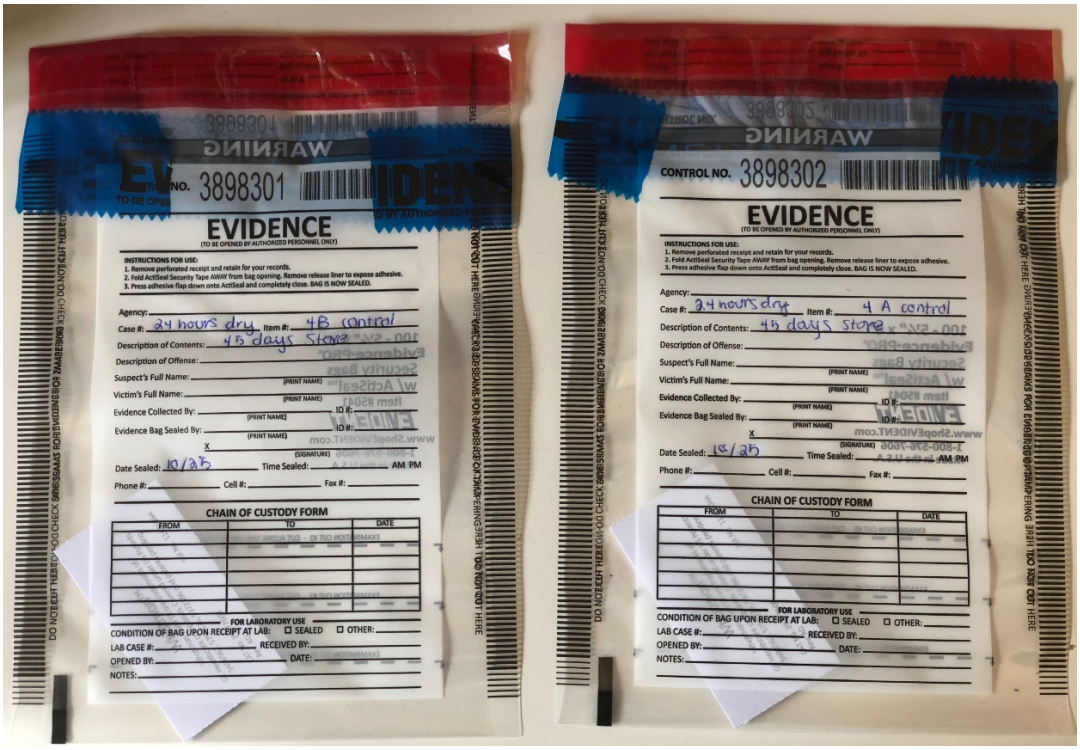

Figure 4.3.2 Whatman card sealed and ready for storage.

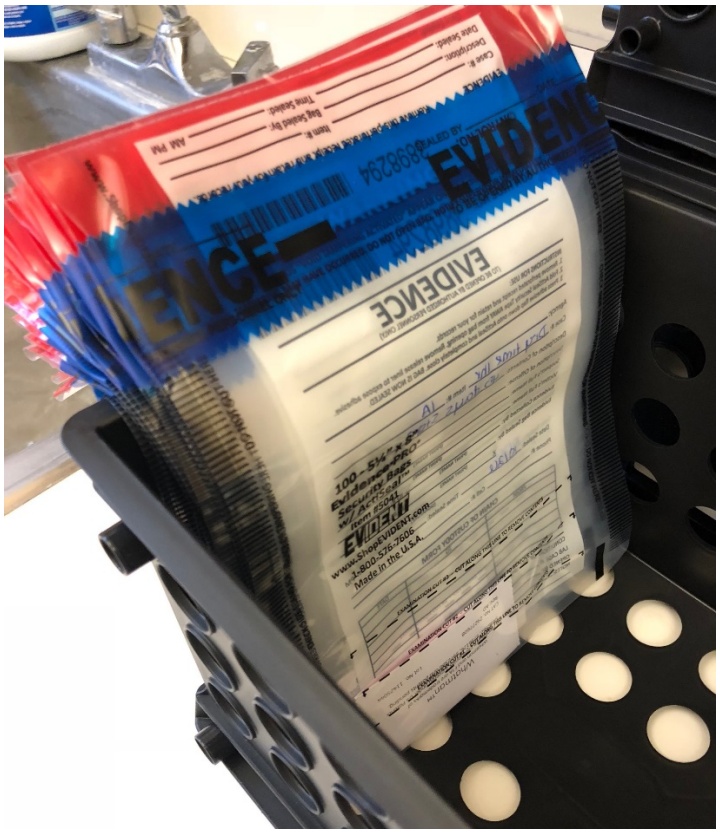

Figure 4.3.3 How the Whatman cards were stored. A container for each storage time.

4.4 Buccal Swab Extraction

DNA extraction was done following an XIT Genomic DNA from Buccal Cells protocol. The extractions were done based on storage time, resulting in three separate sets. Each extraction consisted of 24 samples.

The evidence envelopes were removed from storage after the allotted time and unsealed using scissors. Each sample was designated a number 1-24, this corresponded to a new 1.5 ml tube, which was labeled with a number 1-24. New DNA free gloves were used between handling each tube during this stage. Using a pipette, 400 μl XIT™ Lysis Buffer was added to each tube, then the corresponding buccal swab was placed in the solution for 10 minutes. To ensure the collected DNA from the sample remained in the tube. The buccal swab was tapped on the inside

of the tube until there were no liquid droplets and all the liquid was off the swab. The buccal swab was then placed back into its designated envelope. Then 10 μl LongLife™ Proteinase K was added to each tube and the tube mixed by inverting 25 times. The tubes were incubated at 37°C for an hour and periodically inverted. After incubation, 90 μl XIT™ Protein Precipitation Buffer was added to each sample followed by inverting the tube 10 times, then followed by a 5-minute centrifuge at 13,000 rpms, the tubes were centrifuge until the supernatant was clear. The supernatant was removed from the original tube and into a new corresponding 1.5ml tube labeled 1-24ℒ. Next, 400 μl isopropanol was added to the supernatant and inverted 20 times followed by a 10 minute centrifuge at 13,000rpm. Now the supernatant was discarded using a pipette and 200 μl 70% ethanol to each tube followed by inverting the tube twice and a final 11-minute centrifuge at 13,000rpm. After the final centrifuge, the supernatant was discarded and the tube dried while open and on its side on a paper towel. Then 50 μl TE buffer was added to the dried tube followed by an hour incubation at 55°C. The last step required an overnight incubation at room temperature followed by storing the samples in the laboratory refrigerator.

During the extraction of the 72-hour buccal swabs samples 1-12 supernatant was mistakenly discarded after the first centrifuge rather than transferred into a new tube. These samples were then centrifuged again this time transferring the supernatant to a new tube. The supernatant of samples 13-24 was transferred into a new tube after the first centrifuge.

4.5 Whatman Card Extraction

DNA extraction was done following an Illustra tissue and cells genomicPrep Mini Spin Kit protocol. The extractions were done based on storage time, resulting in three separate sets. Each extraction consisted of 24 samples to ensure the procedures were correctly performed.

The evidence bags were removed from storage after the allotted time and unsealed using scissors. Each sample was designated a number 1-24, this corresponded to a new 1.5 ml tube, which was labeled with a number 1-24. Using a sterile single hole punch a 6 mm disc was removed from the Whatman card and placed into a corresponding 1.5 ml tube. Phosphate-buffered saline (PBS) was diluted with nine parts water and one-part PBS. Then 1 ml of PBS was added to each 1.5 ml tube followed by one-minute centrifuge at 13,000 x g. Next, the disc was macerated using a sterile pipet tip followed by a ten second spin at 2,000 x g. Then 50 μL of buffer 1 was added to each sample along with 10 μL of proteinase K and then vortexed for fifteen seconds. The proteinase K first had to be rehydrated with 1.5 ml of nuclease-free water. Then the samples incubated for one hour at 56°C. After incubating, the samples were centrifuged for ten seconds at 2,000 x g to pull the disc material to the bottom. Then 5 μL of RNase A (buffer 4) was added, followed by a fifteen-minute room temperature incubation. Next, 500 μL of buffer 4 was added and each sample vortexed for fifteen-seconds and a ten-minute room temperature incubation. Then the samples were pipetted into a mini-column that was placed in a collection tube followed by a one-minute centrifuge at 11,000 x g. Next, the flowthrough was discarded from the collection tube. Then another 500 μL of buffer 4 was added to the column followed by another one-minute centrifuge at 11,000 g and the flowthrough discarded. Next, 500 μL of buffer 6 was added to the column followed by a three-minute centrifuge at 11,000 x g. Then the column was transferred to a 1.5 ml tube and the collection tube discarded. Next, 50 μL buffer 5 was added to the column followed by a one-minute incubation at room temperature. Finally, the samples were centrifuged for one-minute at 11,000 x g and the column discarded.

4.6 Qubit

The Qubit® dsDNA HS Assay was used to read the concentration of DNA in the samples. New 0.5mL tubes were labeled on the lid 1-26, 1-24 for the samples 25-26 for the standard. Next, 190 μL of Qubit® was added to tubes 25-26, and 195μL of Qubit® added to tubes 1-24. Then 5 μL of the corresponding sample was added to tubes 1-24. Then 10 μL of Qubit® dsDNA HS Standard #1 was added to tube 25, and 10 μL of Qubit® dsDNA HS Standard #2 was added to tube 26. Next, each tube was vortexed for 3 seconds before incubating for 2 minutes at room temperature. Following this, a Qubit4 was used to measure the concentration of DNA in each sample.

The first Qubit test using the dsDNA Assay for these samples was unable to detect DNA in any of the samples. The original sample was directly tested to determine if there was quantifiable DNA, based on the Qubit there was quantifiable DNA. Therefore, quantifiable DNA should have been detected on the samples with known DNA. With this, the samples were tested using a Qubit test with high sensitivity. With this test the Qubit was able to detect two samples with DNA, these samples had known DNA. Since DNA was undetected with the first Qubit test but detected with the high sensitivity test, all other storage samples were tested using the high sensitivity Qubit.

4.7 PCR

Polymerase chain reaction (PCR) was used to amplify species-specific mitochondrial DNA. Species-specific primers were used for this study. The pig samples used primes CO2susF2 (5′GCCTAAATCTCCCCTCAATGGTA -3′) and CO2susR2 (5′AGAAAGAGGCAAATAGAT TTTCG -3′; Silverman, 2018) and the human samples used primers 15986F (Coordinates

according to the Cambridge Reference Sequence: 15986-16010) and 16404R (Coordinates according to the Cambridge Reference Sequence: 16383-16404; Kemp et al., 2006). During the PCR process, the samples were separated into groups to prevent contamination: samples unexposed to pig DNA, positive pig DNA, blank human DNA, and positive human DNA. Between samples groups, the area and pipettes was bleached down. Two master mixes were created one for the pig samples and another for the human samples. The master mix was created based on the initial n=1 (Table 1) and then adjusted to the number of samples needed. Next, the PCR samples were prepared with each sample consisting of 13.37 μL master mix and 3 μL DNA. These samples were then placed in the thermocycler at 52-60 °C. Then the samples were visualized using gel electrophoresis.

If a blank sample had amplified DNA, then the samples were retested. During the second PCR, none of them amplified DNA.

Table 1 Master mix

H_2O	dNTPs	Buffer	$MgCl_2$	Primer Forward	Primer Reverse	Taq
8.76 μL	2.4 μL	1.5 μL	0.45 μL	0.18 μL	0.18 μL	0.08 μL

4.8 Fisher exact test

Statistical Package for the Social Science (SPSS) software was used to run descriptive statistical analysis on the data (IBM Corp. 2013). Using SPSS, the comparison of two variables were collected for two different tabulations contamination with storage time and then with sample dry time.

5.0 Results

5.1 Buccal Swabs: 72 Hours Storage

The Qubit was unable to detect trace DNA in any of the samples using the non-high sensitivity kit. Nine samples had a known concentration of DNA introduced to the buccal swab before storage. Samples 1, 3, 5, 9, 11, 13, 17, 19, and 21 were introduced to DNA before storage. The original DNA sample 116Aa had 0.05ng/ml DNA and this is the concentration of DNA the samples were introduced to.

The high sensitivity Qubit test was able to detect trace DNA in two of the twenty-four samples (Table 2-4). The Qubit was unable to detect DNA in table 2, when samples 1,3, and 5 were introduced to DNA before storage. Table 3 shows sample 11 and 13 with quantifiable DNA; these samples were introduced to DNA before storage. The Qubit was unable to detect DNA in the other samples from table 4, consisting of known blank buccal swabs, the controls, and sample 9 with known DNA. The Qubit was unable to detect DNA in table 4 when samples 17, 19, and 21 were introduced to DNA before storage.

Table 2 Buccal Swabs High Sensitivity Qubit: Dry time none, Storage time 72 hours

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 3 Buccal Swabs High Sensitivity Qubit: Dry time 24 hours, Storage time 72 hours

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
Out of range	Out of range	0.038 ng/µl	Out of range	0.0372 ng/µl	Out of range	Out of range	Out of range

Table 4 Buccal Swabs High Sensitivity Qubit: Dry time one hour, Storage time 72 hours

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

5.2 Buccal Swabs: 14 Days Storage

The A buccal swabs showed varying degrees of condensation upon removal from storage. Condensation was visible inside the transport tube of the non dry time A swabs 1-3 (Figure 5.2.1). Condensation was slightly visible inside the transport tube of the one-hour dry time A swabs 1-3. No condensation was visible inside the transport tube of the 24-hour dry time A swabs 1-3.

The high sensitivity test using the Qubit was able to detect trace DNA in six of the twenty-four samples (Table 5-7). Table 5 shows samples 1,3, and 5 with quantified DNA present in the samples, these were the sample introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 5, consisting of the known blank buccal swabs and the controls. Table 6 shows samples 9 and 11 with quantified DNA present in the samples, and these were the samples introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 6, consisting of the known blank buccal swabs, the controls, and sample 13 with known DNA. Table 7 shows sample 21 with quantified DNA present in the samples, and this was a sample introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 7, consisting of known blank buccal swabs, the controls, and samples 17 and 19 with known DNA.

Table 5 Buccal Swabs: Dry time None, Storage time 14 Days

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
0.0508 ng/µl	Out of range	0.108 ng/µl	Out of range	0.0344 ng/µl	Out of range	Out of range	Out of range

Table 6 Buccal Swabs: Dry time one hour, Storage time 14 Days

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
0.0272 ng/µl	Out of range	0.0260 ng/µl	Out of range	Out of range	Out of range	Out of range	Out of range

Table 7 Buccal Swabs: Dry time 24 hours. Storage time 14 Days

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
Out of range	Out of range	Out of range	Out of range	0.0304 ng/µl	Out of range	Out of range	Out of range

Table 8 Buccal Swabs: Dry time none, Storage time 45 days

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
0.0740 ng/µl	Out of range	0.0704 ng/µl	Out of range	0.0344 ng/µl	Out of range	Out of range	Out of range

Table 9 Buccal Swabs: Dry time one hour, Storage time 45 days

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
0.131 ng/µl	Out of range	0.0736 ng/µl	Out of range	0.0788 ng/µl	Out of range	Out of range	Out of range

Table 10 Buccal Swabs: Dry time 24 hours, Storage time 45 days

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
0.0608 ng/µl	Out of range	0.0368 ng/µl	Out of range	0.0780 ng/µl	Out of range	Out of range	Out of range

5.3 Buccal Swabs: 45 Days Storage

The A buccal swabs showed varying degrees of condensation upon removal from storage. Condensation was visible inside the transport tube of the none dry time and one-hour dry time A swabs 1-3 (Figure 5.3.1-5.3.2). No condensation was visible inside the transport tube of the 24-hour dry time A swabs 1-3. The condensation was slightly more for the none dry time samples.

The high sensitivity test using the Qubit was able to detect trace DNA in nine of the twenty-four samples (Table 8-10). Table 8 shows samples 1,3, and 5 with quantified DNA present in the samples, these were the sample introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 8, consisting of the known blank buccal swabs and the controls. Table 9 shows samples 9, 11, and 13 with quantified DNA present in the samples, and these were the samples introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 9, consisting of the known blank buccal swabs and

the controls. Table 10 shows sample 17, 19, and 21 with quantified DNA present in the samples, and this was a sample introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 10, consisting of known blank buccal swabs and the controls.

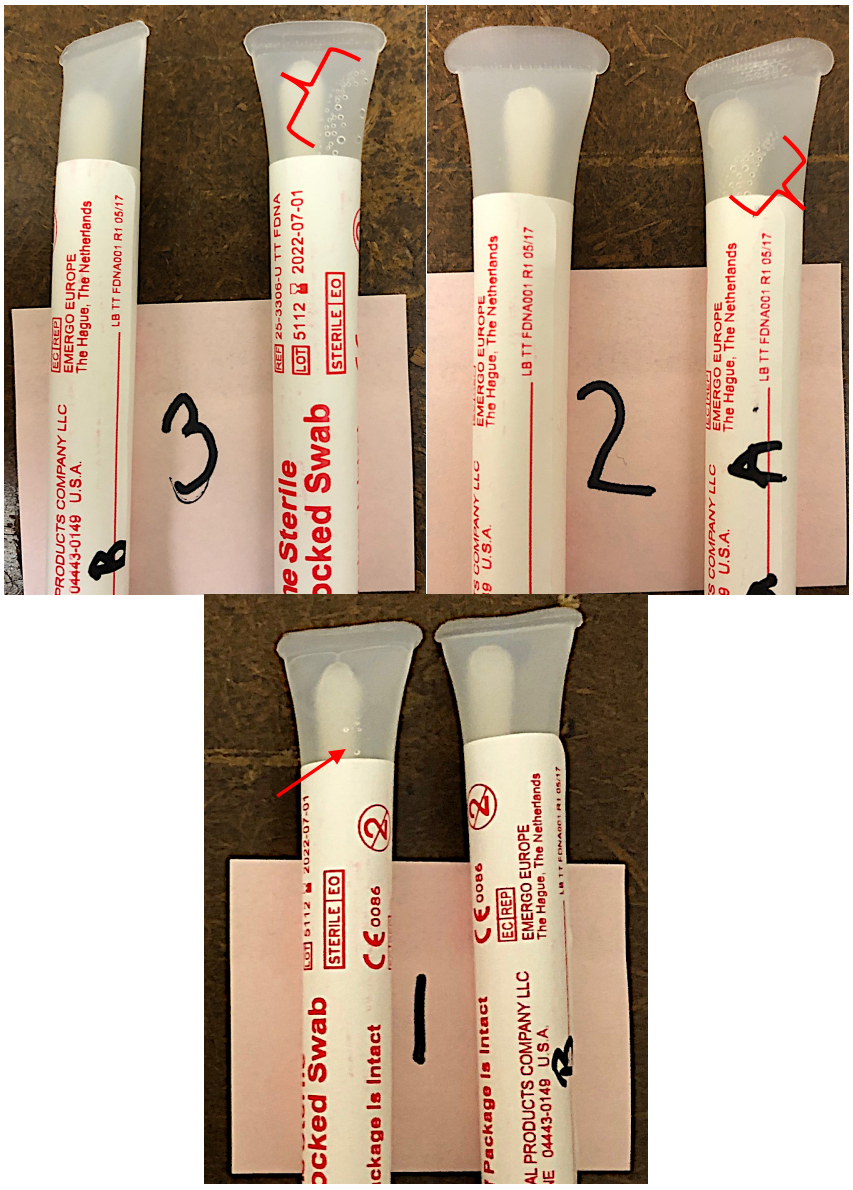

Figure 5.2.1 After 14 days of storage and no dry time A samples show condensation

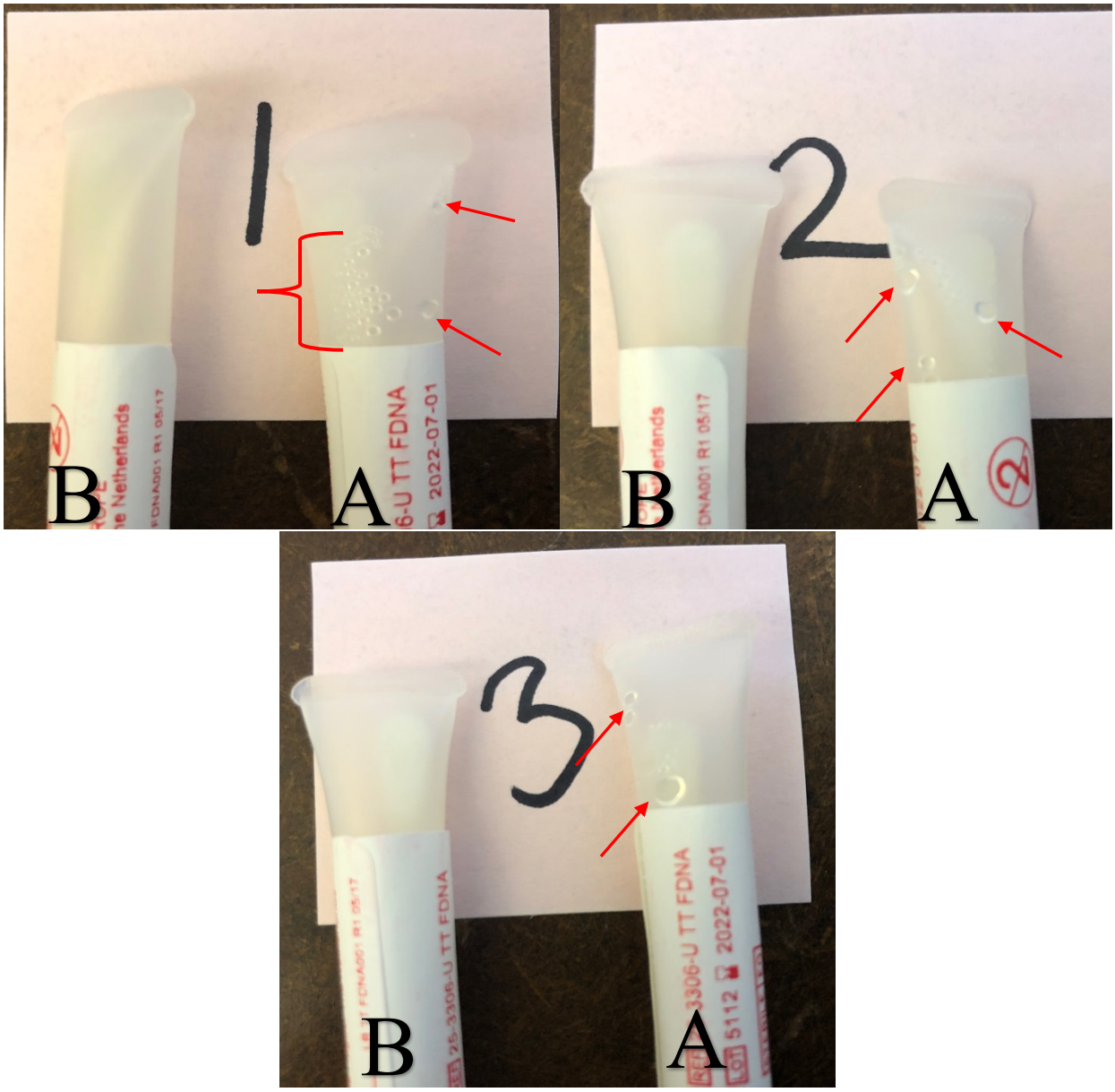

Figure 5.3.1 After 45 days of storage and no dry time A samples show condensation (samples on the right)

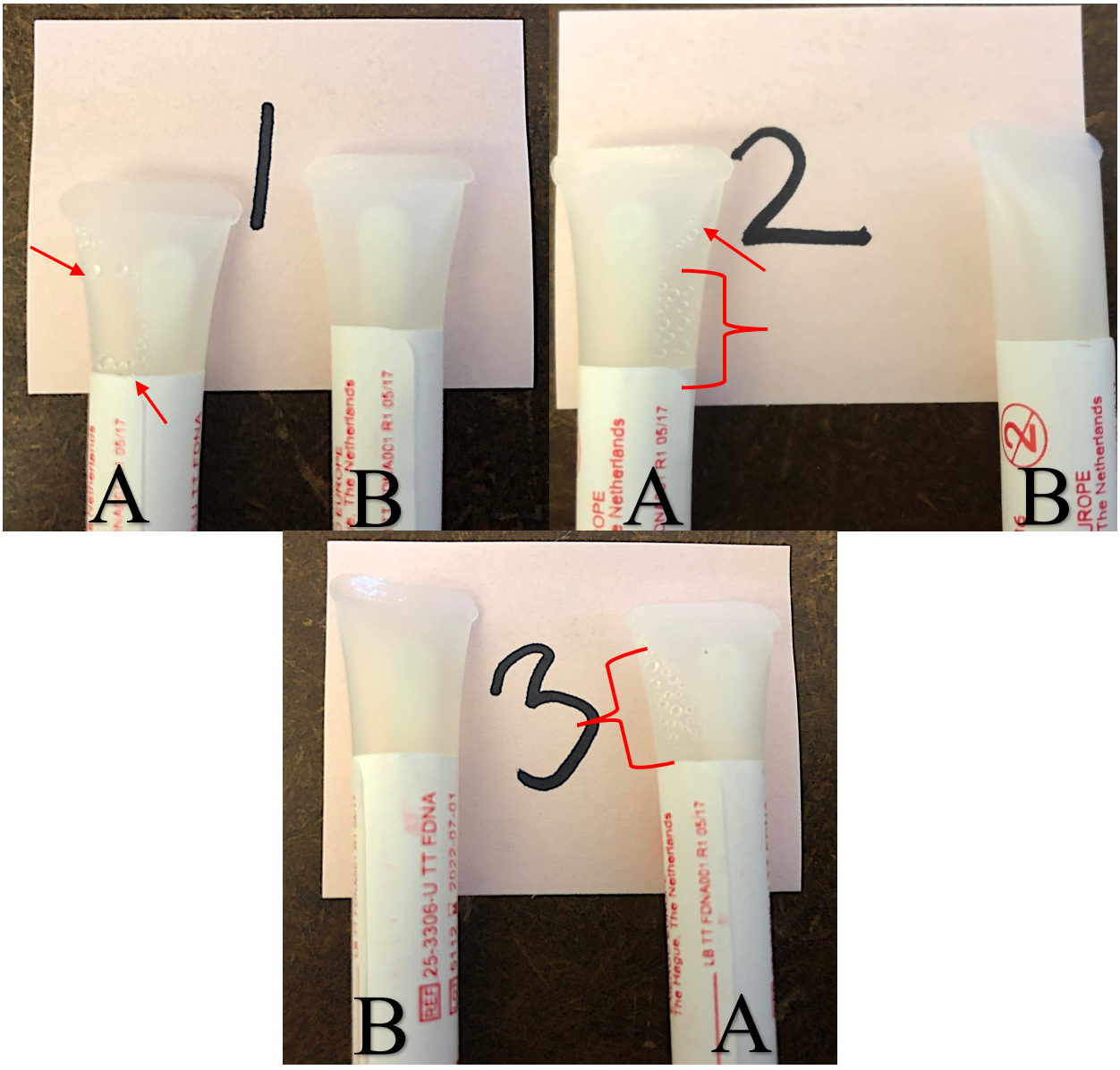

Figure 5.3.2 After 45 days of storage and one hour dry time A samples show condensation.

Table 11 Whatman Cards: Dry time none, Storage time 72 hours

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 12 Whatman Cards: Dry time 1 hour, Storage time 72 hours

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 13 Whatman Cards: Dry time 24 hours, Storage 72 hours

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 14 Whatman Cards: Dry time none, Storage time 14 Days

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 15 Whatman Cards: Dry time 1 hour, Storage time 14 Days

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

5.4 Whatman Cards: 72 Hours Storage

The high sensitivity Qubit was unable to detect trace DNA in any of the samples (Table 11-13). Nine samples had a known concentration of DNA introduced to the Whatman cards before storage. Samples 1, 3, 5, 9, 11, 13, 17, 19, and 21 were introduced to DNA before storage.

5.5 Whatman Cards: 14 Days Storage

The high sensitivity Qubit was unable to detect trace DNA in any of the samples (Table 14-16). Nine samples had a known concentration of DNA introduced to the Whatman cards before storage. Samples 1, 3, 5, 9, 11, 13, 17, 19, and 21 were introduced to DNA before storage.

Table 16 Whatman Cards: Dry time 24 hours, Storage time 14 Days

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 17 Whatman Cards: Dry time none, Storage time 45 days

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

5.6 Whatman Cards: 45 Days Storage

The high sensitivity Qubit was unable to detect trace DNA in any of the samples (Table 17-20). Nine samples had a known concentration of DNA introduced to the Whatman cards before storage. Samples 1, 3, 5, 9, 11, 13, 17, 19, and 21 were introduced to DNA before storage.

5.7 Cheek Buccal Swabs: 49 Days Storage

The high sensitivity test using the Qubit was able to detect trace DNA in nine of the twenty-four samples (Table 20-22). Table 20 shows samples 1,3, and 5 with quantified DNA present in the samples, these were the sample introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 20, consisting of the known blank buccal swabs and the controls. Table 21 shows samples 9, 11, and 13 with quantified DNA present in the samples, and these were the samples introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 21 consisting of the known blank buccal swabs and the controls. Table 22 shows sample 17, 19, and 21 with quantified DNA present in the samples, and this was a sample introduced to DNA before storage. The Qubit was unable to detect DNA for the other samples from table 22, consisting of known blank buccal swabs and the controls.

Table 18 Whatman Cards: Dry time 1 hour, Storage time 45 days

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 19 Whatman Cards: Dry time 24 hours, Storage time 45 days

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range	Out of range

Table 20 Buccal Swabs: Dry time none, Storage time 49 days

1: 1A	2: 1B	3: 2A	4: 2B	5: 3A	6: 3B	7: 4A	8: 4B
12.0 ng/µl	Out of range	9.08 ng/µl	Out of range	3.46 ng/µl	Out of range	Out of range	Out of range

Table 21 Buccal Swabs: Dry time one-hour, Storage time 49 days

9: 1A	10: 1B	11: 2A	12: 2B	13: 3A	14: 3B	15: 4A	16: 4B
3.64 ng/µl	Out of range	8.00 ng/µl	Out of range	14.3 ng/µl	Out of range	Out of range	Out of range

Table 22 Buccal Swabs: Dry time 24 hours, Storage time 49 days

17: 1A	18: 1B	19: 2A	20: 2B	21: 3A	22: 3B	23: 4A	24: 4B
4.48 ng/µl	Out of range	12.7 ng/µl	Out of range	5.64 ng/µl	Out of range	Out of range	0.113 ng/µl

5.8 PCR

PCR Run: Pig DNA

The first run of the PCR for blank pig DNA samples was able to detect DNA in twenty-one of the ninety samples. The detected DNA comprised of nine buccal swabs and twelve Whatman cards (Table 23). None of the blank samples stored for 14 days detected DNA. DNA was detected from all three dry times. The DNA detected from the different dry times were roughly equivalent. The second run of the PCR for the blank pig DNA and positive pig DNA did not work for an undetermined reason. The third PCR run detected DNA in the pig DNA samples

(Figure 5.8.1). The final PCR confirmed eighteen blank pig DNA samples were contaminated, comprised of eight buccal swabs and ten Whatman cards, with a total of six samples being controls (Table 24). The contaminated controls consisted of two 72 hours and four 45 days storage samples along with two non dry time, three one-hour dry times, and one 24 hours dry time.

PCR Rung: Human DNA

The PCR for the blank human DNA and positive human DNA did not work for an undetermined reason. The human DNA PCR samples were tested four times, with each test resulting in indeterminate results. A known human sample was also tested to ensure there were problems with the PCR and human DNA was able to be detected.

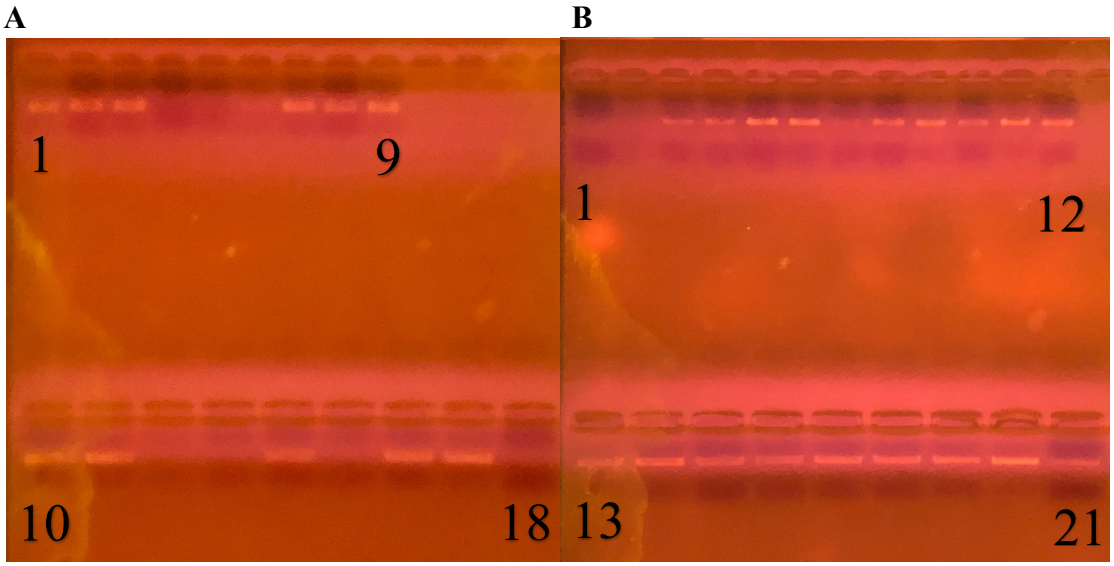

Figure 5.8.1 Gel Electrophoresis 3rd results of pig DNA A) Known pig DNA samples 1,11, and 21 from each storage time: (1-3) 14 days buccal swabs; (4-6) 72 hours buccal swabs; (7-9) 45 days buccal swabs; (10-12) 45 days Whatman cards; (13-15) 72 hours Whatman cards; (16-18) 14 days Whatman cards. B) Known pig DNA blank samples: (1-3) 72 hours Whatman cards; (4-7) 72 hours buccal swabs; (8-12) 45 days buccal swabs; (13-21) 45 days Whatman cards

Table 23 The results of the known blank buccal swabs and Whatman cards after the first PCR for the pig DNA. The left number indicates the PCR sample number and the right number indicates the storage sample number. See Appendix for Gel electrophoresis

#*	(#)	#	#s	#𝒹	# 𝒹•
Whatman Cards 72 hour Samples	Whatman Cards 14 Day Samples	Whatman Cards 45 Day Samples	Buccal Swabs 72 hour Samples	Buccal Swabs 14 Day Samples	Buccal Swabs 45 Day Samples

1:2*	**2:4***	**3:6***	**4:7***	**5:8***	**6:10***	**7:12***	**8:14***	**9:15***	**10:16***
Blank	Blank	Band	Blank	Blank	Blank	Blank	Blank	Blank	Blank
11:18*	**12:20***	**13:22***	**14:23***	**15:24***	**16:(2)**	**17:(4)**	**18:(6)**	**19:(7)**	**20:(8)**
Blank	Band	Blank	Blank	Band	Blank	Blank	Blank	Blank	Blank
21:(10)	**22:(12)**	**23:(14)**	**24:(15)**	**25:(16)**	**26:(18)**	**27:(20)**	**28:(22)**	**29:(23)**	**30:(24)**
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
31:2𝒹	**32:4𝒹**	**33:6𝒹**	**34:7𝒹**	**35:8𝒹**	**36:10𝒹**	**37:12𝒹**	**38:14𝒹**	**39:15𝒹**	**40:16𝒹**
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
41:18𝒹	**42:20𝒹**	**43:22𝒹**	**44:23𝒹**	**45:24𝒹**	**46: 2s**	**47:4s**	**48:6s**	**49:7s**	**50:8s**
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Band	Blank	Blank
51:10s	**52:12s**	**53:14s**	**54:15s**	**55:16s**	**56:18s**	**57:20s**	**58:22s**	**59:23s**	**60:24s**
Blank	Blank	Band	Band	Blank	Blank	Band	Blank	Blank	Blank
61: 2𝒹•	**62: 4𝒹•**	**63: 6𝒹•**	**64: 7𝒹•**	**65: 8𝒹•**	**66: 10𝒹•**	**67: 12 𝒹•**	**68: 14 𝒹•**	**69: 15 𝒹•**	**70: 16 𝒹•**
Band	Blank	Blank	Blank	Band	Blank	Band	Blank	Blank	Blank
71: 18𝒹•	**72: 20𝒹•**	**73: 22𝒹•**	**74: 23𝒹•**	**75: 24𝒹•**	**76: 2**	**77: 4**	**78: 6**	**79: 7**	**80: 8**
Blank	Band	Band	Blank	Blank	Band	Band	Blank	Blank	Band
81:10	**82:12**	**83:14**	**84:15**	**85:16**	**86:18**	**87:20**	**88:22**	**89:23**	**90:24**
Band	Blank	Band	Band	Band	Band	Band	Blank	Blank	Blank

Table 24 The breakdown of the contaminated blank pig DNA. A) storage time B) dry time C) control

A

STORAGE TIME:	72 HOURS	14 DAYS	45 DAYS
BUCCAL SWAB	3		5
WHATMAN CARD	1		9

B

DRY TIME:	NONE	ONE HOUR	24 HOURS
BUCCAL SWAB	3	3	2
WHATMAN CARD	3	4	3

C

CONTROL:	A: OPENED	B: UNOPENED
BUCCAL SWAB	1	1
WHATMAN CARD	1	3

5.9 Statistics

Fisher exact test: Storage time

Storage time and DNA contamination were compared for both the buccal swabs and the Whatman cards (Figure 5.9.1). The test determined the association between storage time and DNA contamination. The p-value for the buccal swabs was 0.054 which is slightly greater than significance level ($\alpha = 0.05$). While the p-value for buccal swabs the Whatman cards was 0.000 which is less than significance level ($\alpha = 0.05$).

Fisher exact test: Dry time

Dry time and DNA contamination were compared for both the buccal swabs and the Whatman cards (Figure 5.9.2). The test determines the association between sample dry time and DNA contamination. The p-value for both the buccal swabs and the Whatman cards were greater than significance level ($\alpha = 0.05$).

A

Chi-Square Tests: Buccal Swabs

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5.833[a]	2	.054
Likelihood Ratio	8.543	2	.014
Linear-by-Linear Association	.204	1	.652
N of Valid Cases	45		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 3.00.

B

Chi-Square Tests: Whatman Cards

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	18.771[a]	2	.000
Likelihood Ratio	20.135	2	.000
Linear-by-Linear Association	12.069	1	.001
N of Valid Cases	45		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 3.33.

Figure 5.9.1 Chi-square test using the fisher exact test indicating the association between two variables significance level α = 0.05 A) buccal swabs and storage time B) Whatman cards and storage time

Chi-Square Tests: Buccal Swabs

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.944[a]	3	.584
Likelihood Ratio	1.952	3	.583
Linear-by-Linear Association	1.761	1	.184
N of Valid Cases	45		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 1.80.

Chi-Square Tests: Whatman Card

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.000[a]	3	1.000
Likelihood Ratio	.000	3	1.000
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	45		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 2.00.

Figure 5.9.2 Chi-square test using the fisher exact test indicating the association between two variables significance level α = 0.05 A) buccal swabs and dry time B) Whatman cards and dry time

6.0 Discussion

6.1 Condensation

Storage conditions can potentially cause damaging effects of genetic evidence. Bacteria and mold can grow if evidence is stored improperly. This growth would obstruct any DNA results and render the evidence useless. Depending on how the genetic evidence is packaged, the bacterial growth could spread throughout storage.

Condensation was found within some of the buccal swab transparent tubes when removed from storage. There were varying degrees of condensation within the tubes depending on the length of storage. For just 72 hours of storage, there was no visible condensation in any of the buccal swab transparent tubes. Condensation was visible after fourteen days of storage and increased concentration over time. The sample drying time affected the condensation within the packaging. Samples that dried for 24 hours showed no signs of condensation, and it only occurred with one-hour and no dry times. In the transparent tubes with no dry time, the condensation was visible, having the most liquid from the sample trapped within the transparent tube. The condensation could potentially lead to a higher chance of cross-contamination or bacterial growth. In the transparent tubes with an hour dry time, the condensation was slightly visible having some moisture from the sample trapped within the transparent tube. After 45 days there was significant condensation visible in both dry times of none and one-hour. Similar to the 14 days samples, there was no visible condensation for the samples that dried for 24 hours.

Whatman cards showed no signs of condensation. Unlike the buccal swabs, any condensation would be reabsorbed by the Whatman cards. Bacteria and mold growth are still possible.

6.2 Dry time

The quantifiable DNA from the samples may give further insight into how to dry samples before storage. The longer the samples dry before storage, the higher the probability of DNA degradation. Consistently throughout the storage times, known DNA was undetected from the 24 hours dry time buccal swabs. DNA was detected from one-hour dry time swabs; however, slight condensation was still present. Therefore, one hour is not enough time for a sample to dry. Three to four hours of dry time should be sufficient to prevent condensation for both buccal swabs and Whatman cards.

How does dry time affect contamination during storage? Based on the PCR contamination occurred during all three dry times. The dry times shared the same number of cross-contaminations. Therefore, there is no significance in dry time regarding cross-contamination during storage.

6.3 Environmental Factors

The environmental factors should be taken into consideration for the storage results. These results are based on storage at room temperature in Missoula, Montana autumn weather. The results of this research could potentially have different results in other environments. For examples, potentially more contamination in a more humid environment.

6.4 Storage time: 72 hours

There might have been an error during the extraction process of the 72 hours buccal samples. The extraction requires the samples to be centrifuged at high speeds. However, the

centrifuged used for this fist extraction did not reach the speed required. So, a different centrifuge was used for the other extractions.

Cross-contamination was undetected within the 72 hours buccal swab and Whatman card samples. If DNA did migrate through the packaging, there was no quantifiable DNA to detect using the high sensitivity Qubit assay. Therefore, there is no significant cross-contamination for 72 hours storage of PurFlock Ultra DNA-Free Swabs within a plastic transport tube and Whatman cards. This means the integrity of the DNA remains intact during short storage time. Despite there being no quantifiable DNA, the samples were tested further for DNA using PCR. The gel electrophoresis detected four blank pig samples with DNA out of the thirty 72 hours samples. Three of the detected samples were buccal swabs, and one was a Whatman card. Since the 72 hours buccal swabs were the first extracted during the study, it is possible the detected contaminations are from human error during the extraction process. If the contamination is not due to extraction error, then the possibility of cross-contamination during storage times less than 72 hours is not significant.

6.5 Storage time: 14 days

Cross-contamination was undetected within the 14 days buccal swab and Whatman card samples. The Qubit detected DNA in most of the buccal swab samples with known DNA; however, only one of the 24-hour dry time samples detected DNA, and it is unlikely that cross-contamination could occur within those samples. The Qubit did not detect any DNA with Whatman cards. If DNA did migrate through the packaging, there was no quantifiable DNA to detect using the high sensitivity Qubit. Therefore, there is no significant cross-contamination for 14 days storage of PurFlock Ultra DNA-Free Swabs within a plastic transport tube and Whatman

cards. This means the integrity of the DNA remains intact concerning storage time. Despite there being no quantifiable DNA, the samples were tested further for DNA using PCR. The gel electrophoresis did not detect any cross-contamination during the 14 days storage. Based on the Qubit and PCR results 14 days storage is not a significant amount of time for cross-contamination during storage.

6.6 Storage time: 45 days

Most of the cross-contamination occurred within the 45 days buccal swab and Whatman card samples. The Qubit did not detect DNA is in any of the blank samples. Despite there being no quantifiable DNA, the samples were tested further for DNA using PCR. The gel electrophoresis detected fourteen contaminated samples of thirty blank 45 days samples. These contaminations consist of more than half the blank samples, though the Whatman card samples consist of nine of the fourteen contaminations. During storage, contamination is unavoidable under the current long-term storage protocols for buccal swabs and Whatman cards. Compared to the other storage times, 45 days is a significant amount of time for cross-contamination to occur during storage.

6.7 Buccal Swabs

The PurFlock Ultra DNA-Free Swabs were used for the study. These swabs do not require additional packaging aside for storage envelopes. Protocols suggest genetic evidence should not be packaged in plastic. However, the PurFlock swabs uses thin transparent tubing to store samples. From the forty-five blank buccal swab samples eight were contaminated with pig DNA. These results are comparable to the Whatman cards. The 14 days and 45 days samples had

condensation within the packaging. If the box packaging was used to store the buccal swabs, then more contamination might have occurred. Additional precautions will be required for long-term storage for buccal swabs to prevent cross-contamination during storage.

6.8 Whatman Card

Across the three different storage times, no quantifiable DNA came from the Whatman card based on the Qubit. However, the buccal swabs used the same DNA for those samples with quantifiable results. A diluted sample became even further reduced during the extraction process. The Whatman cards were introduced to 50 μl DNA prior to storage, but the extraction only used a 6mm disc from the sample. There are five extractions procedures available for the Whatman cards. The extraction procedure for this research was chosen based on the total nanograms of extracted DNA given a 6mm disc and cost; therefore, the Illustra tissue and cells genomicPrep Mini Spin Kit was the best fit. Based on the results the extraction method used was insufficient for the sample.

Though the Qubit did not detect quantifiable DNA in any of the samples, the PCR detected DNA. From the forty-five blank Whatman card samples, ten were contaminated with pig DNA. These results are comparable to the buccal swabs. Although the cards are more easily exposed to contamination, the cards are equally contaminated during storage as the buccal swabs. Due to the extraction method and contamination rate another collection method is suggested.

6.9 Human DNA Samples

The human cheek samples provided insight into the storage process, but not what was expected. The samples were in storage for 49 days and the last to be extracted. In comparison to the pig samples, the Qubit results were high with all known DNA samples reading over 3.0ng/ μl and the pig samples reading below 0.2ng/ μl. After running four PCRs of the human cheek samples, none of the samples worked. Different thermocycler temperatures and sample volumes were tried to get the PCR samples to work, but all failed. This may indicate possible inhibitors arose during the storage process. The Qubit results only indicate if quantifiable DNA is in the sample but does not indicate if the quantifiable DNA is the anticipated DNA. The combined results from the Qubit and the PCR indicate the storage conditions are unsuitable for these samples.

6.10 Significance

Cross-contamination did occur during storage for the pig proxy DNA, but not as expected. It was expected that an increase in dry time would reduce the probability of cross-contamination. The study shows that dry time does not influence the probability of storage cross-contamination because it occurs at equal rates. The fisher exact test shows that the longer samples are left to dry there is no decrease in the potential of contamination based on the p-values being above significance level $\alpha = 0.05$. Therefore, there is no significance to dry time in regard to cross-contamination during storage.

Cross-contamination was expected to increase with storage time. The results of this study support this expectation. The longer a sample is in storage, the more contamination will occur. The close proximity of the samples during storage increased the chance for cross-contamination.

If the human DNA samples did not have inhibitors, then it is expected similar results would have occurred. The fisher exact test shows that the longer Whatman card samples are left in storage, the more likely Whatman cards are to be contaminated, at a statistically significant level. Therefore, 45 days is a significant amount of time for cross-contamination to occur during storage. However, the fisher exact test shows that the longer buccal swab samples are left in storage, the more likely buccal swabs are to be contaminated at a slightly insignificant level. Using a larger sample size could increase the statistical significance of buccal swab contamination in storage overtime.

7.0 Conclusion

Cross-contamination was detected in an alarming number of blank buccal swab and Whatman card samples during storage. The prevention of contamination is important for crime scene investigations. Protocols are currently in place to prevent contamination throughout the investigation process. With this new insight on storage cross-contamination, new protocols will need to be implemented. There should be no cross-contamination during short-term storage. The longer genetic evidence is stored the greater the chance for cross-contamination to occur. But prevention protocols should be applied to short-term storage for consistency and allowing for an easy transfer to long-term storage. Ideally, these protocols would explicitly indicate the meaning of short-term storage to ensure the best storage method is being used at the proper time.

There are a few limitations for this study to take into consideration. The first limitation is the storage environment. There are four storage environments: frozen, refrigerated, temperature controlled, and room temperature. Only room temperature was used during this study. The second limitation is the collection methods. Only two collection methods were used and there are many ways evidence can be collected. There can also be variations between types of collection methods based on the material, i.e. buccal swabs. Until further research, the results of this study do not provide insight into other storage environments or collection methods. The third limitation is the sample size. The sample size for each storage time was twenty-four comprised of three different dry times tested in triplicate along with a control.

The study amplifies for pig mitochondrial DNA whereas FBI protocols require human Combined DNA Index System (CODIS) markers. Pig DNA shows the potential for basic amplification. Since the study used pig DNA as a proxy for human DNA and contamination was detected, now further research needs to be done using human DNA. Because without using

CODIS markers, it is impossible to know if similar results would be achieved. Regardless the species DNA carryover is still shown indicating the potential for cross-contamination during storage.

Future research is needed to further understand storage cross-contamination. This study only tested two collection methods and one storage condition. Different collection methods, packaging materials, and storage conditions need to be tested. Potentially there could be more or less cross-contamination during storage with other variables.

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Appendix

A1 The identification number and extracted DNA used of Buccal Swab Storage 72 Hours

1: 1A	**2: 1B**	**3: 2A**	**4: 2B**	**5: 3A**	**6: 3B**	**7: 4A**	**8: 4B**
116 B	Blank	116 B	Blank	116 B	Blank	Control Exposed	Control Unopened
9: 1A	**10: 1B**	**11: 2A**	**12: 2B**	**13: 3A**	**14: 3B**	**15: 4A**	**16: 4B**
116 Aa	Blank	116 Aa	Blank	116 Aa	Blank	Control Exposed	Control Unopened
17: 1A	**18: 1B**	**19: 2A**	**20: 2B**	**21: 3A**	**22: 3B**	**23: 4A**	**24: 4B**
116 Bb	Blank	116 Bb	Blank	116 Bb	Blank	Control Exposed	Control Unopened

A 2 The identification number and extracted DNA used of Buccal Swab Storage 14 Days

1: 1A	**2: 1B**	**3: 2A**	**4: 2B**	**5: 3A**	**6: 3B**	**7: 4A**	**8: 4B**
116 A	Blank	116 Aa	Blank	116 Bb	Blank	Control Exposed	Control Unopened
9: 1A	**10: 1B**	**11: 2A**	**12: 2B**	**13: 3A**	**14: 3B**	**15: 4A**	**16: 4B**
116 Bb	Blank	116 Bb	Blank	116 Bb	Blank	Control Exposed	Control Unopened
17: 1A	**18: 1B**	**19: 2A**	**20: 2B**	**21: 3A**	**22: 3B**	**23: 4A**	**24: 4B**
116 A	Blank	116 B	Blank	116 Aa	Blank	Control Exposed	Control Unopened

A 3 The identification number and extracted DNA used of Buccal Swab Storage 45 Days

1: 1A	**2: 1B**	**3: 2A**	**4: 2B**	**5: 3A**	**6: 3B**	**7: 4A**	**8: 4B**
116 A	Blank	116 A	Blank	116 A	Blank	Control Exposed	Control Unopened
9: 1A	**10: 1B**	**11: 2A**	**12: 2B**	**13: 3A**	**14: 3B**	**15: 4A**	**16: 4B**
116 Aa	Blank	116 Aa	Blank	116 Aa	Blank	Control Exposed	Control Unopened
17: 1A	**18: 1B**	**19: 2A**	**20: 2B**	**21: 3A**	**22: 3B**	**23: 4A**	**24: 4B**
116 A	Blank	116 A	Blank	116 A	Blank	Control Exposed	Control Unopened

A 4 The identification number and extracted DNA used of Whatman Cards Storage 72 Hours

1: 1A	**2: 1B**	**3: 2A**	**4: 2B**	**5: 3A**	**6: 3B**	**7: 4A**	**8: 4B**
117 A	Blank	117 A	Blank	117 A	Blank	Control Exposed	Control Unopened
9: 1A	**10: 1B**	**11: 2A**	**12: 2B**	**13: 3A**	**14: 3B**	**15: 4A**	**16: 4B**
117 A	Blank	117 A	Blank	117 A	Blank	Control Exposed	Control Unopened
17: 1A	**18: 1B**	**19: 2A**	**20: 2B**	**21: 3A**	**22: 3B**	**23: 4A**	**24: 4B**
117 A	Blank	117 A	Blank	117 A	Blank	Control Exposed	Control Unopened

A 5 The identification number and extracted DNA used of Whatman Cards Storage 14 Days

1: 1A	**2: 1B**	**3: 2A**	**4: 2B**	**5: 3A**	**6: 3B**	**7: 4A**	**8: 4B**
117 A	Blank	117 A	Blank	1117 A	Blank	Control Exposed	Control Unopened
9: 1A	**10: 1B**	**11: 2A**	**12: 2B**	**13: 3A**	**14: 3B**	**15: 4A**	**16: 4B**
117 A	Blank	117 A	Blank	117 A	Blank	Control Exposed	Control Unopened
17: 1A	**18: 1B**	**19: 2A**	**20: 2B**	**21: 3A**	**22: 3B**	**23: 4A**	**24: 4B**
117 A	Blank	117 A	Blank	117 A	Blank	Control Exposed	Control Unopened

A 6 The identification number and extracted DNA used of Whatman Cards Storage 45 Days

1: 1A	**2: 1B**	**3: 2A**	**4: 2B**	**5: 3A**	**6: 3B**	**7: 4A**	**8: 4B**
116 B	Blank	116 B	Blank	116 B	Blank	Control Exposed	Control Unopened
9: 1A	**10: 1B**	**11: 2A**	**12: 2B**	**13: 3A**	**14: 3B**	**15: 4A**	**16: 4B**
116 B	Blank	116 B	Blank	116 B	Blank	Control Exposed	Control Unopened
17: 1A	**18: 1B**	**19: 2A**	**20: 2B**	**21: 3A**	**22: 3B**	**23: 4A**	**24: 4B**
116 B	Blank	116 B	Blank	116 B	Blank	Control Exposed	Control Unopened

A7 The breakdown of each variable grouping of dry time and storage

Dry Time	Storage Time: 72 Hours	14 Days	45 Days
None	3 Blank 3 DNA 2 Control	3 Blank 3 DNA 2 Control	3 Blank 3 DNA 2 Control
1 Hour	3 Blank 3 DNA 2 Control	3 Blank 3 DNA 2 Control	3 Blank 3 DNA 2 Control
24 Hours	3 Blank 3 DNA 2 Control	3 Blank 3 DNA 2 Control	3 Blank 3 DNA 2 Control

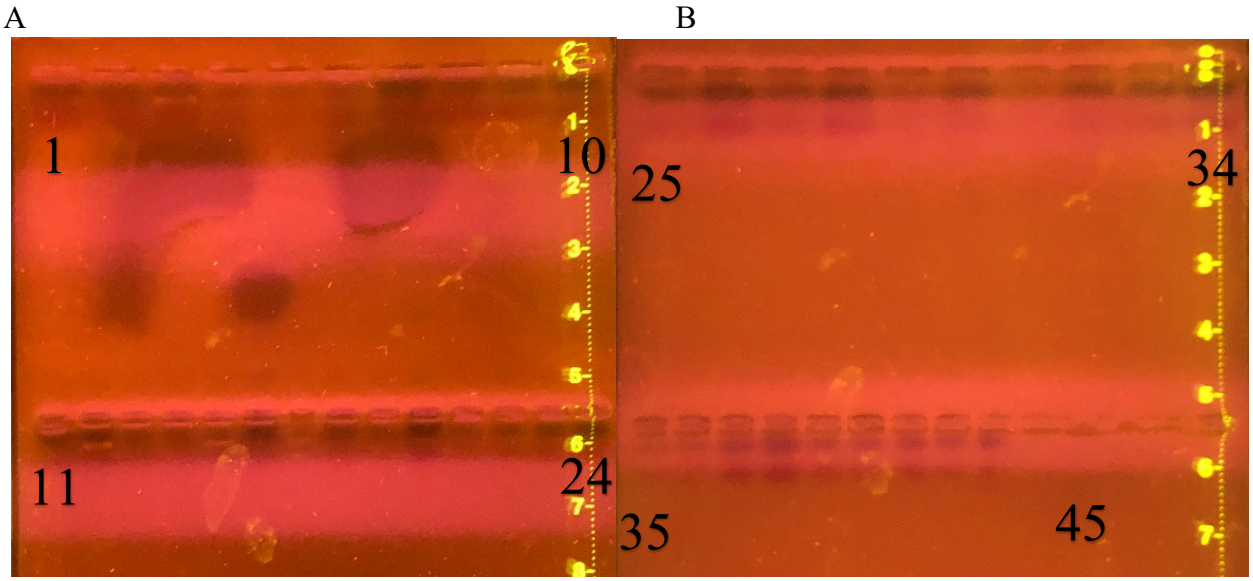

Figure A1 A) Gel Electrophoresis Pig Blank 1B) Gel Electrophoresis 2 Pig Blank

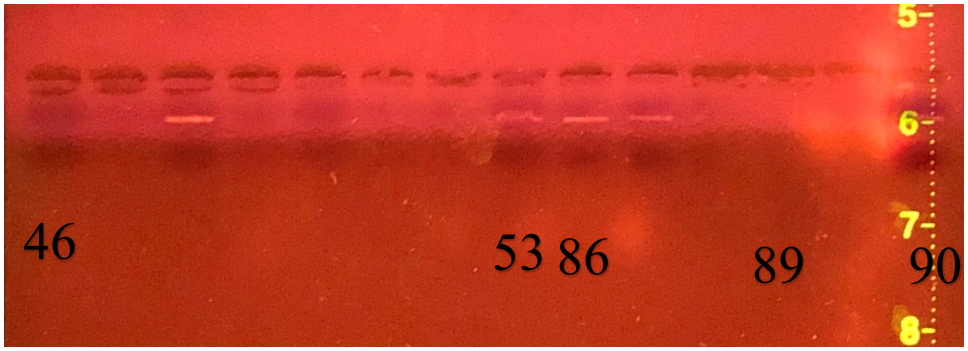

Figure A2 Gel Electrophoresis 3 Pig Blank

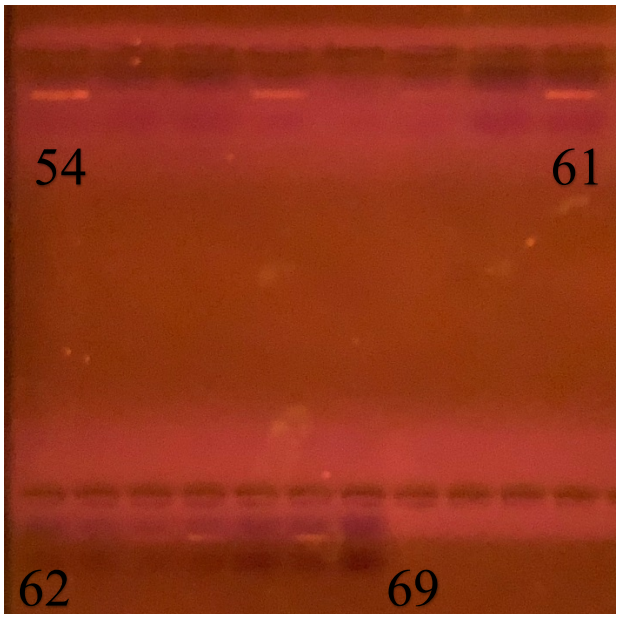

Figure A3 Gel Electrophoresis 4 Pig Blank

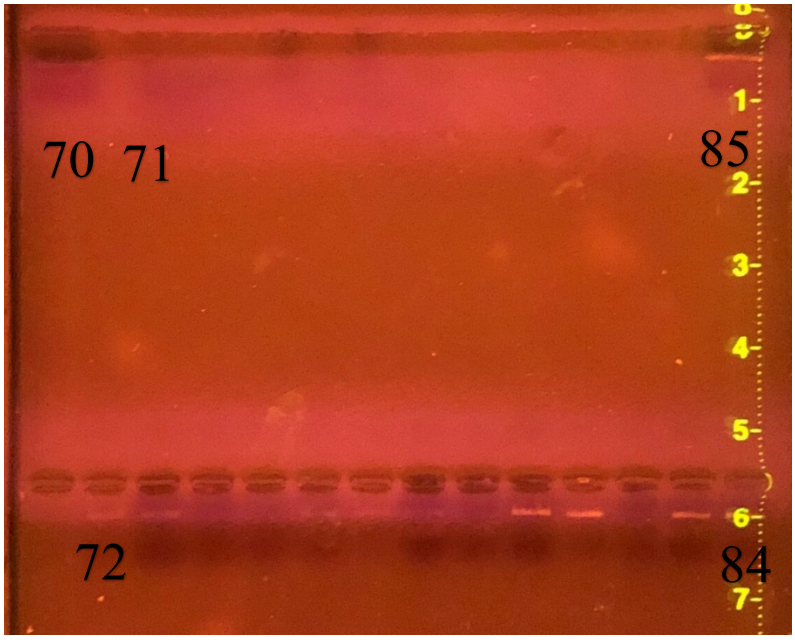

Figure A4 Gel Electrophoresis 5 Pig Blank

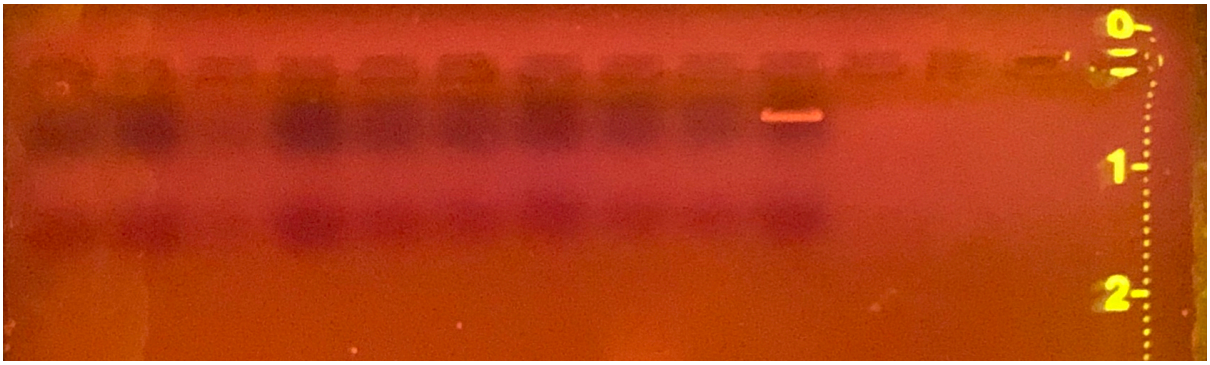

Figure A5 Gel Electrophoresis Human Samples