The Sequential Analysis of DNA Interpretation and Fingerprint Ridge Patterns on Porous Paper Evidence

By

SARAH HEGENBART

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Approved:

Ashley Hall, Chair

Allison Ehrlich

Robert Poppenga

Committee in Charge

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Abstract

In forensic investigations, when porous substances are submitted for analysis, either fingerprint or DNA analysis can be performed. The purpose of this study was to see if it is possible to perform both fingerprint and DNA analysis on the same piece of evidence and to determine the sequence of analysis that produces the best results. Studies have focused on what fingerprint methods affect DNA analysis but have yet to focus on how DNA analysis affects fingerprint enhancement quality. There are many methods to enhance the visibility of fingerprints on porous substances, but this study chose to use ninhydrin and 1,2-indanedione. In this study, three volunteers deposited their DNA and latent prints onto five different paper substrates (money, copy paper, cardboard, cardstock, and thermal paper). The samples then went through one of the following sequences of analysis: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first and then fingerprint enhancement with ninhydrin method, and 4) DNA analysis first and then fingerprint enhancement with 1,2-indanedione. The results show that the DNA analysis process significantly decreased the fingerprint enhancement quality while the fingerprint enhancement process with either ninhydrin or 1,2-indandione methods does not significantly decrease the quality and quantity of DNA. These results are important because both fingerprint enhancement and DNA analysis can be performed on the same paper substrates.

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1. Introduction

The Locard Exchange Principle states that when two items come in contact, they will exchange material (Locard 1930). This principle is integral to forensic science such as both fingerprint identification and DNA analysis (Tang et al., 2020). When an individual touches a surface they leave behind both their fingerprint and their DNA, (Wickenheiser 2002; van Oorschot and Jones, 1997). Forensic Science encompasses many scientific fields to examine evidence and draw conclusions. To obtain the best results in order to make an identification, it is imperative to determine the sequence of forensic processing for each piece of evidence. As both DNA and fingerprint analysis can be destructive processes, it is important to understand which process should be performed first to obtain the most information from evidence without destroying evidence in the process.

1.1. Fingerprint background

Our fingers and parts of our hands and feet contain friction ridges that are unique to the individual (Trozzi et al., 2000). Therefore, fingerprints can help in investigations to identify an individual. The primary ridges begin to form on the 13th gestational week and are fully formed by the 17th gestational week (Babler, 1991; Glover et al., 2023). The secondary ridge formations then begin to develop (Babler, 1991; Wertheim and Maceo, 2002; Glover et al., 2023). Fingerprints can have one of three primary ridge patterns, also known as classification patterns: loop, whorl, or arch. The secondary ridge formations are identification characteristics, also known as minutiae, including ridge ending, bifurcation, and dot which disrupt the continuous flow of the fingerprint ridges at different locations on the fingerprint pattern (Lennard, 2005; Kaur et al., 2022). Identification or exclusion can be made by the type of classification pattern,

the minutia types, their orientations, and their relative positions on the fingerprint (Stoney and Thorton, 1986). Fingerprints are left behind due the production of sweat primarily from three glands: 1) eccrine sudoriferous gland, 2) apocrine sudoriferous gland, and 3) sebaceous gland (Anderson et al., 1998). These sweat glands begin to form in the 14th gestational week (Babler, 1991; Wertheim and Maceo, 2002). The eccrine and apocrine glands exude sweat from friction ridge pores and sebaceous secretions are transferred from other body locations that the fingers contact (Bathrick et al., 2022). On average within 24 hours, 700 to 900 grams of secretions are produced from the eccrine gland and excreted primarily to the palms of the hands and soles of the feet (Kaiser and Drack, 1974; Anderson et al., 1998). The most important component of the eccrine secretions for fingerprint enhancement is the amino acids (Hamilton, 1965; Oró and Skewes, 1965; Hadorn et al., 1967). The most abundant amino acid is serine, followed by glycine, alanine, and aspartic acid (Mekkaoui and Halamek, 2019). Proteins and lipids are also found in eccrine sweat (Uyttendaele et al., 1977; Nakayashiki, 1990). The apocrine gland secretes sweat at the armpits and pubic area. Due to the secretions often mixing with sebaceous secretions, the content is not well understood (Shelley, 1951; Anderson et al., 1998). The sebaceous glands are located throughout the body at locations with body hair. They are not present at the palms of the hands nor the soles of the feet (Anderson et al., 1998). The sebaceous secretions are primarily made up of lipids (Goode and Morris, 1983). The components of the fingerprints (amino acids, and lipids) are targeted to enhance the latent fingerprint to be visualized (Hamilton, 1965; Oró and Skewes, 1965; Hadorn et al., 1967; Uyttendaele et al., 1977; Nakayashiki, 1990; McDiarmid, 1992). When an individual touches a surface, these secretions leave behind the impressions of the ridges from the fingers, hands, and feet. (Bathrick et al., 2022). Fingerprints can be found on a multitude of surfaces, categorized as porous,

nonporous, and semiporous. It is important to understand the characteristics of these surfaces as they will determine what type of procedure is used to enhance the fingerprint (Trozzi et al., 2000). For porous surfaces (i.e., paper) methods that target amino acids are ideal because they are always present in sweat and have a high affinity for cellulose (Speaks, 1970; Almog, 2001; Champod et al., 2004). Upon contact with paper, the amino acids permeate the paper and, due to the high affinity for cellulose, the amino acids adhere to the paper and do not migrate from the initial deposition location (Knowles, 1978; Almog, 2001; Champod et al., 2004; Hansen and Joullié, 2005). There are many different fingerprint methods for porous surfaces such as: ninhydrin, 1,2-indanedione, 1,8-Diazafluoren-9-one (DFO), 5-Methylthioninhydrin (5-MTN), vacuum metal deposition, silver nitrate, and silver physical developer (Forgeot, 1891; Theys et al., 1968; Pounds et al., 1990; Cantu et al., 1993; Ramotowski et al., 1997; Cantu, 2001; Champod et al., 2004). Ninhydrin, 1,2-indanedione, DFO, and 5-MTN all react with amino acids in the fingerprint residue (Ruhemann, 1910; Grigg et al., 1990; Wilkinson, 2000; Elber et al., 2000). Vacuum metal reacts with the fatty acids in the fingerprint residue (Stroud et al., 1971). Silver nitrate reacts with the chloride ions in the salt from eccrine secretions (Dean, 1985). Silver physical developer reacts with both fatty acids and lipids in fingerprint sweat residue (Sodhi and Kaur, 2016).

1.2. Ninhydrin fingerprint method

One common method used for the analysis of fingerprints on porous surfaces is ninhydrin. Ninhydrin and its derivatives are used to detect primary and secondary amines, such as amino acids found in eccrine sweat along with peptides and proteins (Hark et al., 2001; Das and Banik, 2021). Ninhydrin is a great enhancement method because it reacts nonspecifically to the different amino acids, meaning no matter the type of amino acid present, ninhydrin will still

have a reaction (Champod, 2004). Ninhydrin reagent will produce Ruhemann's purple when the α-amino group of the primary amino acids in the fingerprint residue react with the colorless crystal ninhydrin molecule (McCaldin, 1960; Bottom et al., 1978; Grigg et al., 1989; Jasuja et al., 2009; Perrett et al., 2015). This purple color allows the examiner to identify the fingerprint more readily (Bottom et al., 1978; Grigg et al., 1989; Lennard 2005; Jasuja et al., 2009; Azman, 2020). The byproducts of this reaction are aldehyde derivatives coming from the amino acids, and carbon dioxide (Yemm et al., 1955; Friedman and Williams, 1974). The fingerprint can then be examined and photographed in white light. (Kent, 2013). The intensity of the purple depends on the number of amino acids present (Perrett et al., 2014). The ninhydrin reagent can be applied to the porous surface by dipping, spraying, brushing, or fuming the surfaces (Odén and van Hofsten, 1954; Speaks, 1964). When performing DNA analysis after fingerprint enhancement, the dipping technique can contaminate the DNA if reusing the same fingerprint enhancement solution for each new sample (Bhoelai et al., 2011). For the best results, the pH should be between 4.5 and 5.2, and in a humid environment (Grigg et al., 1989; Champod et al., 2004). One disadvantage for ninhydrin is its inability to be performed with receipt paper, also known as thermal paper (Yadav, 2019). The ninhydrin solution reacts with thermal paper, turning the thermal paper black (Schwarz, & Klenke, 2010).

1.3. 1,2-Indanedione fingerprint method

Another common fingerprint analysis method for porous surfaces is 1,2-indanedione. 1,2-Indandione reagent can be created with either ethyl acetate or acetic acid. Like ninhydrin, 1,2-indanedione reacts with the amino acids present in the latent print (Almog, 2001; Petrovskaia et al., 2001; Sirchie, 2014). Unlike ninhydrin, fingerprints from 1,2-indanedione fluoresce without

further enhancement processes (Elad et al., 2017). Blue (455nm) or cyan (505nm) light sources are paired with either red or orange filters for fingerprint analysis, (Ramotowski et al., 1997; Sirchie, 2014). The fluorescence is dependent on the abundance and type of amino acid present on the fingerprint (Mekkaoui and Halamek, 2019). The 1,2-indanedione reagent can be applied to the surface by dipping or spraying (Roux et al., 2000). When performing DNA analysis after fingerprint enhancement, the dipping technique can contaminate the DNA if reusing the same fingerprint enhancement solution for each new sample (Bhoelai et al., 2011). For the best results, the temperature should be 100°C and the relative humidity (Rh) be 60% (Almog et al., 1998; Roux et al., 2000; Wiesner et al., 2001). Unlike ninhydrin, 1,2-indanedione is particularly useful for analysis on receipt paper (Hong et al., 2017).

1.4. DNA background

Touch DNA is when an individual touches another person or an item and subsequently leaves behind their DNA (Lacerenza et al., 2016). The origin of the DNA found within touch samples are not completely known, but it is likely from the following contributions: shed corneocytes, transferred or endogenous nucleated epithelial cells, fragmented cells and nuclei, and lastly cell-free DNA (Kita et al., 2008; Hanson et al., 2012). The skin has several different layers in the epidermis. As the keratinocytes move from the granular layer outward towards the cornified layer, the keratinocytes lose their nuclei through apoptosis. The DNA is subsequently degraded by enzymes during keratinization of cells (Kita et al., 2008). The DNA found on the anucleate corneocytes may come from the stripped DNA of the keratinized cells (Alessandrini et al., 2003; Lacerenza et al., 2016). DNA can also be found in eccrine and sebaceous secretions. When an individual touches a surface, they leave some of their sebaceous secretions on the

surface (Kita et al., 2008; Ostojic and Wurmbach, 2017).The amount of DNA present from a latent print varies based on many factors, such as intensity and length of contact, moisture on either the skin or the surface, number of times touching the surface or other surfaces, the type of surface, the individual's DNA shedding amount, handwashing, surface friction and the presence of bodily fluid. Both friction and pressure are the most important factors. (Alessandrini et al., 2003; Bhoelai et al., 2011; Warshauer et al., 2012; Burrill et al., 2019).

1.4. DNA analysis

The DNA left behind when touching a surface, such as paper, can be collected and analyzed to determine the identification of an individual based on their unique DNA profile (Ostojic and Wurmbach, 2017). Short Tandem Repeat (STR) markers are polymorphic DNA loci that contain a short nucleotide sequence that is repeated (Tautz, 1993). STRs are unevenly distributed throughout the human genome making up about 3% of the genome. The noncoding regions of the genome hold most of the STRs (Koreth et al., 1996; Ellegren, 2000). There are a variety of commercial DNA PCR amplification kits that target STRs. Promega® PowerPlex® Fusion 6C PCR Amplification system, manufactured by Promega® of Wisconsin, United States, is one available kit. It amplifies twenty-seven loci, including a sex identification locus. Further, out of the 23 autosomal loci, Fusion 6C contains all thirteen of the original core CODIS loci for uploading profiles into Combined DNA Index System (CODIS), seven expanded core CODIS loci, and three loci specific to the Y chromosome (Promega®, 2023).

1.5. Purpose of this study

Multiple studies have focused on the analysis steps that affect the quality and quantity of DNA when fingerprint enhancement is performed prior to DNA analysis, but these studies have not investigated how DNA analysis techniques affect fingerprint enhancement (Balogh et al., 2003; Tsai et al., 2016; (Zaghloul, 2019; Carlin et al., 2023). Fingerprint enhancement methods that rely on immersion of samples, such as ninhydrin, 1,8-Diazafluoren-9-one (DFO), and 1,2-indanedione in acetic acid introduces contamination from the solution used for staining the fingerprint (Bhorelai et al., 2011; Tsai et al., 2016). Not only does ninhydrin introduce contamination, but it also significantly reduces the DNA recovery (Carlin et al., 2023). While 1,2-indanedione is an immersion technique, it does not reduce DNA quantity when ethyl acetate is used rather than acetic acid (Tsai et al., 2016). Methods that include washing steps such as safranin or basic yellow staining had reduced DNA quantities (Bhoelai et al., 2011). The DNA results after fingerprint enhancement also relied on the type of porous surface. When magazines, office papers, and newspapers all underwent fingerprint enhancement and then DNA analysis, magazines had the highest DNA recovery (Zaghloul, 2019). When fingerprint enhancement with ninhydrin method is used on office paper, newspaper, and wood, the DNA quantity is significantly reduced (Zaghloul, 2019; Carlin et al., 2023). The process of swabbing the surface to obtain DNA destroys the fingerprint making it necessary to perform fingerprint enhancement prior to DNA analysis (Zaghloul, 2019). All the described studies above, focused on the effects fingerprint enhancement methods and procedures had on DNA analysis from various paper surfaces. To the best of my knowledge there is no study that has examined how the DNA analysis process affects the quality of fingerprint enhancement. This study focused on understanding the sequence of analysis of fingerprint and DNA and which sequence was less detrimental to the

second analysis. Further, it compared the fingerprint enhancement method ninhydrin versus 1,2-indanedione on several common paper substrates (money, copy paper, cardboard, cardstock, and thermal paper). This study was performed at the Sacramento District Attorney's Crime Laboratory for the DNA analysis portion, and the Sacramento Sheriff's Office for the fingerprint enhancement process. Both ninhydrin and 1,2-indanedione fingerprint methods were used because they are the methods that the Sacramento Sheriff's Office uses on paper substrates. The five paper substrates used were chosen based on the most prevalent substrates examined in both the Sacramento District Attorney's Crime Laboratory and the Sacramento Sheriff's Office.

2. Methods

2.1. Porous substrates

The paper substrates used were one-dollar bills, copy paper, cardstock, brown cardboard, and thermal paper. Each paper substate was cleaned with 10% bleach and 70% ethanol to limit contamination. Each substrate was measured and cut to the size of a dollar bill (5.5cm x 41.0cm).

2.2. Collection of samples

Three volunteers, two females and one male, refrained from washing their hands for an hour prior to touching the samples. Each sample was collected at a one-hour increment, a total of 36 time points for each volunteer, and was placed in a sterile coin pouch. Each paper substrate had an unintentional latent fingerprint with DNA deposited and then an intentional latent print with DNA deposited on the back of the same paper. A buccal swab reference was collected from each of the volunteers. A total of 36 samples were collected including three buccal swab reference samples from the volunteers. There was a total of 36-time intervals with no replicates

from each of the three volunteers. To limit any contamination of inadvertent fingerprints or DNA, gloves were always used, the only exception was the intended depositing of fingerprints on the paper substrates by the volunteers.

The volunteers removed the substrate from the coin pouch and wrote, “give me all of your money”, then placed the sample back into the coin pouch. When the volunteer touched the substate, they were not intentionally trying to deposit their fingerprint and DNA onto the substrate. Further, while they wrote on the paper, they were holding the paper down to steady the paper. When the volunteers steadied the paper, they unintentionally deposited their DNA onto the paper. This served as the unintentional latent print deposit.

The volunteers again removed the substrate from the coin pouch. They pressed their thumb down in the center of the paper on the opposite side of the paper that they had previously written “give me all of your money”. The contact took 1-2 seconds with medium pressure. This served as the intentional latent print deposit.

2.3. Sequence of analysis

Category 1:

Both intentional and unintentional fingerprints and DNA were isolated from the porous substrates by performing fingerprint enhancement first with ninhydrin followed by DNA analysis.

Category 2:

Both intentional and unintentional fingerprints and DNA were isolated from the porous substrates by performing fingerprint enhancement first with 1,2-indanedione followed by DNA analysis.

Category 3:

Both intentional and unintentional fingerprints and DNA were isolated from the porous substrates by performing DNA analysis first followed by fingerprint enhancement with ninhydrin.

Category 4:

Both intentional and unintentional fingerprints and DNA were isolated from the porous substrates by performing DNA analysis first followed by fingerprint enhancement with 1,2-indanedione.

2.4. Fingerprint enhancements

The samples either went through the 1,2 indanedione or the ninhydrin method because these are the enhancement methods that are used by the Sacramento County Sheriff's Office, where the fingerprint portion of the study was performed. The two different methods followed the Sacramento County Sheriff's Office 1,2-indanedione and ninhydrin protocols.

2.5. Ninhydrin fingerprint method

Ninhydrin reagent was created by dissolving 5g of ninhydrin crystals into a spray bottle filled with 757ml of acetone. Four samples at a time were enhanced. Binder clips cleaned with 70% ethanol were used to grab the edge of the sample from the coin manilla envelopes. The binder clips were then attached to a second binder clip hanging from a horizontal wire inside a fume hood. The samples were dampened with the ninhydrin reagent by spraying the front and back of the samples, making sure the ninhydrin reagent covered each of the entire sample. The samples completely dried for 2-5 minutes and then went onto a metal rack inside of the NinCha

M31chamber, manufactured by Attestor Forensics GmbH in Wurzach, Germany. The NinCha M31 chamber was set at 65% relative humidity (rh) and 60°C for 20 minutes. After the 20 minutes, the front and back of the paper substrates were photographed and sent for fingerprint quality assessment. The samples were then placed back in their coin manilla envelope. After each round of fingerprint enhancement, the metal rack was cleaned with 70% ethanol. Receipt paper did not undergo ninhydrin method as per Sacramento County Sheriff's Office protocol.

2.6. 1,2-Indanedione fingerprint method

1,2- Indanedione reagent was created by dissolving 1.5g indanedione crystals into a spray bottle filled with 52.5ml of ethyl acetate and 697.5 ml of 3M™ Novec™ HFE7100 Engineered fluid, manufactured by 3M™ Electronics Markets Materials Division in Minnesota, United States. Five samples at a time were enhanced. Binder clips cleaned with 70% ethanol were used to grab the edge of the sample from the coin manilla envelopes. The binder clips were then attached to a second binder clip hanging from a horizontal wire inside in a fume hood. The samples were dampened with the 1,2-indanedione reagent by spraying the front and back of the samples, making sure the 1,2-indanedione reagent covered each of the entire sample. The samples completely dried for 2-5 minutes and then went onto a metal rack inside of the NinCha M31chamber. The NinCha M31 chamber was set at 100°C for 20 minutes. The prints were examined in the dark with a 532nm light source and an orange filter attached to the camera. The front and the back of the paper substrates were photographed and sent for fingerprint quality assessment. The samples were then placed back in their coin manilla envelope. After each round of fingerprint enhancement, the metal rack was cleaned with 70% ethanol. Receipt paper did not undergo ninhydrin method as per Sacramento County Sheriff's Office protocol.

2.7. Fingerprint assessment

Five latent print examiners certified by the International Association for Identification (IAI) for fingerprints assessed the quality of the single best latent print impressions for each of the 54 unintentional and 54 intentional samples from the pictures previously taken. The examiners followed the following fingerprint quality rankings from 1-5 below:

1. Poor (no visible fingerprint ridges).
2. Low (fingerprint ridges visible and the ridge detail has low contrast and clarity).
3. Medium (fingerprint ridges visible and the ridge detail has medium contrast and clarity).
4. Good (fingerprint ridges visible and the ridge detail has good contrast and clarity).
5. Excellent (fingerprint ridges visible and the ridge detail has excellent contrast and clarity).

2.8. DNA collection

The samples were swabbed with cotton swabs moistened with sterile water. For each sample, two different DNA swabs were collected. The first was swabbing the entire paper substrate, except for the back center of the paper. The second swab was taken from the intentional fingerprint location at the back center of the paper. A total of 108 samples were swabbed from the paper substrates. Each of the cotton swabs were placed in a tube and the swab cut off using a sterile scalpel and left to air dry in a fume hood.

2.9. DNA extraction

The entire portion of the cotton swab samples was placed in a tube along with 475µl of 1:1diluted G2 buffer and 25µl of Proteinase K. The samples were vortexed briefly and incubated at 56°C for 60 minutes on a ThermoMixer heat block at 900rpm, manufactured by Thermo

Fisher Scientific Inc.™ in Massachusetts, United States. The swabs were placed in a spin basket and placed back into the tube. The samples then went on the centrifuge for 5 minutes on high. The samples were then transferred to BioRobot EZ1 Advanced XL sample tubes, manufactured by Qiagen® in Hilden, Germany. DNA was lysed by a Qiagen® DNA Investigator Kit and extracted using an EZ1 XL robot Thirteen samples and 1 reagent blank were placed on the EZ1 XL robot at a time. The EZ1 XL robot followed the trace protocol, and the samples were eluted into 40µl of Tris EDTA (TE) Buffer. The cartridges used for the BioRobot EZ1 Advanced XL were inverted twenty times to mix the magnetic particles before placing on the instrument. The extracts were eluted into elution tubes. The 108 swabs and 3 reference samples were extracted and went through the protocols at a separate time. After each round of sample extraction, the EZ1 XL robot was cleaned by cleaning the piercing unit, worktable, and rack with 70% ethanol and DI water.

2.10. DNA Quantification

Five standards were prepared. Standard 1 was prepared by adding10µL of 100 ng/µl of Quantifiler® THP DNA standard, manufactured by Thermo Fisher Scientific™ in Massachusetts, Germany, and 10µl of Quantifiler® THP DNA dilution buffer to a tube. Standard 1 was then vortexed and centrifuged for 3-5 seconds. Standard 2 was prepared by adding 10 µL of standard 1 and 90 µL of Quantifiler® THP DNA dilution buffer to a second tube. Standard 2 was then vortexed and centrifuged for 3-5 seconds. Standard 3 was prepared by adding 10 µL of standard 2 and 90 µL of Quantifiler® THP DNA dilution buffer to a third tube. Standard 3 was then vortexed and centrifuged for 3-5 seconds. Standard 4 was prepared by adding 10 µL of standard 3 and 90 µL of Quantifiler® THP DNA dilution buffer to a fourth tube. Standard 4 was then

vortexed and centrifuged for 3-5 seconds. Standard 5 was prepared by adding 10 µL of standard 2 and 90 µL of Quantifiler® THP DNA dilution buffer to a fifth tube. Standard 5 was then vortexed and centrifuged for 3-5 seconds. The PCR Reaction Mix and Primer Mix were centrifuged for 3-5 seconds. A master mix was prepared by adding 1,610µL of the Quantifiler® Trio PCR Reaction Mix and 1,288 µL of the Quantifiler® Trio PCR Primer Mix to a sixth tube. The master mix was created by pipetting the solution up and down several times. Two µL duplicate sets of the five standards were added to two 96 well plates. Two µL duplicate sets of Quantifiler® THP DNA dilution buffer, utilized as No Template Controls (NTC), were then added to their labeled wells within the two 96 well plates. Two µL of samples and reagent blanks were then added to their own labeled wells within the two 96 well plates. The first plate had all the samples that went through fingerprint enhancement first and the second plate had the samples that went through DNA analysis first. Eighteen µL of master mix was added to each of the wells containing standards, NTCs, samples, and reagent blanks on both plates. An Optical Adhesive Cover was placed over the each 96 well plate and sealed, making sure there were no air bubbles. DNA was quantified by Real-Time PCR using ThermoFisher Quantifiler® Trio Quantitation Kit on the Quant Studio™ 5 instrument, manufactured by Thermo Fisher Scientific™, in Massachusetts, United States.

The parameters were:

95°C for 2 minutes

For 40 cycles, 95°C for 9 seconds and 60°C for 30 seconds

The samples were then concentrated to a final volume of 15µl using the Eppendorf® Vacufuge® Plus, manufactured by Eppendorf, in Hamburg Germany.

2.11. DNA Amplification/PCR

PowerPlex® Fusion 6C 5X Master Mix and PowerPlex® Fusion 6C 5X Primer Set were centrifuged for 3-5 seconds. A master mix was prepared by adding 690 µL of the PowerPlex® Fusion 6C 5X Master Mix and 690 µL of the PowerPlex® Fusion 6C 5X Primer Set to a tube and pipetting the solution up and down several times. Ten µL of master mix was transferred from the master mix tube into a PCR tube for each of the following: allelic ladder, reagent blank, positive control, negative control, samples, and reagent blanks. Fifteen µL of allelic ladder, reagent blank, positive control (1.0 ng of DNA using Fusion 6C), negative control (Promega® Amplification grade water), the concentrated DNA samples, and reagent blanks were transferred into their PCR tubes. The DNA samples were amplified using the Promega® PowerPlex® Fusion 6C PCR Amplification Kit and ThermoFisher™ Veriti ™Thermocycler, manufactured in Massachusetts, United States. The parameters were:

96°C for 1 minute, then:

96°C for 5 seconds

60°C for 1 minute

For 29 cycles, then:

60°C for 10 minutes

4°C hold

2.12. DNA Typing

Two 96 well plates were used. The first plate had all the samples that went through fingerprint enhancement first and the second plate had the samples that went through DNA analysis first. There were either allelic ladders, positive control, negative control, blank, samples, or reagent blanks in each well. Each well with an allelic ladder, positive control, negative control, or sample had 0.5 µl of WEN ILS 500X and 9.5 µl Hi-Di™ Formamide X. The blank had 10 µl of formamide and 1 µl of WEN ILS 500 X. 1 µl of allelic ladder, positive control, negative control, and sample were added to their wells. The 96 well plate was covered with a septum and centrifuged for 20 seconds. Then the 96 well plate was denatured at 95°C for 3 minutes on a thermal cycler. The plate was then snap cooled by placing it into a 96 well freezer block and placing a weight on top of the plate for 3 minutes. DNA was typed using the 3500xL Genetic Analyzer, manufactured by Thermo Fisher Scientific™ in Massachusetts, United States. The injection parameter was 1.2 kV for 24 seconds. The run parameter was 13kV for 1500 seconds. The dye set was Promega® J6 manufactured by in Wisconsin, United States.

2.13. DNA Analysis

GeneMapper™ Software version 1.6 was used to assess the quality and quantity of DNA. GeneMapper™ assigned the alleles above the analytical threshold of 100 relative fluorescence units (RFU)s and the number of loci with detected alleles were counted out of a total of 23 possible autosomal loci. Next, the loci that did contain possible alleles, but did not reach the analytical threshold were counted. The samples were compared with the volunteer's profile to determine the number of the volunteer's alleles present in the sample profiles. Each profile was

deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile in the sample's profile.

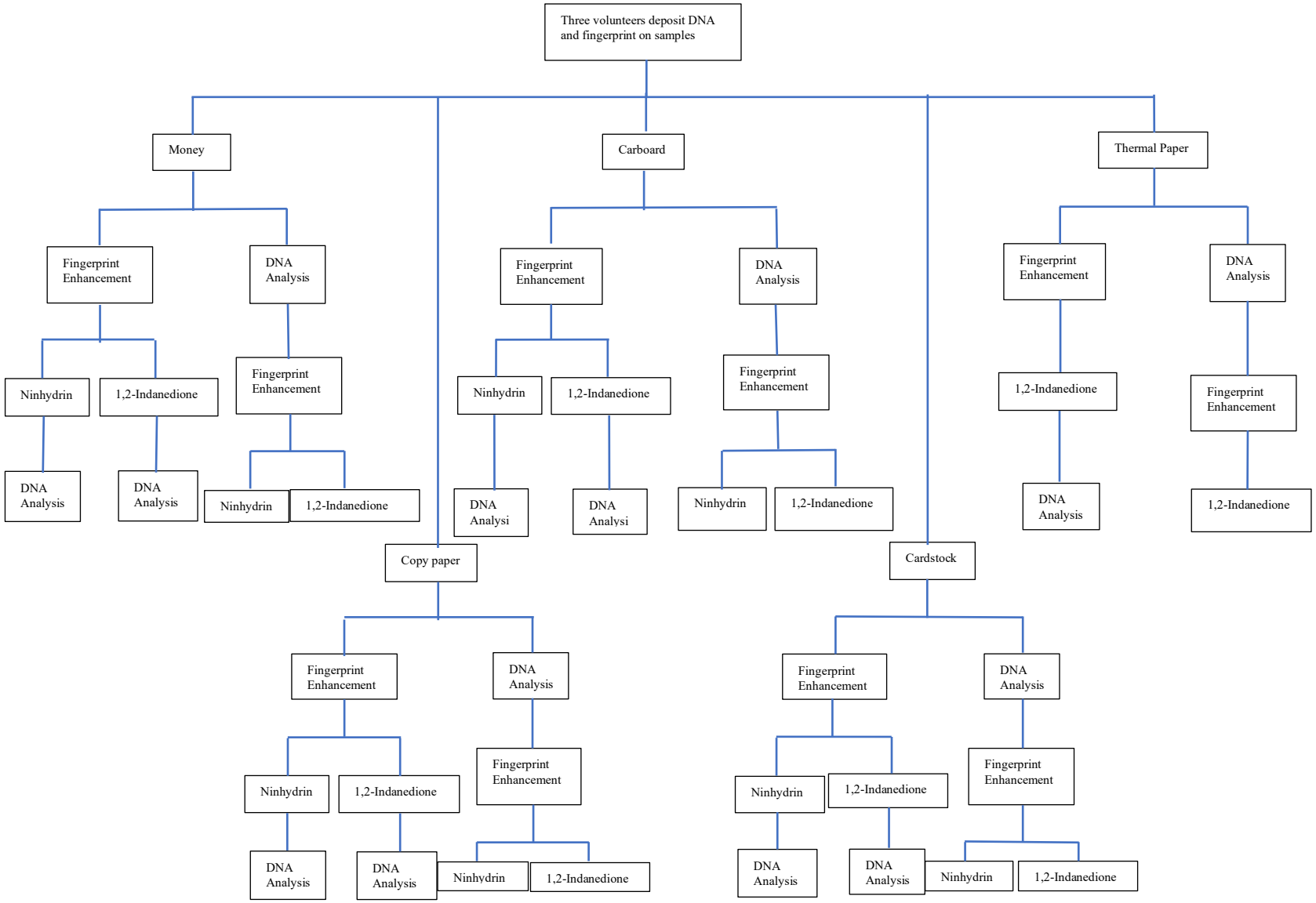

Figure 1: Flow chart of analysis for each of the paper substrates.

3. Statistical analysis

One-way ANOVA was performed on the fingerprint quality, qPCR results, and DNA profiles. If One-way ANOVA suggested a significance, then Tukey-Kramer's Post Honest Significant Difference (HSD) test was used to determine the category that was significant.

4. Results

4.1. DNA concentrations and profile analysis

4.1.1. Fingerprint enhancement first versus DNA analysis first

4.1.1.1. DNA concentrations

The DNA concentration for each sample that went through fingerprint enhancement first then DNA analysis or DNA analysis first then fingerprint enhancement was calculated. When DNA analysis was performed prior to fingerprint enhancement, the mean DNA concentration was 0.121519±0.257523 ng/µl. When fingerprint enhancement was performed prior to DNA analysis, the mean DNA concentration was 0.086713±0.150405 ng/µl. The p-value was 0.393028. There was no significant difference in the DNA concentrations for the two sequences of analysis. There were three outliers for fingerprint enhancement first and three outliers for DNA analysis first (Figure 2A). Outliers were determined by using excel® version 2310.

4.1.1.2. Number of loci with called alleles in the DNA profiles

When GeneMapper software analyzes the DNA profile, each allele above the analytical threshold of 100 RFUs gets called. The number of loci that contained a called allele was calculated for each profile. The total number of loci possible was 23. Samples that went through fingerprinting first had a higher mean number of loci with called alleles of 10.72222±9.483683

loci with 22.22% of the samples having all 23 loci with called alleles. DNA analysis prior to fingerprinting analysis had a mean of 10.11111±9.205549 loci with 16.67% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.734698. The difference in the number of loci with called alleles was not significant when comparing the two sequences of analysis.

4.1.1.3. Number of loci with alleles present in the DNA profiles

Despite the alleles not reaching the analytical threshold of 100 RFUs, and thus not called by GeneMapper, the alleles are still present in the profile. Samples that reach the analytical threshold indicate that they can definitively be distinguished from the instruments background noise (background signals). The alleles that do not reach the analytical threshold and were calculated in this study were chosen because their signal was visually above the background noise, but just missed the 100 RFU analytical threshold. The number of loci with alleles present include all of the loci with alleles called, and the number of loci that have alleles that are visibly above the noise, but not above the 100 RFU analytical threshold. The number of loci with alleles present in the profile were manually counted. The total number of loci possible was 23. Like the loci with alleles above the analytical threshold and called by GeneMapper, fingerprinting prior to DNA showed a higher number of loci with alleles present in the DNA profiles. The mean value for fingerprinting prior to DNA was 16±8.547316 loci with 38.89% of the samples having all 23 loci with called alleles, while DNA prior to fingerprinting had a mean of 15.22222±9.248491 loci with 40.74% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.650864. There was no significant difference between the two analysis pathways for the samples based on the number of loci that showed the presence of an allele in the sample.

4.1.1.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the profiles were compared to each of the volunteer's reference profiles. The number of correct alleles observed in each of the profiles was quantified and the comparison of fingerprinting and DNA analysis sequences was performed. The samples that went through fingerprint enhancement first and then DNA analysis had a higher mean of 28.68519±17.6473 alleles with 27.78% of the samples having all alleles present in the profile. For those samples with DNA analysis first then fingerprint enhancement there was a mean of 27.40741±17.68116 alleles with 12.96% of the samples having all alleles present in the profile. The ANOVA p-value was 0.707762. There was no significant difference between the two analysis pathways for the samples based on the number of alleles that matched each of the volunteer's DNA profiles.

4.1.1.5. Interpretability of the DNA profiles

Each profile was deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile in the sample to assess profile quality. A "1" was given to profiles that were interpretable. A "-1" was given to profiles that were not interpretable. Fingerprinting prior to DNA had a mean quality of 0.074074±1.006617 DNA profile quality with 53.70% of the samples deemed interpretable and 46.30% not interpretable. For samples with DNA analysis prior to fingerprinting, a mean quality of -0.18519±0.991931 DNA profile quality was calculated with 40.74% samples deemed interpretable and 59.26% of the samples uninterpretable. The ANOVA p-value was 0.180502. There was no significant difference in the quality of DNA profiles when comparing the sequence of analysis.

Table 1: DNA concentrations for first analysis performed. Samples either went through fingerprint enhancement first or DNA analysis first. Both analyses had 54 samples.

Fingerprint	DNA
0.0988 ng/µl	0.0608 ng/µl
0.0036 ng/µl	1.27305 ng/µl
0.0365 ng/µl	0.34505 ng/µl
0.0071 ng/µl	0.01155 ng/µl
0.01875 ng/µl	0 ng/µl
0.1113 ng/µl	0.1026 ng/µl
0.004 ng/µl	0.0504 ng/µl
0.0624 ng/µl	0.06715 ng/µl
0.1425 ng/µl	0.04485 ng/µl
0 ng/µl	0.0266 ng/µl
0.0432 ng/µl	0.0406 ng/µl
0.1296 ng/µl	0.10585 ng/µl
0.0576 ng/µl	0.0154 ng/µl
0.012 ng/µl	0.5206 ng/µl
0.0222 ng/µl	0.1575 ng/µl
0 ng/µl	0.01095 ng/µl
0.03 ng/µl	0 ng/µl
0.1218 ng/µl	0.032 ng/µl
0 ng/µl	0 ng/µl
0 ng/µl	0.0076 ng/µl
0.35625 ng/µl	0.0805 ng/µl
0.019 ng/µl	0.008 ng/µl
0 ng/µl	0 ng/µl
0.3337 ng/µl	0.0345 ng/µl
0.07125 ng/µl	0.1679 ng/µl
0.06545 ng/µl	1.3536 ng/µl
0.5476 ng/µl	0.24885 ng/µl

Fingerprint	DNA
0.01752 ng/µl	0.0675 ng/µl
0.0315 ng/µl	0.0432 ng/µl
0.12375 ng/µl	0.028 ng/µl
0.0231 ng/µl	0.06035 ng/µl
0.0333 ng/µl	0.0345 ng/µl
0.0657 ng/µl	0.0684 ng/µl
0.05325 ng/µl	0.0925 ng/µl
0.154 ng/µl	0.0324 ng/µl
0.028 ng/µl	0.1584 ng/µl
0.02765 ng/µl	0.02485 ng/µl
0.01775 ng/µl	0.03195 ng/µl
0.1566 ng/µl	0.2808 ng/µl
0.007 ng/µl	0.02925 ng/µl
0.02835 ng/µl	0.3145 ng/µl
0.1224 ng/µl	0.15975 ng/µl
0 ng/µl	0.0152 ng/µl
0.0072 ng/µl	0 ng/µl
0.50765 ng/µl	0.01035 ng/µl
0.0148 ng/µl	0 ng/µl
0.0142 ng/µl	0 ng/µl
0.0432 ng/µl	0.1517 ng/µl
0.0075 ng/µl	0 ng/µl
0.02625 ng/µl	0.01035 ng/µl
0.0925 ng/µl	0.0134 ng/µl
0.00375 ng/µl	0.00325 ng/µl
0 ng/µl	0 ng/µl
0.781 ng/µl	0.16555 ng/µl

Table 2: DNA ANOVA results for first analysis performed. The samples either went through fingerprint enhancement first or DNA analysis first. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint	54	4.68252	0.150405	0.086713		
DNA	54	6.56205	0.257523	0.121519		
					0.393028	Not significant

B)

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint	54	579	9.483683439	10.722222		
DNA	54	546	9.205549324	10.111111		
					0.734698	Not significant

C)

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint	54	864	8.547315589	16		
DNA	54	822	9.248491293	15.222222		
					0.650864	Not significant

D)

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint	54	1549	17.64730358	28.685185		
DNA	54	1480	17.68115778	27.407407		
					0.707762	Not significant

E)

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint	54	4	1.006616823	0.0740741		
DNA	54	-10	0.991931108	-0.185185		
					0.180502	Not significant

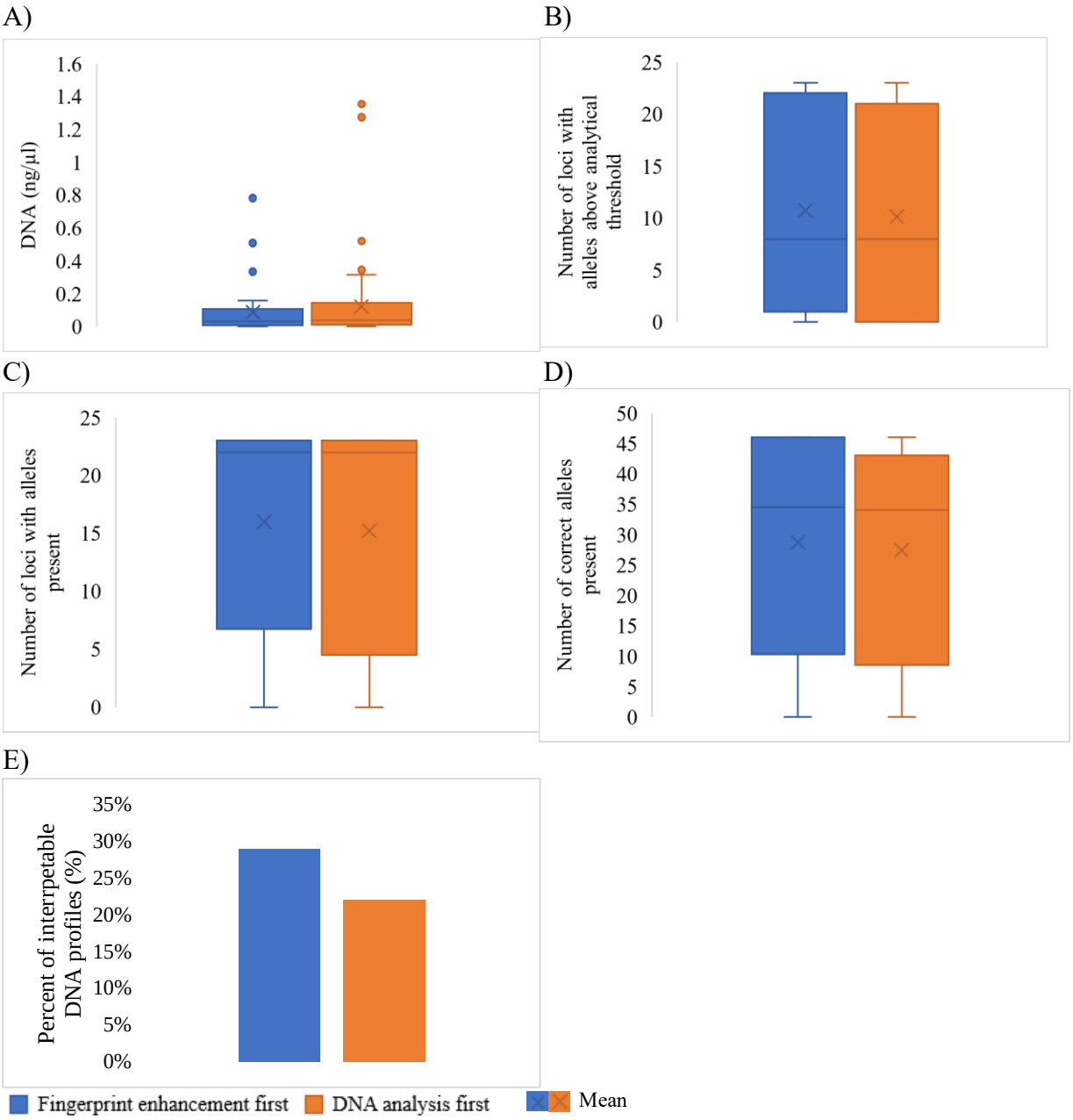

Figure 2: DNA comparison for first analysis performed. The samples either went through fingerprint enhancement first or DNA analysis first. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Both fingerprint enhancement and DNA analysis had 54 samples.

4.1.2. Ninhydrin versus 1,2-indanedione fingerprinting methods

4.1.2.1. DNA concentrations

The DNA concentration for each sample was calculated. When the ninhydrin fingerprint method was used prior to DNA analysis, the mean DNA concentration was 0.067096±0.097144 ng/µl. When the 1,2-indanedione fingerprint method was used prior to DNA analysis, the mean DNA concentration was 0.102407±0.182443 ng/µl. The ANOVA p-value for the comparison of ninhydrin versus 1,2-indanedione fingerprint methods was 0.396417. There was no significant difference in the DNA concentrations between the two fingerprint methods when fingerprint enhancement is performed prior to DNA analysis. There was one outlier for fingerprint enhancement first with ninhydrin and three outliers for fingerprint enhancement first with 1,2-indanedione (Figure 3A). Outliers were determined by using excel® version 2310.

4.1.2.2. Number of loci with called alleles in the DNA profiles

The number of loci that contained a called allele was calculated for each profile. The total number of autosomal loci possible was 23. Fingerprint enhancement with 1,2-indanedeione resulted in the highest mean number of loci with alleles reaching the analytical threshold with a mean of 12.1±8.941766 loci and 20.00% of the samples having all 23 loci with called alleles. Fingerprint enhancement with ninhydrin had a mean of 9.00±10.04338 loci with 25.00% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.23616. The difference in the number of loci with called alleles was not significant when comparing the two fingerprint enhancement methods.

4.1.2.3. Number of loci with alleles present in the DNA profiles

The number of loci with alleles present in the profiles were manually counted. The total number of loci possible was 23. Similar to the results of the number of loci with called alleles, fingerprint enhancement with 1,2-indanedione had a higher mean number of loci with alleles present. 1,2-Indanedione had a mean of 17.76667±7.811059 loci with 43.33% of the samples having all 23 loci with called alleles. Ninhydrin had a mean of 13.79167±9.069678 loci with 33.33% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.089603. There was no significant difference in the number of loci with alleles present in the DNA profiles when comparing the two fingerprint enhancement methods. There were two outliers for fingerprint enhancement first with 1,2-indanedione (Figure 3C). Outliers were determined by using excel® version 2310.

4.1.2.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the DNA profiles were compared to each of the volunteer's reference profiles. The number of correct alleles found in each of the profiles was compared for both the ninhydrin and the 1,2-indandeione fingerprint methods. The 1,2-indanedione fingerprint method had the higher mean of 31.86667±16.65416 alleles with 30.00% of the samples having all alleles present. In comparison, the ninhydrin fingerprint method had a mean of 24.70833±18.39064 alleles with 25.00% of the samples having all alleles present. The ANOVA p-value was 0.140059. This difference in the number of alleles was not significant.

4.1.2.5. Interpretability of the DNA profiles

Each DNA profile was deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile. A "1" was given to profiles that were interpretable. A "-1" was given to profiles that were not interpretable. Fingerprinting with 1,2-indanedione had a higher fingerprint quality than fingerprinting with ninhydrin. The former had a mean of 0.266667±0.980265 DNA profile quality. The latter had a mean of -0.16667±1.00722 DNA profile quality. Ninhydrin had 41.67% of the samples being interpretable and 58% of them uninterpretable. The 1,2-indanedione method had 63.33% of the samples being interpretable and 37% of them uninterpretable. The ANOVA p-value was 0.116857. There was no significant difference in the number of interpretable DNA profiles when comparing ninhydrin and 1,2-indanedione fingerprinting methods.

Table 3: DNA concentrations for fingerprint methods. Comparison of ninhydrin and 1,2-indanedione fingerprint methods when fingerprint enhancement was performed prior to DNA analysis. Ninhydrin had 24 samples while 1,2-indanedione had 30 samples.

Ninhydrin	**1,2-Indanedione**
0.0988 ng/µl	0.07125 ng/µl
0.0036 ng/µl	0.06545 ng/µl
0.0365 ng/µl	0.5476 ng/µl
0.0071 ng/µl	0.01752 ng/µl
0.01875 ng/µl	0.0315 ng/µl
0.1113 ng/µl	0.12375 ng/µl
0.004 ng/µl	0.0231 ng/µl
0.0624 ng/µl	0.0333 ng/µl
0.1425 ng/µl	0.0657 ng/µl
0 ng/µl	0.05325 ng/µl
0.0432 ng/µl	0.154 ng/µl
0.1296 ng/µl	0.028 ng/µl
0.0576 ng/µl	0.02765 ng/µl
0.012 ng/µl	0.01775 ng/µl
0.0222 ng/µl	0.1566 ng/µl
0 ng/µl	0.007 ng/µl
0.03 ng/µl	0.02835 ng/µl
0.1218 ng/µl	0.1224 ng/µl
0 ng/µl	0 ng/µl
0 ng/µl	0.0072 ng/µl
0.35625 ng/µl	0.50765 ng/µl
0.019 ng/µl	0.0148 ng/µl
0 ng/µl	0.0142 ng/µl
0.3337 ng/µl	0.0432 ng/µl
	0.0075 ng/µl
	0.02625 ng/µl
	0.0925 ng/µl
	0.00375 ng/µl
	0 ng/µl
	0.781 ng/µl

Table 4: DNA ANOVA results for fingerprint methods. Comparison of ninhydrin and 1,2-indanedione fingerprint methods when fingerprint enhancement was performed prior to DNA analysis. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Fingerprint method, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Fingerprint						
Ninhydrin	24	1.6103	0.097144199	0.0670958		
1,2-Indanedione	30	3.07222	0.182442955	0.1024073		
					0.396417	Not significant

B)

Fingerprint method, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Fingerprint						
Ninhydrin	24	216	10.04338415	9		
1,2-Indanedione	30	363	8.941765621	12.1		
					0.236159793	Not significant

C)

Fingerprint method, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Fingerprint						
Ninhydrin	24	331	9.069677942	13.791667		
1,2-Indanedione	30	533	7.811059063	17.766667		
					0.089603157	Not significant

D)

Fingerprint method, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Fingerprint						
Ninhydrin	24	593	18.39063837	24.708333		
1,2-Indanedione	30	956	16.65415622	31.866667		
					0.140059214	Not significant

E)

Fingerprint method, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Fingerprint						
Ninhydrin	24	-4	1.00722031	-0.166667		
1,2-Indanedione	30	8	0.980265036	0.2666667		
					0.116857148	Not significant

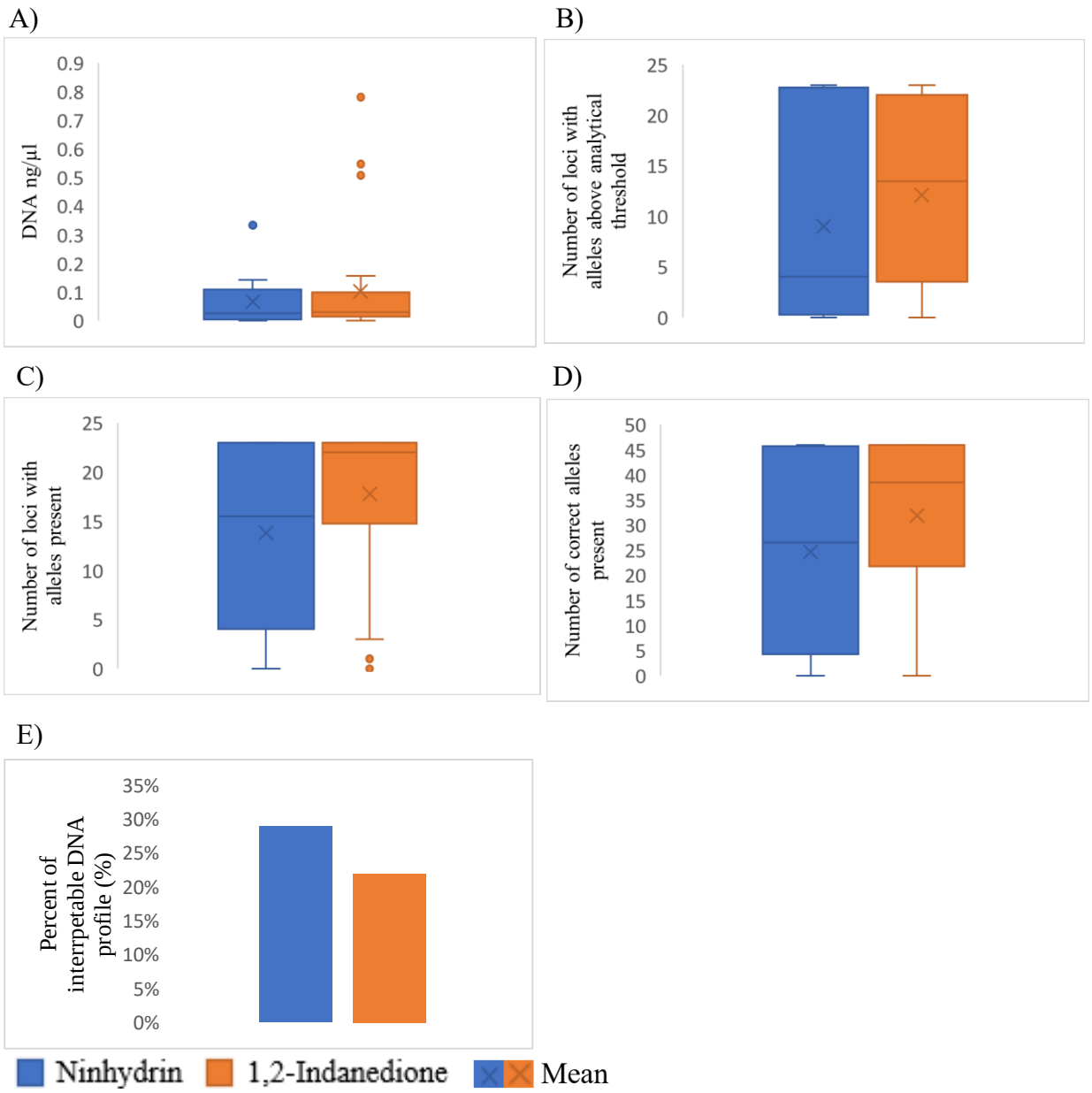

Figure 3: DNA comparisons of fingerprint enhancement methods. Comparison of ninhydrin and 1,2-indanedione fingerprint methods when fingerprint enhancement was performed prior to DNA analysis. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Fingerprint enhancement with ninhydrin had 24 samples while fingerprint enhancement with 1,2-indanedione had 30 samples. The ninhydrin method had less samples because thermal paper cannot undergo ninhydrin fingerprint enhancement method.

4.1.3. Sequence of analysis for money samples

4.1.3.1. DNA concentrations

The DNA concentration for each sample was calculated. The three DNA analysis pathways money went through were: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, and 3) DNA analysis first. There was no significant difference of DNA concentrations when comparing these three analysis pathways. The pathway with the highest mean DNA concentration was DNA analysis first, with a mean DNA concentration of 0.387188±0.456085 ng/µl. Fingerprint enhancement with 1,2-indanedione had a mean DNA concentration of 0.140342±0.203414 ng/µl. Fingerprinting first with ninhydrin method showed the lowest mean DNA concentration of 0.03845±0.035188 ng/µl. The ANOVA p-value was 0.11855. The difference of DNA concentrations for each of the money analysis pathways was not significant. There was one outlier for fingerprint enhancement first with 1,2-indanedione and two outliers for DNA analysis first (Figure 4A). Outliers were determined by using excel® version 2310.

4.1.3.2. Number of loci with called alleles in the DNA profiles

The number of loci that contained a called allele was calculated for each DNA profile. The total number of loci possible was 23. DNA analysis first had the highest mean of 15.66667±8.700401 loci with 33.33% of the samples having all 23 loci with called alleles. Fingerprint enhancement first with 1,2-indanedione had the next highest mean of 15.33333±8.640988 with 33.33% of the samples having all 23 loci with called alleles. Lastly, fingerprint enhancement first with ninhydrin had a mean of 3.5±3.507136 loci with 0.00% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.0123637. There is a

significant difference in the number of loci with called alleles for money when comparing the analysis pathways. The Tukey-Kramer's HSD test was performed to determine which of the comparisons were significantly different than the others. Any Q value above 3.96 was considered significant. The comparison of fingerprint first with ninhydrin versus DNA performed prior to the fingerprint enhancement had a Q value of 4.429475. For money samples DNA analysis first had significantly more loci with called alleles than fingerprint enhancement first with ninhydrin.

4.1.3.3. Number of loci with alleles present in the DNA profiles

The number of loci with alleles present in the DNA profiles were manually counted. While the number of loci with alleles above the analytical threshold had a significant difference, this was not the case for the number of loci with alleles present. The ANOVA p-value was 0.106177. DNA analysis first had the highest mean of 20.25±5.986728 loci with 50.00% of the samples having all 23 loci with alleles present, followed by fingerprinting with 1,2-indanedione method first with a mean of 19±8.854377 loci with 67.00% of the samples having all 23 loci with alleles present. Fingerprint enhancement with ninhydrin method first had the lowest mean of 12.16667±8.376555 loci with 17.00% of the samples having all 23 loci with alleles present. Since the ANOVA p-value was above 0.05 there was no significant difference in the number of loci with alleles present in the DNA profiles for each money analysis pathway. There was one outlier for fingerprint enhancement first with 1,2-indanedione and two outliers for DNA analysis first (Figure 4C). Outliers were determined by using excel® version 2310.

4.1.3.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the DNA profiles were compared to each of the volunteer's reference DNA profiles. The number of correct alleles found in each of the DNA profiles were quantified and the different analysis pathways the money samples went through were compared. The number of correct alleles present on the money samples did not show a significant difference between fingerprint enhancement with ninhydrin prior to DNA analysis, fingerprint enhancement with 1,2-indanedione prior to DNA analysis, and DNA analysis prior to fingerprinting. DNA analysis first had the highest mean of 34.83333±12.22392 alleles with 8.33% of the samples having all alleles present in the DNA profile, followed by fingerprinting with 1,2-indanedione with a mean of 32.33333±16.52473 alleles with 33.33% of the samples having all alleles present in the DNA profile. Fingerprinting with ninhydrin had the lowest mean number of alleles matching the volunteer's DNA profile with a mean of 18.5±12.94218 alleles with 0.00% of the samples having all alleles present in the DNA profile. The ANOVA p-value was 0.070375. There was no significant difference in the number of correct alleles in the DNA profiles for each money analysis pathway. There was one outlier for DNA analysis first (Figure 4D). Outliers were determined by using excel® version 2310.

4.1.3.5. Interpretability of the DNA profiles

Each DNA profile was deemed interpretable or not interpretable based on the degree of the DNA profile mixture and the ability to identify the volunteer's DNA profile in the sample's DNA profile. A "1" was given to DNA profiles that were interpretable. A "-1" was given to DNA profiles that were not interpretable. Fingerprinting with ninhydrin had a mean of -1±0 DNA profile quality with 0% of the samples being interpretable. Fingerprinting with 1,2-indanedione

had a mean DNA quality of -0.66667±0.816497 DNA profile quality. DNA analysis had a mean DNA quality of -0.66667±0.778499 DNA profile quality. For both fingerprinting with 1,2-indanedione first and DNA analysis 16.67% of the samples were interpretable while 83.33% of the samples were not interpretable. The ANOVA p-value was 0.599118. The differences in the DNA profile quality for each of the money analysis pathways were not significant.

Table 5: DNA concentrations for money samples. The three different sequences of analysis for money were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. Both fingerprint enhancement methods had 6 samples while DNA analysis had 12 samples.

Fingerprint ninhydrin	**Fingerprint 1,2-indanedione**	**DNA**
0.0988 ng/µl	0.07125 ng/µl	0.0608 ng/µl
0.0036 ng/µl	0.06545 ng/µl	1.27305 ng/µl
0.0365 ng/µl	0.5476 ng/µl	0.34505 ng/µl
0.0576 ng/µl	0.007 ng/µl	0.0154 ng/µl
0.012 ng/µl	0.02835 ng/µl	0.5206 ng/µl
0.0222 ng/µl	0.1224 ng/µl	0.1575 ng/µl
		0.1679 ng/µl
		1.3536 ng/µl
		0.24885 ng/µl
		0.02925 ng/µl
		0.3145 ng/µl
		0.15975 ng/µl

Table 6: DNA ANOVA results for money samples. The three different sequences of analysis for money were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Money, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	0.2307	0.035187597	0.03845		
1,2-Indanedione	6	0.84205	0.203413769	0.1403417		
DNA	12	4.64625	0.456084707	0.3871875		
					0.11855	Not significant

B)

Money, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	21	3.507135583	3.5		
1,2-Indanedione	6	92	8.640987598	15.333333		
DNA	12	188	8.700400548	15.666667		
					0.012637	Significant

C)

Money, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	73	8.376554582	12.166667		
1,2-Indanedione	6	114	8.854377448	19		
DNA	12	243	5.986727745	20.25		
					0.106177	Not significant

D)

Money, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	111	12.94217911	18.5		
1,2-Indanedione	6	194	16.52472894	32.333333		
DNA	12	418	12.22392091	34.833333		
					0.070375	Not significant

E)

Money, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	-6	0	-1		
1,2-Indanedione	6	-4	0.816496581	-0.666667		
DNA	12	-8	0.778498944	-0.666667		
					0.599118	Not significant

Table 7: DNA Tukey-Kramer's HSD test results for money samples. The three different sequences of analysis for money were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. Comparison of the number of loci with called alleles in the DNA profiles for each sequence of analysis.

Money first sequence comparison	**Absolute difference**	**Standard error**	**Q Tukey**	**Q critical**	**Significance**
Ninhydrin vs. 1,2-indanedione	11.83333333	3.171675237	3.730941	3.96	Not significant
Ninhydrin vs. DNA	12.16666667	2.746751328	4.429475	3.96	Significant
1,2-indanedione vs. DNA	0.333333333	2.746751328	0.121355	3.96	Not significant

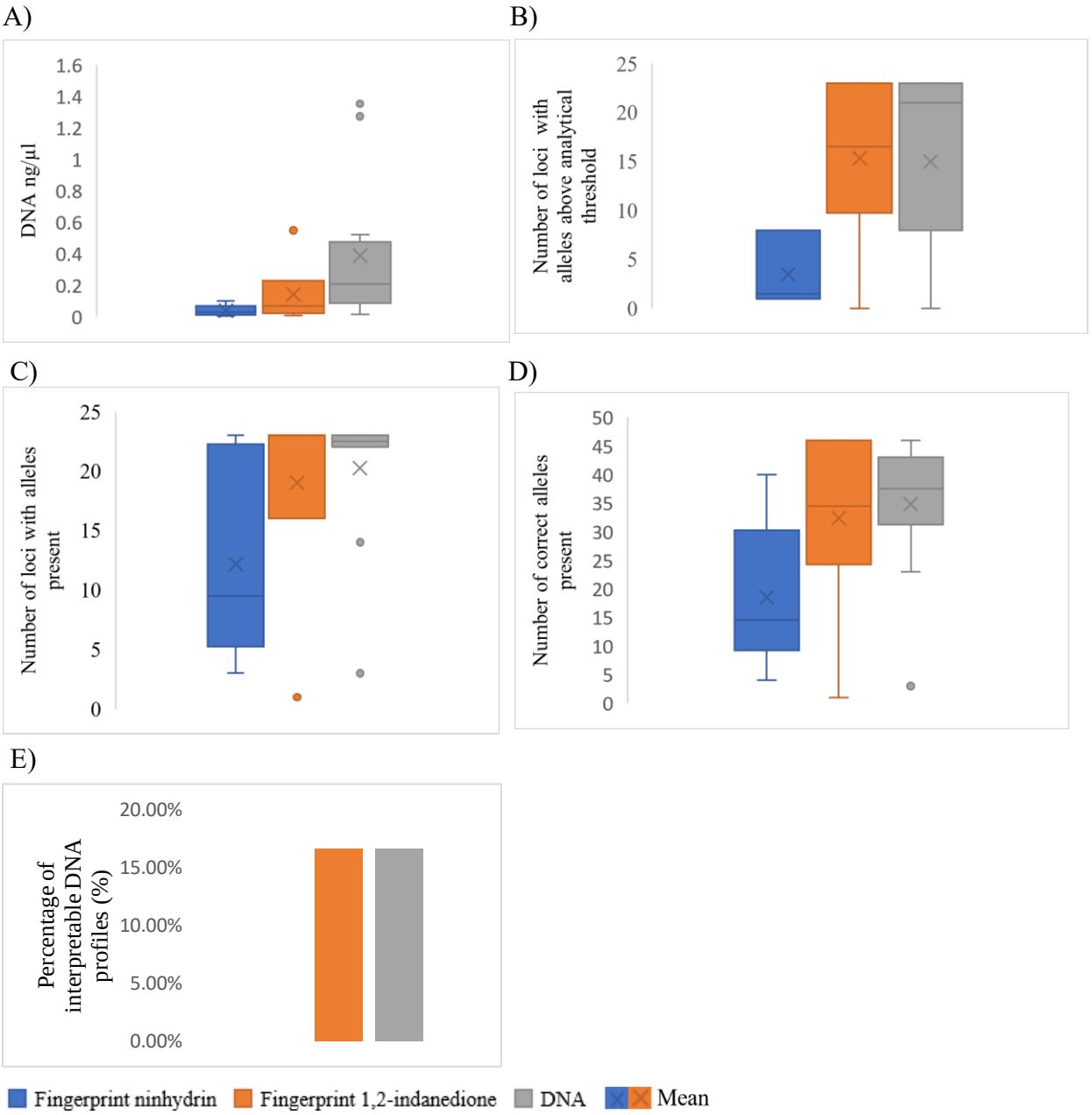

Figure 4: DNA comparison for money samples. The three different sequences of analysis for money were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Both fingerprinting with ninhydrin and fingerprinting with 1,2-indanedione had 6 samples while DNA prior to fingerprint enhancement had 12 samples.

4.1.4. Sequence of analysis for copy paper samples

4.1.4.1. DNA concentrations

The DNA concentration for each sample was calculated. The three pathways the copy paper went through for DNA analysis were: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, and 3) DNA analysis first. The DNA concentrations of these three pathways of analysis were compared. There was no significant difference between the three pathways tested on copy paper. Fingerprinting with 1,2-indanedione prior to DNA analysis showed the highest mean DNA concentration of 0.114603±0.197773 ng/µl. Fingerprinting with ninhydrin prior to DNA analysis had the second highest mean DNA concentration of 0.048158±0.054055 ng/µl. DNA analysis prior to fingerprinting analysis showed the lowest mean DNA concentration of 0.026779±0.031252 ng/µl. The ANOVA p-value was 0.250503. The difference in the DNA concentrations was not significant for each of the copy paper analysis pathways. There was one outlier for DNA analysis first (Figure 5A). Outliers were determined by using excel® version 2310.

4.1.4.2. Comparing the number of loci with called alleles in the DNA profiles

The number of loci that contained a called allele was calculated for each DNA profile. The total number of autosomal loci was 23. For the copy paper samples there was no significant difference between the different analysis pathways when examining the number of loci that have called alleles by GeneMapper software. This was indicated by the ANOVA p-value of 0.556644. Fingerprinting with 1,2-indanedione had the highest mean number of loci of 9.666667±10.32796 loci with 16.67% of the samples having all 23 loci with called alleles, followed by fingerprint enhancement first with ninhydrin had a mean of 9.166667±10.9438 loci with 33.33% of the

samples having all 23 loci with called alleles. Finally, DNA analysis first had a mean of 5.5±6.640099 loci with 0.00% of the samples having all 23 loci with called alleles. There was one outlier for DNA analysis first (Figure 5B). Outliers were determined by using excel® version 2310.

4.1.4.3. Number of loci with alleles present in the DNA profiles

The number of loci with alleles present in the DNA profiles were manually counted. The total number of loci possible was 23. Fingerprinting first with 1,2-indanedione had the highest mean of 15.5±9.20326 loci with 33.33% of the samples having all 23 loci with called alleles. The second highest was fingerprint enhancement first with ninhydrin with a mean of 14.66667±10.13246 loci with 33.33% of the samples having all 23 loci with called alleles. DNA analysis first had the lowest mean of 11.16667±9.183318 mean loci with 16.67% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.596234. The difference in the number of loci with alleles present in the DNA profiles for each of the copy paper analysis pathways was not significant.

4.1.4.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the DNA profiles were compared to each of the volunteer's reference DNA profiles. The number of correct alleles found in each of the DNA profiles were quantified and the different analysis pathways the copy paper samples went through were compared. Fingerprinting with 1,2-indanedione had the highest mean value of correct alleles present in the copy paper DNA profiles. The mean value was 28.16667±19.70195 alleles with 33.33% of the samples having all alleles present in the DNA profiles. Fingerprinting with

ninhydrin showed the next highest mean of 26.16667±19.78299 alleles with 16.67% of the samples having all alleles present in the DNA profiles. The lowest value was DNA prior to fingerprinting with a mean of 20.08333±16.00828 alleles with 8.33% of the samples having all alleles present in the DNA profiles. The ANOVA p-value was 0.619827. There was no significant difference in the number of alleles that matched the volunteer's DNA profiles for each of the copy paper analysis pathways.

4.1.4.5. Interpretability of the DNA profiles

Each DNA profile was deemed interpretable or not interpretable based on the degree of the DNA profile mixture and the ability to identify the volunteer's DNA profile in the sample's DNA profile. A "1" was given to DNA profiles that were interpretable. A "-1" was given to DNA profiles that were not interpretable. Copy paper showed slightly better results than money when comparing the DNA profile quality. Both fingerprinting with ninhydrin and fingerprinting with 1,2-indanedione had a mean of 0.333333±1.032796 DNA profile quality. They both had 66.67% of their samples interpretable while 33.33% of them were uninterpretable. DNA analysis prior to fingerprinting had a mean of -0.5±0.904534 DNA profile quality with 25% of the samples being interpretable and 75% of them uninterpretable. The ANOVA p-value was 0.132964. There was no significant difference in the DNA profile quality for each of the copy paper analysis pathways.

Table 8: DNA concentrations for copy paper samples. The three different sequences of analysis for copy paper were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. Both fingerprint enhancement methods had 6 samples while DNA analysis had 12 samples.

Fingerprint ninhydrin	**Fingerprint 1,2-indanedione**	**DNA**
0.0071 ng/µl	0.01752 ng/µl	0.01155 ng/µl
0.01875 ng/µl	0.0315 ng/µl	0 ng/µl
0.1113 ng/µl	0.12375 ng/µl	0.1026 ng/µl
0 ng/µl	0 ng/µl	0.01095 ng/µl
0.03 ng/µl	0.0072 ng/µl	0 ng/µl
0.1218 ng/µl	0.50765 ng/µl	0.032 ng/µl
		0.0675 ng/µl
		0.0432 ng/µl
		0.028 ng/µl
		0.0152 ng/µl
		0 ng/µl
		0.01035 ng/µl

Table 9: DNA ANOVA results for copy paper samples. The three different sequences of analysis for copy paper were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Copy paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	0.28895	0.054054698	0.0481583		
1,2-Indanedione	6	0.68762	0.197772638	0.1146033		
DNA	12	0.32135	0.03125152	0.0267792		
					0.250503	Not significant

B)

Copy paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	55	10.94379581	9.1666667		
1,2-Indanedione	6	58	10.32795559	9.6666667		
DNA	12	66	6.640098575	5.5		
					0.556644	Not significant

C)

Copy paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	88	10.1324561	14.666667		
1,2-Indanedione	6	93	9.203260292	15.5		
DNA	12	134	9.183318209	11.166667		
					0.596234	Not significant

D)

Copy paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	157	19.78298933	26.166667		
1,2-Indanedione	6	169	19.70194576	28.166667		
DNA	12	241	16.00828384	20.083333		
					0.619827	Not significant

E)

Copy paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	2	1.032795559	0.3333333		
1,2-Indanedione	6	2	1.032795559	0.3333333		
DNA	12	-6	0.904534034	-0.5		
					0.132964	Not significant

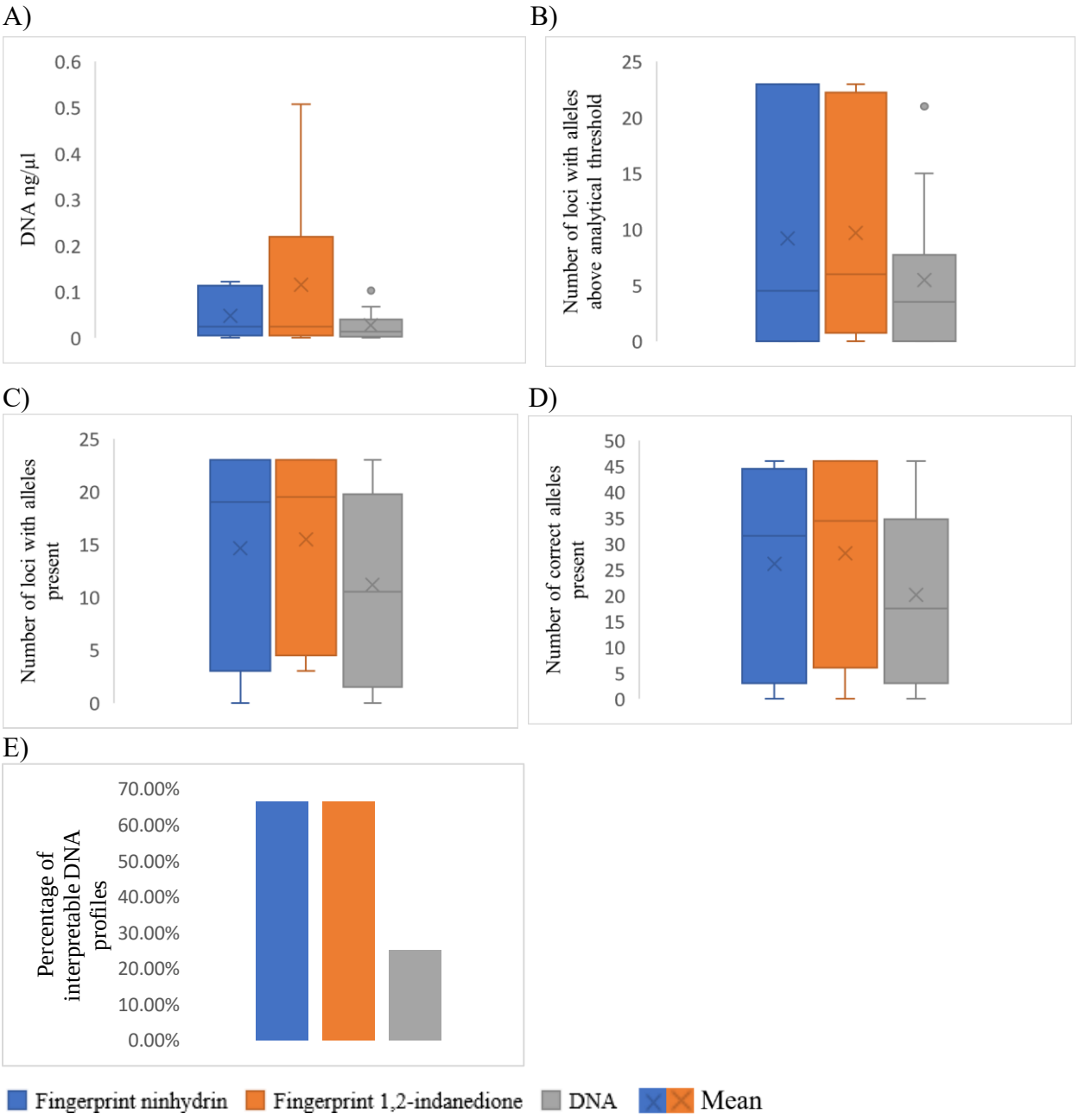

Figure 5: DNA comparison for copy paper samples. The three different sequences of analysis for copy paper were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Both fingerprinting with ninhydrin and fingerprinting with 1,2-indanedione had 6 samples while DNA prior to fingerprint enhancement had 12 samples.

4.1.5. Sequence of analysis for cardboard samples

4.1.5.1. DNA concentrations

The DNA concentration for each sample was calculated. The three pathways the cardboard went through for DNA analysis were: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, and 3) DNA analysis first. Fingerprinting with ninhydrin prior to DNA analysis showed the highest mean DNA concentration of 0.094192±0.139927 ng/µl. DNA analysis prior to fingerprinting analysis showed the second highest mean DNA concentration of 0.047121±0.044188 ng/µl. Fingerprinting with 1,2-indanedione prior to DNA analysis had the lowest mean DNA concentration of 0.032383±0.019768 ng/µl. The ANOVA p-value was 0.342661. The difference in the DNA concentrations for each of the copy paper analysis pathways was not significant.

4.1.5.2. Number of loci with called alleles in the DNA profiles

The number of loci that contained a called allele was calculated for each DNA profile. The total number of autosomal loci possible was 23. Cardboard showed no significant differences in the number of loci with called alleles by GeneMapper for each of the cardboard analysis pathways. The ANOVA p-value of 0.829985. Fingerprinting with the 1,2-indanedione method showed the highest mean loci value of 13.66667±8.041559 loci with 16.67% of the samples having all 23 loci with called alleles. The next highest was fingerprinting with ninhydrin with a mean of 11.5±12.24337 loci with 33.33% of the samples having all 23 loci with called alleles followed by DNA analysis first with a mean of 10.66667±9.267081 loci with 16.67% of the samples having all 23 loci with called alleles.

4.1.5.3. Number of loci with alleles present in the DNA profiles

The number of loci with alleles present in the DNA profiles were manually counted. The total number of loci possible was 23. When comparing the number of loci with alleles present, fingerprint analysis with 1,2-indanedione first showed the highest mean loci of 21.16667±3.544949 with 50.00% of the samples having all 23 loci with called alleles, followed by DNA analysis first with a mean of 16.41667±8.371579 with 58.33% of the samples having all 23 loci with called alleles. Lastly fingerprinting with ninhydrin had a mean of 13.66667±10.3473 loci with 50.00% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.283276. There was no significant difference between the number of loci with alleles present in the DNA profiles for each of the copy paper analysis pathways. There was one outlier for fingerprint enhancement first with 1,2-indanedione (Figure 6C). Outliers were determined by using excel® version 2310.

4.1.5.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the DNA profiles were compared to each of the volunteer's reference DNA profiles. The number of correct alleles found in each DNA profile was quantified and the different analysis pathways the cardboard samples went through were compared. Fingerprinting first with 1,2-indanedione had the highest mean of 38.16667±8.727352 alleles with 16.67% of the samples having all alleles present in the DNA profile. DNA analysis had the next highest mean of 29.75±16.4876 alleles with 8.33% of the samples having all alleles present in the DNA profile. Lastly, fingerprinting with ninhydrin prior to DNA analysis had the lowest mean of 26±21.77154 alleles with 33.33% of the samples having all alleles present in the DNA profile. The ANOVA p-value for these three categories was 0.432738. There was no significant

difference in the number of alleles that matched the volunteer's DNA profiles for each of the copy paper analysis pathways. There was one outlier for fingerprint enhancement first with 1,2-indanedione (Figure 6D). Outliers were determined by using excel® version 2310.

4.1.5.5. Interpretability of the DNA profiles

Each profile was deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile in the sample's profile. A "1" was given to profiles that were interpretable. A "-1" was given to profiles that were not interpretable. When comparing the quality of the DNA profiles for the different analysis pathways the cardboard samples underwent, fingerprinting with 1,2-indanedione had the highest quality. The quality was 0.666667±0.816497 DNA profile quality with 83.33% of the samples being interpretable and 16.67% of them being uninterpretable. DNA analysis first had the next highest with a mean quality of 0.1666667±1.029857 DNA profile quality with 58.33% of the samples being interpretable and 41.67% of them uninterpretable. Fingerprinting with ninhydrin had the lowest mean quality of 0.0±1.095445 DNA profile quality with 50% of the samples being interpretable and 50% of them uninterpretable. The ANOVA p-value of 0.484603. The differences in the quality of DNA profiles for each cardboard analysis pathways were not significant.

Table 10: DNA concentrations for cardboard samples. The three different sequences of analysis for cardboard were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. Both fingerprint enhancement methods had 6 samples while DNA analysis had 12 samples.

Fingerprint ninhydrin	**Fingerprint 1,2 indanedione**	**DNA**
0.004 ng/µl	0.0231 ng/µl	0.0504 ng/µl
0.0624 ng/µl	0.0333 ng/µl	0.06715 ng/µl
0.1425 ng/µl	0.0657 ng/µl	0.04485 ng/µl
0 ng/µl	0.0148 ng/µl	0 ng/µl
0 ng/µl	0.0142 ng/µl	0.0076 ng/µl
0.35625 ng/µl	0.0432 ng/µl	0.0805 ng/µl
		0.06035 ng/µl
		0.0345 ng/µl
		0.0684 ng/µl
		0 ng/µl
		0 ng/µl
		0.1517 ng/µl

Table 11: DNA ANOVA results for cardboard samples. The three different sequences of analysis for cardboard were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Cardboard, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	0.56515	0.139926875	0.0941917		
1,2-Indanedione	6	0.1943	0.019767794	0.0323833		
DNA	12	0.56545	0.044187783	0.0471208		
					0.342661	Not significant

B)

Cardboard, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	69	12.24336555	11.5		
1,2-Indanedione	6	82	8.041558721	13.666667		
DNA	12	128	9.267080871	10.666667		
					0.829985	Not significant

C)

Cardboard, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	82	10.34730239	13.666667		
1,2-Indanedione	6	127	3.544949459	21.166667		
DNA	12	197	8.371578903	16.416667		
					0.283276	Not significant

D)

Cardboard, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	156	21.77154106	26		
1,2-Indanedione	6	229	8.727351641	38.166667		
DNA	12	357	16.48759865	29.75		
					0.432738	Not significant

E)

Cardboard, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	0	1.095445115	0		
1,2-Indanedione	6	4	0.816496581	0.6666667		
DNA	12	2	1.029857301	0.1666667		
					0.484603	Not significant

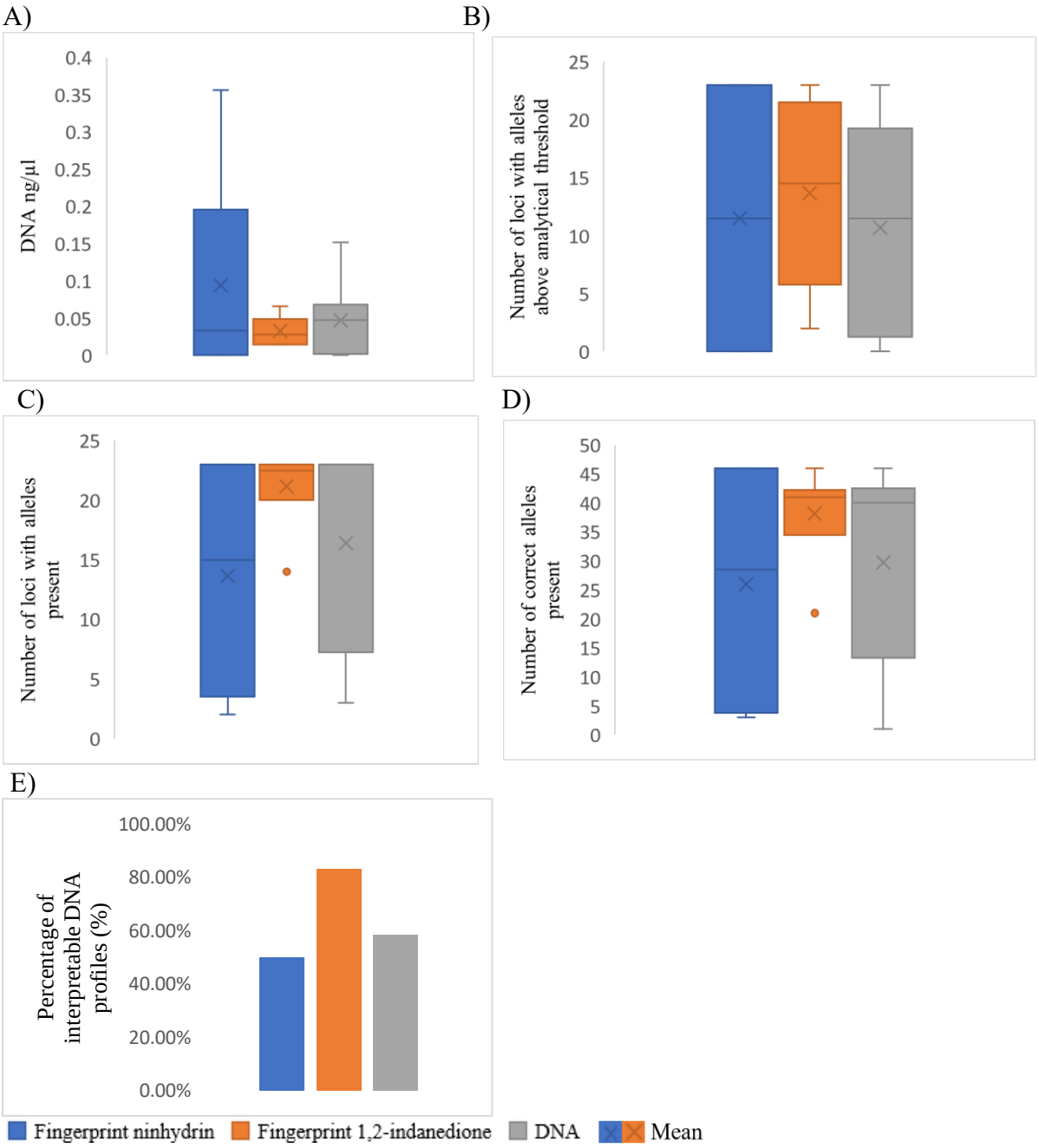

Figure 6: DNA comparison for cardboard samples. The three different sequences of analysis for cardboard were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Both fingerprinting with ninhydrin and fingerprinting with 1,2-indanedione had 6 samples while DNA prior to fingerprint enhancement had 12 samples.

4.1.6. Sequence of analysis for cardstock samples

4.1.6.1. DNA concentrations

The DNA concentration for each sample was calculated. The three pathways the cardboard went through for DNA analysis were: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, and 3) DNA analysis first. The DNA concentrations of these three pathways of analysis were compared. Fingerprinting with ninhydrin prior to DNA analysis showed the highest mean DNA concentration of 0.087583±0.129888 ng/µl. The second highest mean DNA concentration was fingerprinting with 1,2-indanedione prior to DNA analysis with a mean DNA concentration of 0.06025±0.05451 ng/µl. The lowest mean DNA concentration was DNA analysis prior to fingerprinting analysis with a mean value of 0.04355±0.049579 ng/µl. The ANOVA p-value was 0.533957. There was no significant difference in the DNA concentrations for each of the cardstock analysis pathways.

4.1.6.2. Number of loci with called alleles in the DNA profiles

The number of loci that contained a called allele was calculated for each profile. The total number of autosomal loci possible was 23. There was no significant difference in the number of loci with called alleles in the DNA profile for each of the cardstock analysis pathways when comparing fingerprint analysis first with ninhydrin, fingerprint analysis first with 1,2-indanedione, and DNA analysis first. The ANOVA p-value was 0.830044. Tukey-Kramer's HSD test was not necessary, and the differences in the number of loci with called alleles was not significant. Fingerprinting first with 1,2-indanedione showed the highest mean loci of 12±8.966605 loci with 0.00% of the samples having all 23 loci with called alleles, followed by fingerprinting analysis first with ninhydrin with a mean of 11.83333±11.33872 loci with 33.33%

of the samples having all 23 loci with called alleles. DNA analysis first showed the lowest mean loci of 9.416667±9.792932 loci with 8.33% of the samples having all 23 loci with called alleles.

4.1.6.3. Number of loci with alleles present in the DNA profiles

The number of loci with alleles present in the profile were manually counted. Similar to the number of loci with alleles marked by GeneMapper, the number of loci with alleles present in the profile follow the same trend of fingerprinting with 1,2-indanedione having the highest mean of 18±7.949843 loci with 33.33% of the samples having all 23 loci with called alleles, followed by fingerprinting with ninhydrin first with a mean of 14.66667±9.667816 loci with 33.33% of the samples having all 23 loci with called alleles. Lastly, DNA analysis had the lowest mean of 12.83333±10.64154 loci with 33.33% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.583451. The difference in the number of loci with alleles present in the profile for each of the copy paper analysis pathways was not significant.

4.1.6.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the profile were compared to each of the volunteer's reference profiles. The number of correct alleles found in each of the profiles were quantified and the different analysis pathways the cardstock samples went through compared. Fingerprinting first with 1,2-indanedione had the highest mean of 34.83333±17.38294 alleles with 33.33% of the samples having all alleles present in the profile. Fingerprinting with ninhydrin had the next highest mean of 28.16667±21.39548 alleles with 50.00% of the samples having all alleles present in the profile. Lastly, DNA analysis prior to fingerprinting had the lowest mean of 23.83333±21.3023 alleles with 16.67% of the samples having all alleles present in the profile.

The ANOVA p-value for these three categories was 0.568371. There was no significant difference in the number of alleles that matched the volunteer's profile for each of the cardstock analysis pathways.

4.1.6.5. Interpretability of the DNA profiles

Each profile was deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile. A "1" was given to profiles that were interpretable. A "-1" was given to profiles that were not interpretable. Fingerprinting with 1,2-indanedione had the highest quality of 0.666667±0.816497 DNA profile quality with 83.33% of the samples being interpretable and 16.67% of them uninterpretable. DNA analysis first had the next highest with a mean DNA profile quality of 0.166667±1.029857 DNA profile quality with 58.33% of the samples being interpretable and 41.67% of them uninterpretable. Fingerprinting with ninhydrin had the lowest mean quality of 0.0±1.095445 DNA profile quality with 50.0% of the samples being interpretable and 50.0% of them uninterpretable. The ANOVA p-value was 0.484603. There was no significant difference in the DNA profile quality for each of the cardstock analysis pathways.

Table 12: DNA concentrations for cardstock samples. The three different sequences of analysis for cardstock were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. Both fingerprint enhancement methods had 6 samples while DNA analysis had 12 samples.

Fingerprint ninhydrin	Fingerprint 1,2-indanedione	DNA
0 ng/µl	0.05325 ng/µl	0.0266 ng/µl
0.0432 ng/µl	0.154 ng/µl	0.0406 ng/µl
0.1296 ng/µl	0.028 ng/µl	0.10585 ng/µl
0.019 ng/µl	0.0075 ng/µl	0.008 ng/µl
0 ng/µl	0.02625 ng/µl	0 ng/µl
0.3337 ng/µl	0.0925 ng/µl	0.0345 ng/µl
		0.0925 ng/µl
		0.0324 ng/µl
		0.1584 ng/µl
		0 ng/µl
		0.01035 ng/µl
		0.0134 ng/µl

Table 13: DNA ANOVA results for cardstock samples. The three different sequences of analysis for cardstock were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Cardstock, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	0.5255	0.129887681	0.0875833		
1,2-Indanedione	6	0.3615	0.054510091	0.06025		
DNA	12	0.5226	0.049578602	0.04355		
					0.533957	Not significant

B)

Cardstock, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	71	11.33872421	11.833333		
1,2-Indanedione	6	72	8.966604709	12		
DNA	12	113	9.792931898	9.4166667		
					0.830044	Not significant

C)

Cardstock, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	88	9.667816024	14.666667		
1,2-Indanedione	6	108	7.949842766	18		
DNA	12	154	10.64154238	12.833333		
					0.583451	Not significant

D)

Cardstock, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	169	21.39548239	28.166667		
1,2-Indanedione	6	209	17.38294183	34.833333		
DNA	12	286	21.3022975	23.833333		
					0.568371	Not significant

E)

Cardstock, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	0	1.095445115	0		
1,2-Indanedione	6	4	0.816496581	0.6666667		
DNA	12	2	1.029857301	0.1666667		
					0.484603	Not significant

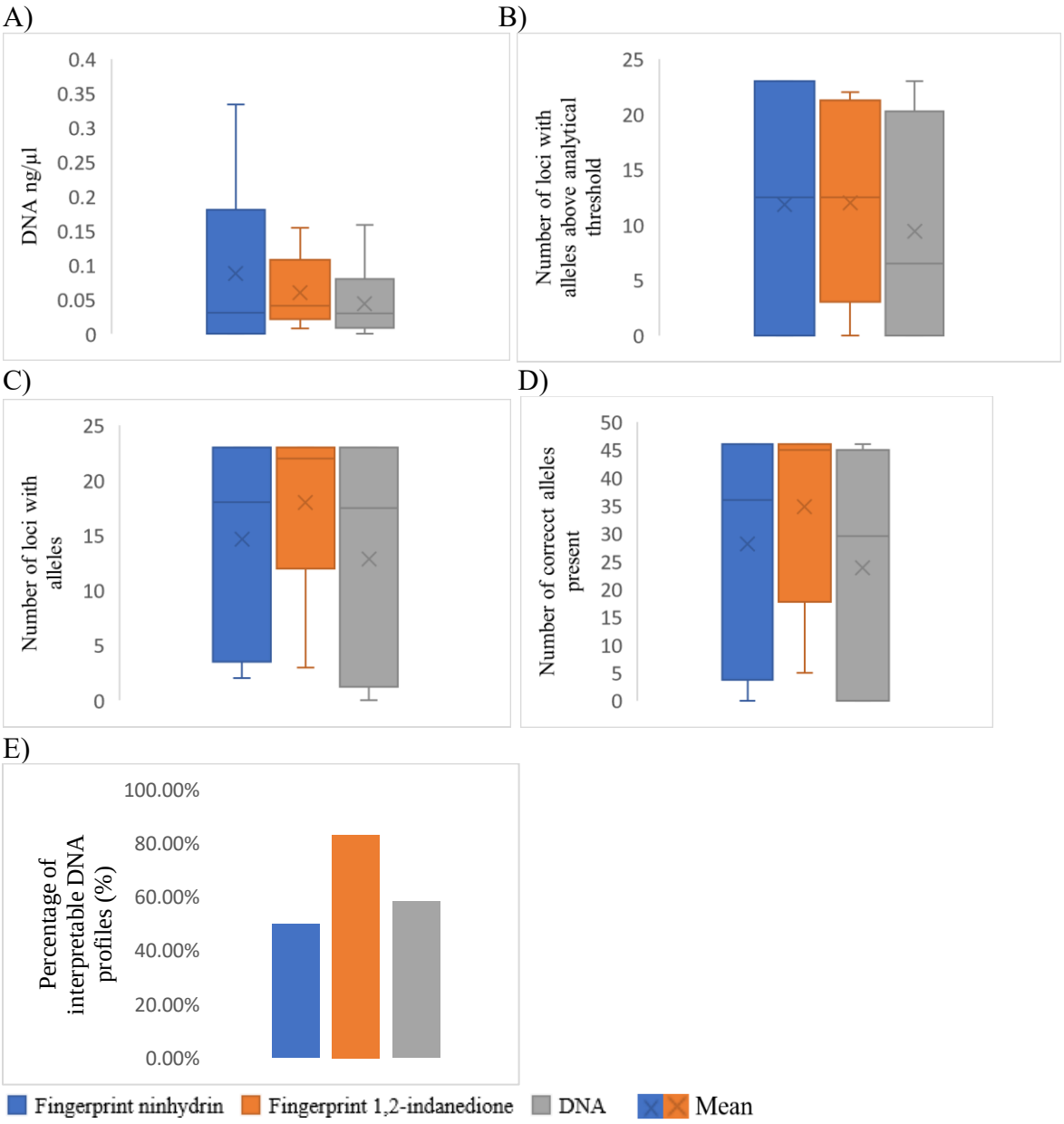

Figure 7: DNA comparison for cardstock samples. The three different sequences of analysis for cardstock were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, and 3) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Both fingerprinting with ninhydrin and fingerprinting with 1,2-indanedione had 6 samples while DNA prior to fingerprint enhancement had 12 samples.

4.1.7. Sequence of analysis for thermal paper samples

4.1.7.1. DNA concentrations

The DNA concentration for each sample was calculated. Thermal paper did not go through the ninhydrin fingerprinting methods. The only methods being compared here are fingerprinting with 1-2-indanedione prior to DNA analysis and DNA analysis prior to fingerprint analysis. Fingerprint enhancement first with 1,2-indanedione had a higher mean DNA concentration of 0.164458±0.307668 ng/µl. DNA analysis prior to fingerprint analysis had a mean DNA concentration of 0.0844±0.114161 ng/µl. The ANOVA p-value was 0.563413. There was no significant difference of DNA concentrations for the two thermal paper analysis pathways. There was one outlier for fingerprint enhancement first with 1,2-indanedione (Figure 8A). Outliers were determined by using excel® version 2310.

4.1.7.2. Number of loci with called alleles in the DNA profiles

The number of loci that contained a called allele was calculated for each profile. The total number of autosomal loci possible was 23. Thermal paper did not go through the ninhydrin fingerprinting methods. The only methods being compared here were fingerprinting with 1,2-indanedione prior to DNA analysis, and DNA analysis prior to fingerprint analysis. The number of loci with alleles above the analytical threshold showed no difference between the two thermal paper analysis pathways. The ANOVA p-value was 0.725144. DNA analysis first had a higher mean of 12±10.33441 loci with 33.33% of the samples having all 23 loci with called alleles. Fingerprint analysis first with 1,2-indanedione had a mean of 9.833333±10.41953 loci with 33.33% of the samples having all 23 loci with called alleles.

4.1.7.3. Number of loci with alleles present in the DNA profiles

The number of loci with alleles present in the profile were manually counted. The only methods being compared here are fingerprinting with 1-2-indanedione prior to DNA analysis and DNA analysis prior to fingerprint analysis. The number of loci present in the profile followed the same trend as the number of loci with called alleles by GeneMapper software. DNA analysis first then fingerprint enhancement had a higher mean of 15.66667±11.02119 loci with 50.00% of the samples having all 23 loci with called alleles. Fingerprinting first with 1,2-indanedione had a lower mean of 15.16667±9.217737 loci with 33.33% of the samples having all 23 loci with called alleles. ANOVA p-value was 0.933751. There was no significant difference in the number of loci with alleles present in the profile for the two thermal paper analysis pathways.

4.1.7.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the profile were compared to each of the volunteer's reference profiles. The number of correct alleles found in each of the profiles were quantified and the different analysis pathways the thermal paper samples went through compared. When comparing the number of alleles that matched the volunteers' profiles for the two categories on thermal paper, DNA prior to fingerprinting had the highest mean of 29.66667±22.66863 alleles with 33.33% of the samples having all alleles present in the profile. Fingerprint enhancement first with 1,2-indanedione had a mean of 25.83333±21.22656 alleles with 33.33% of the samples having all alleles present in the profile. The ANOVA p-value was 0.768577. There was no significant difference in the number of alleles that matches the volunteer's reference profile for each of the thermal paper analysis pathways.

4.1.7.5. Interpretability of the DNA profiles

Each profile was deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile in the sample's profile. A "1" was given to profiles that were interpretable. A "-1" was given to profiles that were not interpretable. Fingerprinting with 1,2-indanedione had a higher DNA profile quality than DNA analysis had on thermal paper. The fingerprint enhancement with 1,2-indanedione had a mean quality of 0.333333±1.032796 DNA profile quality with 66.67% of the samples being interpretable and 33.33% of them uninterpretable. The DNA analysis prior to fingerprinting had a mean quality of 0.0±1.095445 DNA profile quality with 50% of the samples being interpretable and 50% of them uninterpretable. There was no significant difference in the DNA profile quality for the two thermal paper analysis pathways due to the ANOVA p-value being 0.59947.

Table 14: DNA concentrations for thermal paper samples. The two different sequences of analysis for thermal paper were as follows: 1) fingerprint enhancement first with 1,2-indanedione, and 2) DNA analysis first and then fingerprint enhancement. Both sequences had 6 samples.

Fingerprint	**DNA**
0.02765 ng/µl	0.02485 ng/µl
0.01775 ng/µl	0.03195 ng/µl
0.1566 ng/µl	0.2808 ng/µl
0.00375 ng/µl	0.00325 ng/µl
0 ng/µl	0 ng/µl
0.781 ng/µl	0.16555 ng/µl

Table 15: DNA ANOVA results for thermal paper samples. The two different sequences of analysis for thermal paper were as follows: 1) fingerprint enhancement first with 1,2-indanedione, and 2) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Thermal paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
1,2-Indanedione	6	0.98675	0.307668311	0.1644583		
DNA	6	0.5064	0.114161438	0.0844		
					0.563413	Not significant

B)

Thermal paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin	6	59	10.41953294	9.8333333		
1,2-Indanedione	6	72	10.33440855	12		
DNA					0.725144	Not significant

C)

Thermal paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
1,2-Indanedione	6	91	9.217736526	15.166667		
DNA	6	94	11.02119171	15.666667		
					0.933751	Not significant

D)

Thermal paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
1,2-Indanedione	6	155	21.22655569	25.833333		
DNA	6	178	22.66862737	29.666667		
					0.768577	Not significant

E)

Thermal paper, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
1,2-Indanedione	6	2	1.032795559	0.3333333		
DNA	6	0	1.095445115	0		
					0.59947	Not significant

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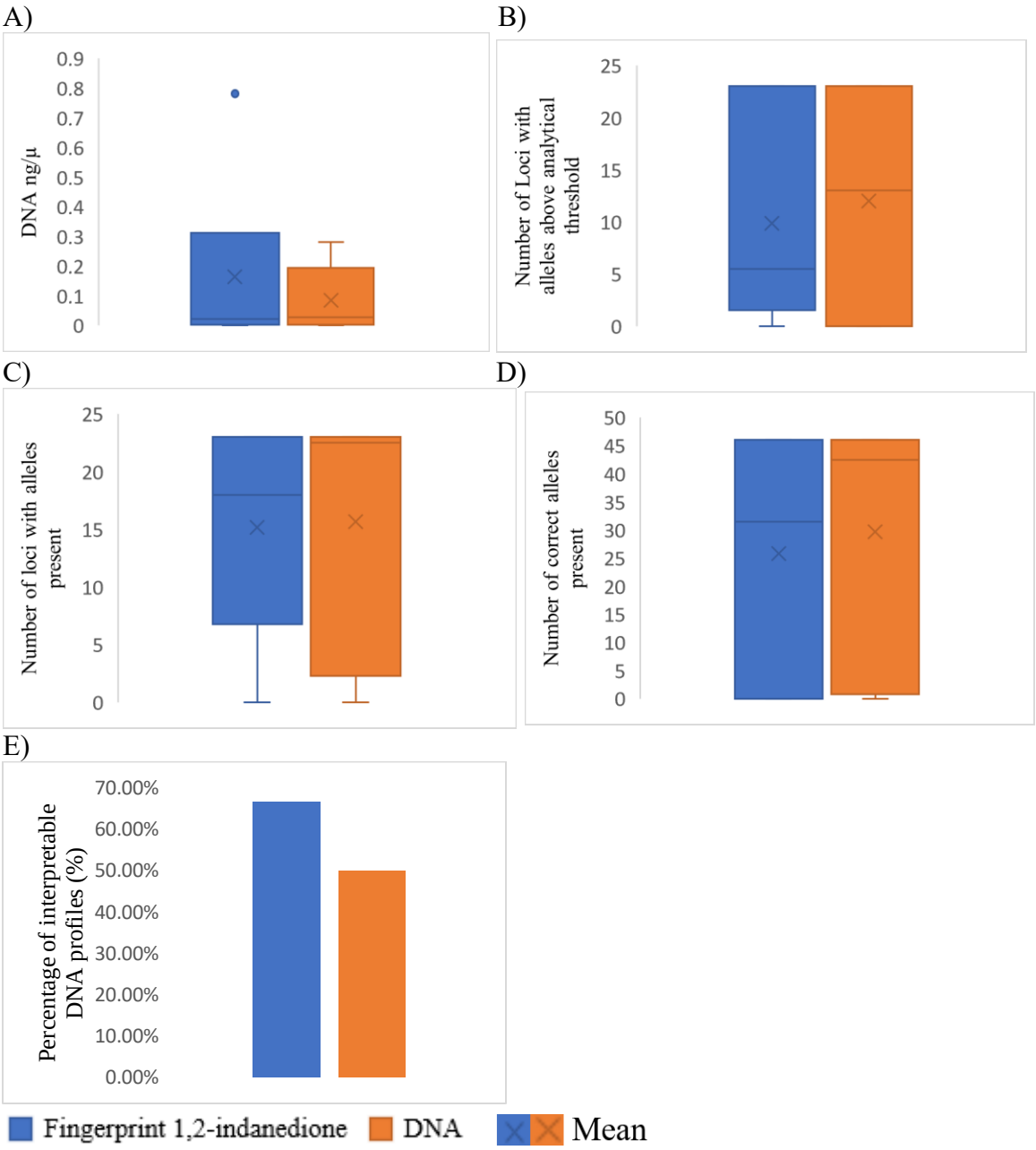


Figure 8: DNA comparison for thermal paper samples. The two different sequences of analysis for thermal paper were as follows: 1) fingerprint enhancement first with 1,2-indanedione, and 2) DNA analysis first and then fingerprint enhancement. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Both fingerprinting with 1,2-indanedione and DNA prior to fingerprint enhancement had 6 samples.

4.1.8. Paper substrates

4.1.8.1. DNA concentrations

The DNA concentrations for each of the paper substrates were calculated. Money had the highest mean DNA concentration of 0.238292±0.0365016 ng/µl. Thermal paper had the second highest mean DNA concentration of 0.105663± 0.225165 ng/µl followed by cardstock 0.058733±0.076335 ng/µl, cardboard 0.055204±0.076431 ng/µl, and lastly copy paper 0.05408±0.104685 ng/µl. When comparing the different paper substrate's DNA concentrations, the ANOVA p-value was 0.006211. There was a significant difference between the paper substrates. The Tukey-Kramer's HSD test was performed to test which paper substrates were significantly different. A Q value above 3.98 was considered significant. Only comparisons with money had significant values: money versus copy paper (Q value of 4.552772), money versus cardboard (Q value of 4.524989), and money versus cardstock (Q value of 4.437766). Money had significantly higher DNA concentrations than copy paper, cardboard, and cardstock. All other paper substrate comparisons showed a Q value lower than 3.98 meaning their DNA concentration differences were not significant. There were two outliers for money, one outlier for copy paper, one outlier for cardboard, one outlier for cardstock, and one outlier for thermal paper (Figure 9A). Outliers were determined by using excel® version 2310.

4.1.8.2 Number of loci with called alleles in the DNA profiles

The number of loci that had alleles above the analytical threshold were calculated. The total possible autosomal loci number was 23. Money had the highest mean number of loci with a mean of 12.54167±9.141302 loci with 25.00% of the sample having all 23 loci with called alleles. Cardboard had the second highest mean of 11.625±9.449235 loci with 20.83% of the

samples having all 23 loci with called alleles. Thermal paper had the third highest mean of 10.91667±9.958627 loci with 33.33% of the samples having all 23 loci with called alleles in the profile. Next highest was cardstock with a mean of 10.66667±9.639893 loci with 12.50% of the samples having all 23 loci with called alleles. Copy paper had the lowest mean of 6.583333±8.245772 loci with 12.50% of the samples having all 23 loci with called alleles. The ANOVA p-value was 0.216677. There was no difference in the number of loci with called alleles when comparing each of the paper substrates. There were two outliers for copy paper (Figure 9B). Outliers were determined by using excel® version 2310.

4.1.8.3. Number of loci with alleles present in the DNA profiles

The number of loci present in the profile, despite not being above the analytical threshold was manually calculated. The total number of loci with alleles present was 23 loci. Money had the highest mean of 17.91667±7.823691 loci with 45.83% of the samples having all 23 loci with an allele present. Cardboard had the second highest mean of 16.91667±8.192874 loci present with 54.17% with the samples having all 23 loci present. Thermal paper had the third highest mean of 15.41667±9.690279 loci with 41.67% of the samples having all 23 loci present. Cardstock had the next highest mean of 14.58333±9.63651 loci present with 33.33% of the samples having all 23 loci present in the profile. Copy paper had the lowest mean number of loci present in the profile with a mane of 13.125±9.228088 loci and 25.00% of the samples having all 23 loci present. The ANOVA p-value was 0.364522. The differences in the number of loci present in the profile for each category were not significant.

4.1.8.4. Number of alleles that matched each of the volunteer's DNA profiles

The alleles present in the profile were compared to each of the volunteer's reference profiles. The number of correct alleles found in each of the profiles were quantified and the different paper substrates were compared. The number of alleles present in the profile that matched the volunteers' profile was calculated. Cardboard had the highest mean of 30.91667±16.44204 alleles with 16.67% of the samples having all the volunteer's alleles present in the DNA profile. Money had the second highest mean of 30.125±14.67418 alleles with 12.50% having all the volunteer's alleles present in the DNA profile, followed by thermal paper mean of 27.75±21.03298 alleles with 33.33% of the samples having all of the volunteer's alleles present in the DNA profile, and cardstock mean of 27.66667±20.08388 alleles with 29.17% of the samples having all of the volunteer's alleles present in the DNA profile. Copy paper had the lowest number of alleles present with a mean of 23.625±17.48244 alleles with 16.67% of the samples having all the volunteer's alleles present in the DNA profile. The ANOVA p-value was 0.651317. The differences in the number alleles present for each of the paper substrates was not significant.

4.1.8.5. Interpretability of the DNA profiles

Each profile was deemed interpretable or not interpretable based on the degree of the profile mixture and the ability to identify the volunteer's profile in the sample's profile. A "1" was given to profiles that were interpretable. A "-1" was given to profiles that were not interpretable. When comparing each of the paper substrates to each other based on the ability to interpret the profile, both cardboard and cardstock had the same mean of 0.25±0.989071 DNA profile quality. They both had 62.50% of the samples being interpretable and 37.50% of them

uninterpretable. Thermal paper had the next highest mean of 0.166667±1.029857 DNA profile quality with 58.33% of the samples being interpretable and 41.67% of them uninterpretable. Copy paper had a mean of -0.08333±1.017955 DNA profile quality with 45.83% of the samples being interpretable and 54.17% of them uninterpretable. Money had the lowest interpretability mean of -0.75±0.675664 DNA profile quality with 12.50% of the samples being interpretable and 87.50% of them uninterpretable. The ANOVA p-value was 0.001554. Tukey-Kramer's HSD test was performed and a Q value above 3.98 was considered significant. Only two comparisons had significant values: money with cardboard (Q value of 5.21199), and money with cardstock (Q value of 5.21199). Cardboard and cardstock had significantly more interpretable DNA profiles than money.

Table 16: DNA concentrations for the paper substrates. Money, copy paper, cardboard, and cardstock each had 24 samples while thermal paper had 12 samples.

Money	**Copy paper**	**Cardboard**	**Cardstock**	**Thermal paper**
0.0988 ng/µl	0.0071 ng/µl	0.004 ng/µl	0 ng/µl	0.02765 ng/µl
0.0036 ng/µl	0.01875 ng/µl	0.0624 ng/µl	0.0432 ng/µl	0.01775 ng/µl
0.0365 ng/µl	0.1113 ng/µl	0.1425 ng/µl	0.1296 ng/µl	0.1566 ng/µl
0.0576 ng/µl	0 ng/µl	0 ng/µl	0.019 ng/µl	0.00375 ng/µl
0.012 ng/µl	0.03 ng/µl	0 ng/µl	0 ng/µl	0 ng/µl
0.0222 ng/µl	0.1218 ng/µl	0.35625 ng/µl	0.3337 ng/µl	0.781 ng/µl
0.07125 ng/µl	0.01752 ng/µl	0.0231 ng/µl	0.05325 ng/µl	0.02485 ng/µl
0.06545 ng/µl	0.0315 ng/µl	0.0333 ng/µl	0.154 ng/µl	0.03195 ng/µl
0.5476 ng/µl	0.12375 ng/µl	0.0657 ng/µl	0.028 ng/µl	0.2808 ng/µl
0.007 ng/µl	0 ng/µl	0.0148 ng/µl	0.0075 ng/µl	0.00325 ng/µl
0.02835 ng/µl	0.0072 ng/µl	0.0142 ng/µl	0.02625 ng/µl	0 ng/µl
0.1224 ng/µl	0.50765 ng/µl	0.0432 ng/µl	0.0925 ng/µl	0.16555 ng/µl
0.0608 ng/µl	0.01155 ng/µl	0.0504 ng/µl	0.0266 ng/µl	
1.27305 ng/µl	0 ng/µl	0.06715 ng/µl	0.0406 ng/µl	
0.34505 ng/µl	0.1026 ng/µl	0.04485 ng/µl	0.10585 ng/µl	
0.0154 ng/µl	0.01095 ng/µl	0 ng/µl	0.008 ng/µl	
0.5206 ng/µl	0 ng/µl	0.0076 ng/µl	0 ng/µl	
0.1575 ng/µl	0.032 ng/µl	0.0805 ng/µl	0.0345 ng/µl	
0.1679 ng/µl	0.0675 ng/µl	0.06035 ng/µl	0.0925 ng/µl	
1.3536 ng/µl	0.0432 ng/µl	0.0345 ng/µl	0.0324 ng/µl	
0.24885 ng/µl	0.028 ng/µl	0.0684 ng/µl	0.1584 ng/µl	
0.02925 ng/µl	0.0152 ng/µl	0 ng/µl	0 ng/µl	
0.3145 ng/µl	0 ng/µl	0 ng/µl	0.01035 ng/µl	
0.15975 ng/µl	0.01035 ng/µl	0.1517 ng/µl	0.0134 ng/µl	

Table 17: DNA ANOVA results for the paper substrates. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Paper substrate	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Money	24	5.719	0.365016229	0.2382917		
Copy paper	24	1.29792	0.104684979	0.05408		
Cardboard	24	1.3249	0.076430878	0.0552042		
Cardstock	24	1.4096	0.076335459	0.0587333		
Thermal paper	15	1.58495	0.225164814	0.1056633		
					0.006211	Significant

B)

Paper substrate	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Money	24	301	9.141302194	12.541667		
Copy paper	24	158	8.245771863	6.5833333		
Cardboard	24	279	9.449235074	11.625		
Cardstock	24	256	9.639892957	10.666667		
Thermal paper	12	131	9.958626533	10.916667		
					0.216677	Not significant

C)

Paper substrate	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Money	24	430	7.82369126	17.916667		
Copy paper	24	315	9.22808803	13.125		
Cardboard	24	406	8.192874246	16.916667		
Cardstock	24	350	9.636509681	14.583333		
Thermal paper	12	185	9.690279416	15.416667		
					0.364522	Not significant

D)

Paper substrate	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Money	24	723	14.67417874	30.125		
Copy paper	24	567	17.48244461	23.625		
Cardboard	24	742	16.44203697	30.916667		
Cardstock	24	664	20.08388207	27.666667		
Thermal paper	12	333	21.03298276	27.75		
					0.651317	Not significant

E)

Paper substrate	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Money	24	-18	0.675663925	-0.75		
Copy paper	24	-2	1.017954755	-0.083333		
Cardboard	24	6	0.98907071	0.25		
Cardstock	24	6	0.98907071	0.25		
Thermal paper	12	2	1.029857301	0.1666667		
					0.001554	Significant

Table 18: DNA Tukey-Kramer's HSD test results for the paper substrates. DNA concentrations (A). Interpretability of the DNA profiles (B).

A)

Paper substrate comparison	**Absolute difference**	**Standard error**	**Q Tukey**	**Q critical**	**Significance**
Money vs. copy paper	0.184211667	0.040461427	4.552772	4.16	Significant
Money vs. cardboard	0.1830875	0.040461427	4.524989	4.16	Significant
Money vs. cardstock	0.179558333	0.040461427	4.437766	4.16	Significant
Money vs. Thermal paper	0.132628333	0.046133125	2.874905	4.16	Not significant
Copy paper vs. cardboard	0.001124167	0.040461427	0.027784	4.16	Not significant
Copy paper vs. cardstock	0.004653333	0.040461427	0.115007	4.16	Not significant
Copy paper vs. Thermal paper	0.051583333	0.046133125	1.118141	4.16	Not significant
Cardboard vs. cardstock	0.003529167	0.040461427	0.087223	4.16	Not significant
Cardboard vs. thermal paper	0.050459167	0.046133125	1.093773	4.16	Not significant
Cardstock vs. thermal paper	0.04693	0.046133125	1.017273	4.16	Not significant

B)

Paper substrate comparison	Absolute difference	Standard error	Q Tukey	Q critical	Significance
Money vs. copy paper	0.666666667	0.191865311	3.47466	3.98	Not significant
Money vs. cardboard	1	0.191865311	5.21199	3.98	Significant
Money vs. cardstock	1	0.191865311	5.21199	3.98	Significant
Money vs. Thermal paper	0.916666667	0.234986056	3.900941	3.98	Not significant
Copy paper vs. cardboard	0.333333333	0.191865311	1.73733	3.98	Not significant
Copy paper vs. cardstock	0.333333333	0.191865311	1.73733	3.98	Not significant
Copy paper vs. Thermal paper	0.25	0.234986056	1.063893	3.98	Not significant
Cardboard vs. cardstock	0	0.191865311	0	3.98	Not significant
Cardboard vs. thermal paper	0.083333333	0.234986056	0.354631	3.98	Not significant
Cardstock vs. thermal paper	0.083333333	0.234986056	0.354631	3.98	Not significant

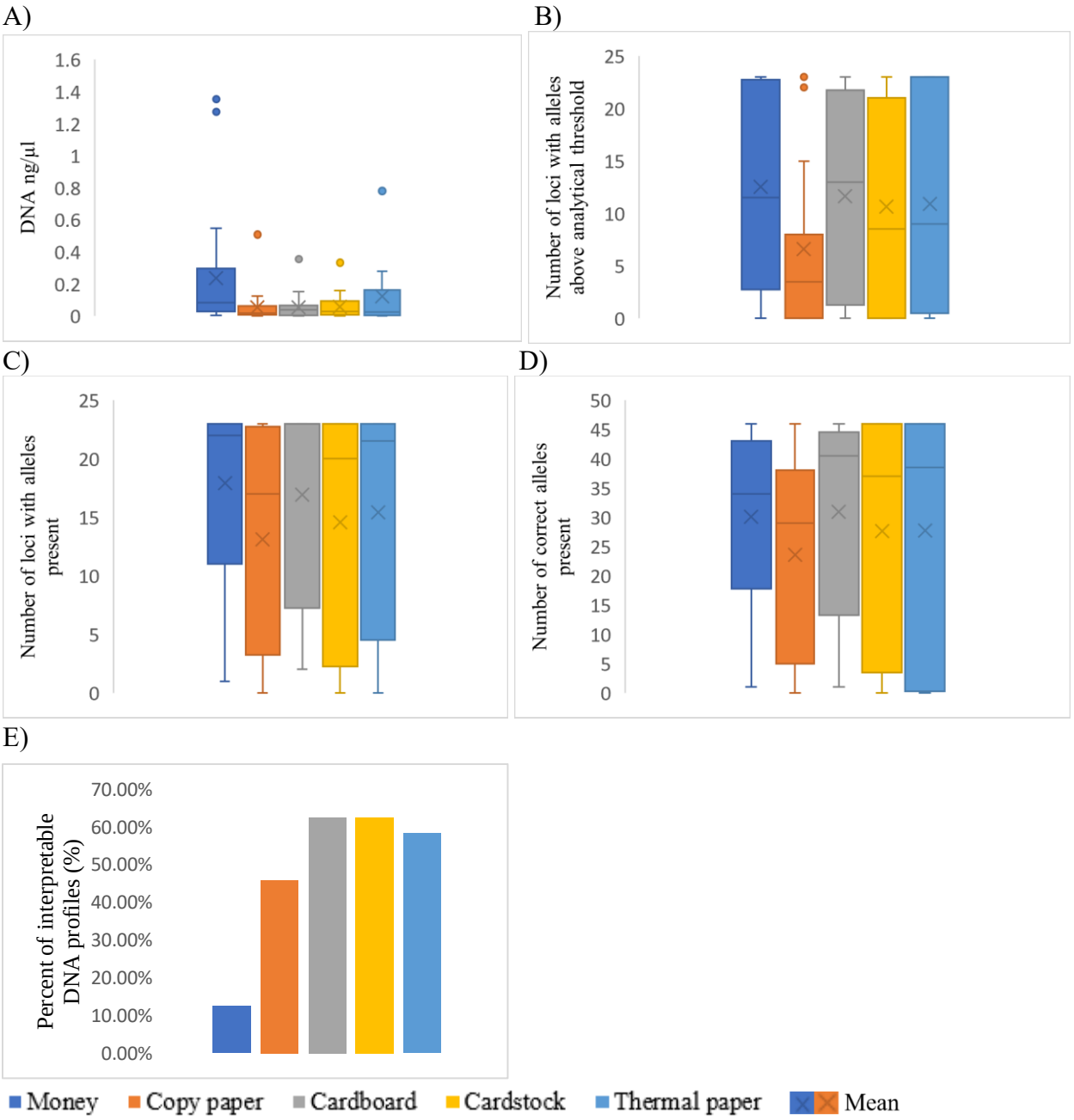


Figure 9: DNA comparisons for the paper substrates. DNA concentrations (A). Number of loci with called alleles in the DNA profile (B). Number of loci with alleles present in the DNA profile (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Money, copy paper, cardboard, and cardstock had 24 samples while thermal paper had 12 samples.

4.1.9. Paper substrates and their sequence of analysis

4.1.9.1. DNA concentrations

When comparing each of the paper substrate's DNA concentrations when they went through fingerprint enhancement first with ninhydrin, cardboard had the highest mean DNA concentration followed by cardstock, copy paper, and money. The ANOVA p-value was 0.713557. The difference in the DNA concentrations for each of the paper substrates going through fingerprinting first with ninhydrin was not significant.

For fingerprinting first with 1,2-indanedione, thermal paper had the highest mean DNA concentration followed by money, copy paper, cardstock, and lastly cardboard. The ANOVA p-value was 0.729469. The difference in the DNA concentrations for each of the paper substrates going through fingerprinting first with 1,2-indanedione was not significant. There was one outlier for money, and one outlier for thermal paper (Figure 10A). Outliers were determined by using excel® version 2310.

When comparing each of the paper substrate's DNA concentrations when they went through DNA analysis first, money had the highest mean DNA concentration followed by thermal paper, cardboard, cardstock, and copy paper. The ANOVA p-value was 0.00086. Tukey-Kramer's HSD test was performed and a Q value above 4.04 was considered significant. Four comparisons were significant: money versus copy paper (Q value of 5.626568), money versus cardboard (Q value of 5.309001), money versus cardstock (Q value of 5.364747), and copy paper versus cardstock (Q value of 5.364747). Money had significantly higher DNA concentrations than copy paper, cardboard, and cardstock. Cardstock had significantly higher DNA concentrations than copy paper. There were two outliers for money, and one outlier for copy paper (Figure 10A). Outliers were determined by using excel® version 2310.

4.1.9.2 Number of loci with called alleles in the DNA profiles

When comparing the number of loci with alleles above the analytical threshold for each of the paper substrates for fingerprinting first with ninhydrin, cardstock had the highest mean loci followed by cardboard, copy paper, and lastly money. The ANOVA p-value was 0.329612. The difference in the number of loci for each of the paper substrates going through fingerprinting first with ninhydrin was not significant.

In the case of fingerprinting first with 1,2-indanedione, money had the highest mean number of loci, followed by cardboard, cardstock, thermal paper, and lastly, copy paper. The ANOVA p-value was 0.797274. The difference in the number of loci for each of the paper substrates going through fingerprinting first with 1,2-indandione was not significant.

In the case of DNA analysis first, money had the highest mean number of loci, followed by thermal paper, cardboard, cardstock and lastly, copy paper. The ANOVA p-value was 0.026653. Turkey t-test was performed and a Q value above 4.04 was considered significant. Only the comparison of money versus copy paper was significant with a Q value of 4.813065. Money had significantly higher number of loci with called alleles by GeneMapper Software than copy paper when DNA analysis was performed first.

4.1.9.3. Number of loci with alleles present in the DNA profiles

When comparing the number of loci with alleles present in the DNA profile for each of the paper substrates for fingerprinting first with ninhydrin, both copy paper and cardstock had the highest mean loci followed by carboard, and money. The ANOVA p-value was 0.964857. The difference in the number of loci for each of the paper substrates going through fingerprinting first with ninhydrin was not significant.

In the case of fingerprinting first with 1,2-indanedione, cardboard had the highest mean number of loci, followed by money, cardstock, copy paper and lastly, thermal paper. The ANOVA p-value was 0.92208. The difference in the number of loci for each of the paper substrates going through fingerprinting first with 1,2-indanedione was not significant. There was one outlier for money, and one outlier for cardboard (Figure 10C). Outliers were determined by using excel® version 2310.

In the case of DNA analysis first, money had the highest mean number of loci with alleles present in the DNA profile, followed by cardboard, thermal paper, cardstock, and lastly, copy paper. The ANOVA p-value was 0.139262. The difference in the number of loci for each of the paper substrates going through DNA analysis first was not significant. There were two outliers for money (Figure 10C). Outliers were determined by using excel® version 2310.

4.1.9.4. Number of alleles that matched each of the volunteer's DNA profiles

When comparing the number of alleles that matched the volunteer's reference profile for each of the paper substrates for fingerprinting first with ninhydrin, cardstock had the highest mean alleles followed by copy paper, cardboard, and lastly money. The ANOVA p-value was 0.831083. The difference in the number of alleles matching the volunteer's profiles for each of the paper substrates was not significant for fingerprinting first with ninhydrin.

In the case of fingerprinting first with 1,2-indanedione, cardboard had the highest mean number of alleles followed by cardstock, money, thermal paper, and lastly copy paper. The ANOVA p-value was 0.868249. The difference in the number of alleles matching the volunteers' profiles for each of the paper substrates was not significant for fingerprinting first with 1,2-

indanedione. There was one outlier for cardboard (Figure 10D). Outliers were determined by using excel® version 2310.

In the case of DNA analysis first, money had the highest mean alleles followed by thermal paper, cardboard, cardstock, and lastly copy paper. The ANOVA p-value was 0.341218. The difference in the number of alleles matching the volunteers' profiles for each of the paper substrates was not significant for DNA analysis first. There was one outlier for money (Figure 10D). Outliers were determined by using excel® version 2310.

4.1.8.5. Interpretability of the DNA profiles

When comparing the percentage of the DNA profiles with interpretable profiles for each of the paper substrates for fingerprinting first with ninhydrin, copy paper had the percentage followed equally by cardboard and cardstock. Lastly, money had no interpretable samples. The ANOVA p-value was 0.107455. The difference in the percentages of interpretable DNA profiles for each of the paper substrates was not significant for fingerprinting first with ninhydrin.

In the case of fingerprinting first with 1,2-indanedione, both cardboard and cardstock had the highest percentage of interpretable profiles followed by thermal paper and copy paper, and lastly money. The ANOVA p-value was 0.100838. The difference in the percentages of interpretable DNA profiles for each of the paper substrates was not significant for fingerprinting first with 1,2-indanedione.

In the case of DNA analysis first, both cardboard and cardstock had the highest percentage of interpretable DNA profiles followed by thermal paper, copy paper, and lastly money. The ANOVA p-value was 0.117672. The difference in the percentages of interpretable DNA profiles for each of the paper substrates was not significant for DNA analysis first.

Table 19: DNA concentrations for the paper substrates and their sequence of analysis. The three different sequences of analysis for money, copy paper, cardboard and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, and 3) DNA analysis first then fingerprinting. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting. Money, copy paper, cardboard, and cardstock each had a total of 24 samples with both fingerprint methods having 6 samples and DNA analysis having 12 samples. Thermal paper had a total of 12 samples with both ninhydrin fingerprint method and DNA analysis each having 6 samples.

First analysis	**Money**	**Copy paper**	**Cardboard**	**Cardstock**	**Thermal Paper**
Ninhydrin	0.0988 ng/µl	0.0071 ng/µl	0.004 ng/µl	0 ng/µl	0.02765 ng/µl
Ninhydrin	0.0036 ng/µl	0.01875 ng/µl	0.0624 ng/µl	0.0432 ng/µl	0.01775 ng/µl
Ninhydrin	0.0365 ng/µl	0.1113 ng/µl	0.1425 ng/µl	0.1296 ng/µl	0.1566 ng/µl
Ninhydrin	0.0576 ng/µl	0 ng/µl	0 ng/µl	0.019 ng/µl	0.00375 ng/µl
Ninhydrin	0.012 ng/µl	0.03 ng/µl	0 ng/µl	0 ng/µl	0 ng/µl
Ninhydrin	0.0222 ng/µl	0.1218 ng/µl	0.35625 ng/µl	0.3337 ng/µl	0.781 ng/µl
1,2-Indanedione	0.07125 ng/µl	0.01752 ng/µl	0.0231 ng/µl	0.05325 ng/µl	0.02485 ng/µl
1,2-Indanedione	0.06545 ng/µl	0.0315 ng/µl	0.0333 ng/µl	0.154 ng/µl	0.03195 ng/µl
1,2-Indanedione	0.5476 ng/µl	0.12375 ng/µl	0.0657 ng/µl	0.028 ng/µl	0.2808 ng/µl
1,2-Indanedione	0.007 ng/µl	0 ng/µl	0.0148 ng/µl	0.0075 ng/µl	0.00325 ng/µl
1,2-Indanedione	0.02835 ng/µl	0.0072 ng/µl	0.0142 ng/µl	0.02625 ng/µl	0 ng/µl
1,2-Indanedione	0.1224 ng/µl	0.50765 ng/µl	0.0432 ng/µl	0.0925 ng/µl	0.16555 ng/µl
DNA	0.0608 ng/µl	0.01155 ng/µl	0.0504 ng/µl	0.0266 ng/µl	
DNA	1.27305 ng/µl	0 ng/µl	0.06715 ng/µl	0.0406 ng/µl	
DNA	0.34505 ng/µl	0.1026 ng/µl	0.04485 ng/µl	0.10585 ng/µl	
DNA	0.0154 ng/µl	0.01095 ng/µl	0 ng/µl	0.008 ng/µl	
DNA	0.5206 ng/µl	0 ng/µl	0.0076 ng/µl	0 ng/µl	
DNA	0.1575 ng/µl	0.032 ng/µl	0.0805 ng/µl	0.0345 ng/µl	
DNA	0.1679 ng/µl	0.0675 ng/µl	0.06035 ng/µl	0.0925 ng/µl	
DNA	1.3536 ng/µl	0.0432 ng/µl	0.0345 ng/µl	0.0324 ng/µl	
DNA	0.24885 ng/µl	0.028 ng/µl	0.0684 ng/µl	0.1584 ng/µl	
DNA	0.02925 ng/µl	0.0152 ng/µl	0 ng/µl	0 ng/µl	
DNA	0.3145 ng/µl	0 ng/µl	0 ng/µl	0.01035 ng/µl	
DNA	0.15975 ng/µl	0.01035 ng/µl	0.1517 ng/µl	0.0134 ng/µl	

Table 20: DNA ANOVA results for the paper substrates and their sequences of analysis. The three different sequences of analysis for money, copy paper, cardboard and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, and 3) DNA analysis first then fingerprinting. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting. DNA concentrations (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E).

A)

Paper substrate, first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin						
Money	6	0.2307	0.035187597	0.03845		
Copy paper	6	0.28895	0.054054698	0.0481583		
Cardboard	6	0.56515	0.139926875	0.0941917		
Cardstock	6	0.5255	0.129887681	0.0875833		
					0.713557	Not significant
1,2-Indanedione						
Money	6	0.84205	0.203413769	0.1403417		
Copy paper	6	0.68762	0.197772638	0.1146033		
Cardboard	6	0.1943	0.019767794	0.0323833		
Cardstock	6	0.3615	0.054510091	0.06025		
Thermal paper	6	0.98675	0.307668311	0.1644583		
					0.729469	Not significant
DNA						
Money	12	4.64625	0.456084707	0.3871875		
Copy paper	12	0.32135	0.03125152	0.0267792		
Cardboard	12	0.56545	0.044187783	0.0471208		
Cardstock	12	0.5226	0.049578602	0.04355		
Thermal paper	6	0.5064	0.114161438	0.0844		
					0.00086	Significant

B)

Paper substrate, first analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Ninhydrin						
Money	6	21	3.507135583	3.5		
Copy paper	6	30	8.876936408	5		
Cardboard	6	69	12.24336555	11.5		
Cardstock	6	71	11.33872421	11.833333		
					0.329612	Not significant
1,2-Indanedione						
Money	6	92	8.640987598	15.333333		
Copy paper	6	58	10.32795559	9.6666667		
Cardboard	6	82	8.041558721	13.666667		
Cardstock	6	72	8.966604709	12		
Thermal paper	6	59	10.41953294	9.8333333		
					0.797274	Not significant
DNA						
Money	12	188	8.700400548	15.666667		
Copy paper	12	45	4.653932843	3.75		
Cardboard	12	128	9.267080871	10.666667		
Cardstock	12	113	9.792931898	9.4166667		
Thermal paper	6	72	10.33440855	12		
					0.026653	Significant

C)

Paper substrate, first analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Ninhydrin						
Money	6	73	8.376554582	12.166667		
Copy paper	6	88	10.1324561	14.666667		
Cardboard	6	82	10.34730239	13.666667		
Cardstock	6	88	9.667816024	14.666667		
					0.964857	Not significant
1,2-Indanedione						
Money	6	114	8.854377448	19		
Copy paper	6	93	9.203260292	15.5		
Cardboard	6	107	8.035338616	17.833333		
Cardstock	6	108	7.949842766	18		
Thermal paper	6	91	9.217736526	15.166667		
					0.92208	Not significant
DNA						
Money	12	243	5.986727745	20.25		
Copy paper	12	134	9.183318209	11.166667		
Cardboard	12	197	8.371578903	16.416667		
Cardstock	12	154	10.64154238	12.833333		
Thermal paper	6	94	11.02119171	15.666667		
					0.139262	Not significant

D)

Paper substrate, first analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Ninhydrin						
Money	6	111	12.94217911	18.5		
Copy paper	6	157	19.78298933	26.166667		
Cardboard	6	156	21.77154106	26		
Cardstock	6	169	21.39548239	28.166667		
					0.831083	Not significant
1,2-Indanedione						
Money	6	194	16.52472894	32.333333		
Copy paper	6	169	19.70194576	28.166667		
Cardboard	6	229	8.727351641	38.166667		
Cardstock	6	209	17.38294183	34.833333		
Thermal paper	6	178	22.66862737	29.666667		
					0.868249	Not significant
DNA						
Money	12	418	12.22392091	34.833333		
Copy paper	12	241	16.00828384	20.083333		
Cardboard	12	299	18.76388375	24.916667		
Cardstock	12	286	21.3022975	23.833333		
Thermal paper	6	178	22.66862737	29.666667		
					0.341218	Not significant

E)

Paper substrate, first analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Ninhydrin						
Money	6	-6	0	-1		
Copy paper	6	2	1.032795559	0.3333333		
Cardboard	6	0	1.095445115	0		
Cardstock	6	0	1.095445115	0		
					0.107455	Not significant
1,2-Indanedione						
Money	6	-4	0.816496581	-0.666667		
Copy paper	6	2	1.032795559	0.3333333		
Cardboard	6	4	0.816496581	0.6666667		
Cardstock	6	4	0.816496581	0.6666667		
Thermal paper	6	2	1.032795559	0.3333333		
					0.100838	Not significant
DNA						
Money	12	-8	0.778498944	-0.666667		
Copy paper	12	-6	0.904534034	-0.5		
Cardboard	12	2	1.029857301	0.1666667		
Cardstock	12	2	1.029857301	0.1666667		
Thermal paper	6	0	1.095445115	0		
					0.117672	Not significant

Table 21: DNA Tukey-Kramer's HSD test results for the paper substrates and their sequence of analysis. The three different sequences of analysis for money, copy paper, cardboard and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, and 3) DNA analysis first then fingerprinting. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting. DNA concentrations when DNA analysis was performed first (A). Number of loci with called alleles in the DNA profiles when DNA analysis was performed first (B).

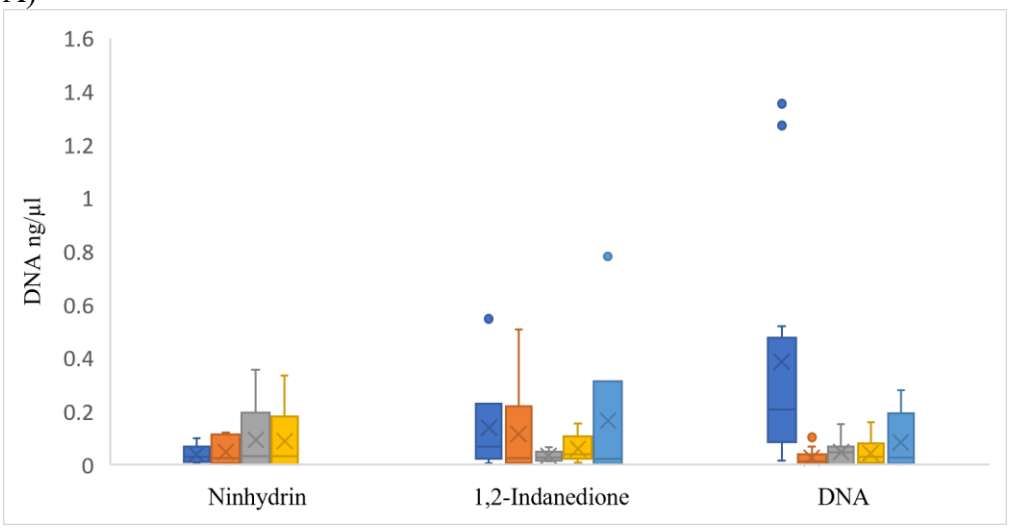
A)

DNA analysis first on paper substrates comparison	**Absolute difference**	**Standard error**	**Q Tukey**	**Q critical**	**Significance**
Money vs. copy paper	0.360408333	0.064054742	5.626568	4.04	Significant
Money vs. cardboard	0.340066667	0.064054742	5.309001	4.04	Significant
Money vs. cardstock	0.3436375	0.064054742	5.364747	4.04	Significant
Money vs. Thermal paper	0.3027875	0.078450716	3.859589	4.04	Not significant
Copy paper vs. cardboard	0.020341667	0.064054742	0.317567	4.04	Not significant
Copy paper vs. cardstock	0.3436375	0.064054742	5.364747	4.04	Significant
Copy paper vs. Thermal paper	0.057620833	0.078450716	0.734484	4.04	Not significant
Cardboard vs. cardstock	0.003570833	0.064054742	0.055747	4.04	Not significant
Cardboard vs. thermal paper	0.037279167	0.078450716	0.475192	4.04	Not significant
Cardstock vs. thermal paper	0.04085	0.078450716	0.520709	4.04	Not significant

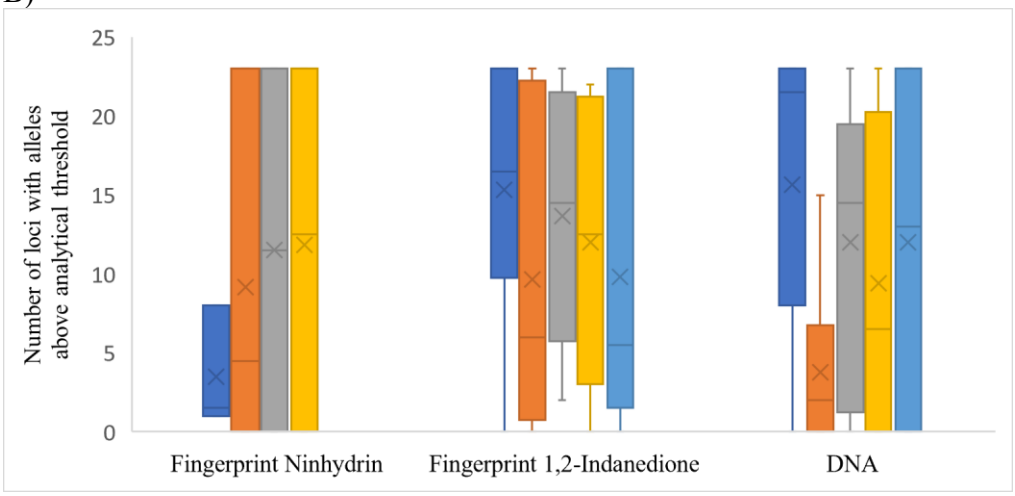
B)

DNA analysis first on paper substrates comparison	Absolute difference	Standard error	Q Tukey	Q critical	Significance
Money vs. copy paper	11.91666667	2.475904287	4.813056	4.04	Significant
Money vs. cardboard	5	2.475904287	2.019464	4.04	Not significant
Money vs. cardstock	6.25	2.475904287	2.52433	4.04	Not significant
Money vs. Thermal paper	3.666666667	3.032351078	1.209183	4.04	Not significant
Copy paper vs. cardboard	6.916666667	2.475904287	2.793592	4.04	Not significant
Copy paper vs. cardstock	5.666666667	2.475904287	2.288726	4.04	Not significant
Copy paper vs. Thermal paper	8.25	3.032351078	2.720661	4.04	Not significant
Cardboard vs. cardstock	1.25	2.475904287	0.504866	4.04	Not significant
Cardboard vs. thermal paper	1.333333333	3.032351078	0.439703	4.04	Not significant
Cardstock vs. thermal paper	2.583333333	3.032351078	0.851924	4.04	Not significant

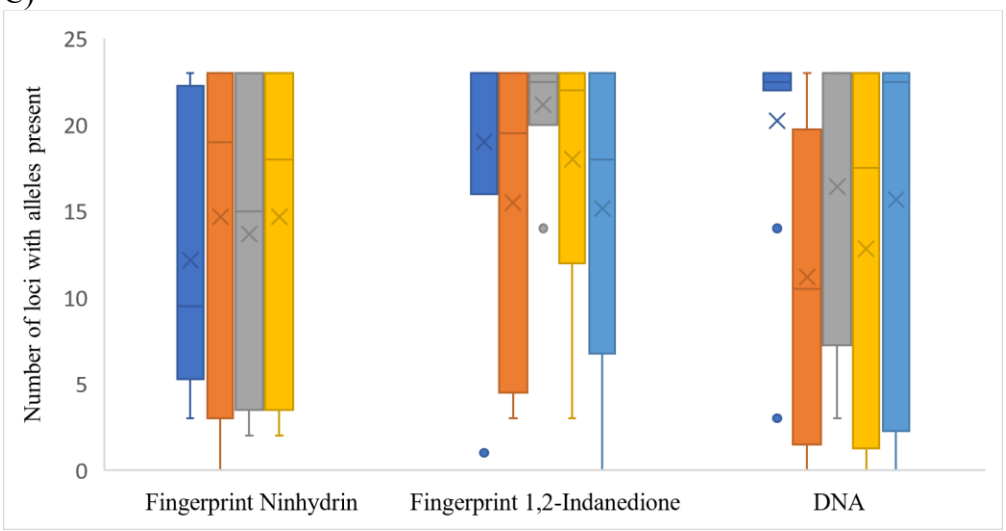
A)


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DNA ng/µl
Ninhydrin
1,2-Indanedione
DNA

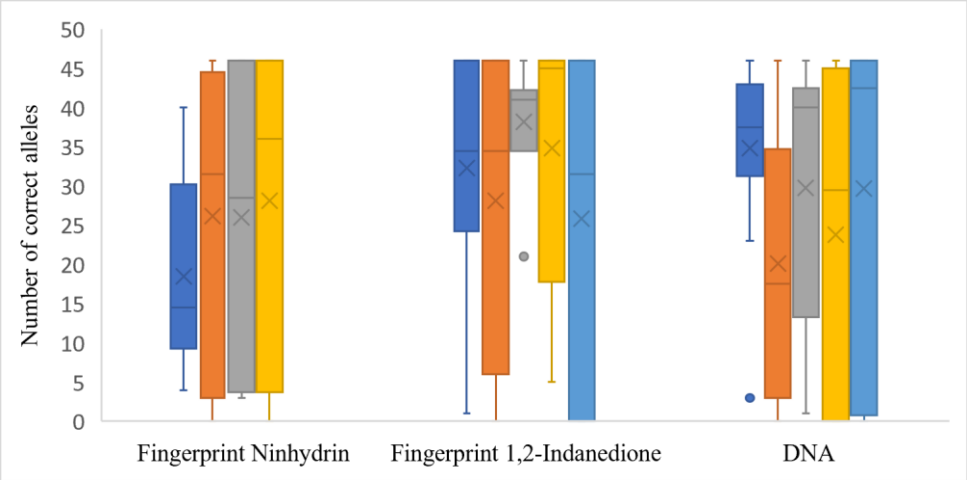
B)


25
20
15
10
5
0
Number of loci with alleles above analytical threshold
Fingerprint Ninhydrin
Fingerprint 1,2-Indanedione
DNA

C)


25
20
15
10
5
0
Number of loci with alleles present
Fingerprint Ninhydrin
Fingerprint 1,2-Indanedione
DNA

D)



E)

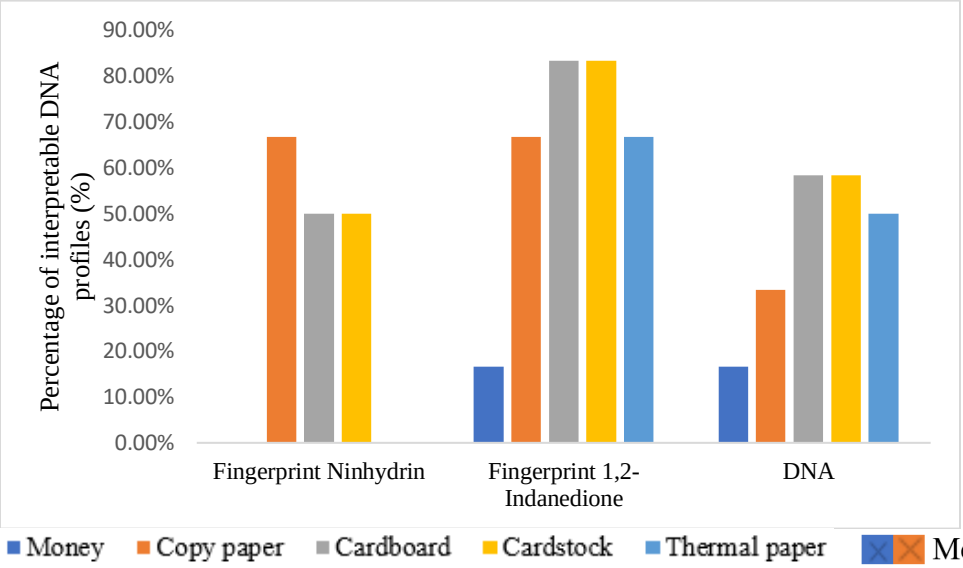


Figure 10: DNA comparison of the paper substrates and their sequence of analysis. The three different sequences of analysis for money, copy paper, cardboard and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, and 3) DNA analysis first then fingerprinting. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting. (A). Number of loci with called alleles in the DNA profiles (B). Number of loci with alleles present in the DNA profiles (C). Number of alleles that matched each of the volunteer's DNA profiles (D). Interpretability of the DNA profiles (E). Fingerprinting first with ninhydrin for money, copy paper, cardboard, and cardstock each had 6 samples. Fingerprinting first with 1,2-indanedione for money, copy paper, cardboard, cardstock, and thermal paper each had 6 samples. DNA first for money, copy paper, cardboard, and card stock each had 12 samples while thermal paper had 6 samples.

4.2. Fingerprint enhancement assessment

4.2.1. Fingerprint enhancement first versus DNA analysis first

Five fingerprint examiners from the Sacramento Sheriff's Office, all certified by the International Association for Identification review and rank of fingerprints, assessed the quality of the fingerprints for 108 samples. The examiners followed the following fingerprint quality rankings from 1-5 below:

1. Poor (no visible fingerprint ridges).
2. Low (fingerprint ridges visible and the ridge detail has low contrast and clarity).
3. Medium (fingerprint ridges visible and the ridge detail has medium contrast and clarity).
4. Good (fingerprint ridges visible and the ridge detail has good contrast and clarity).
5. Excellent (fingerprint ridges visible and the ridge detail has excellent contrast and clarity).

The examiners made their assessments from pictures of fingerprints on the paper substrates. Pictures that should have showcased the fingerprints present on one of the thermal paper samples that went through fingerprint first with 1,2-indaendione and then DNNA analysis were missed. As a result, the examiners were unable to assess the quality of this single sample. Therefore, the statistics for this single sample were omitted from the data. There was a total of 260 fingerprint quality assessments for fingerprinting prior to DNA analysis. Fingerprinting first then DNA analysis had a mean of 2.034615±1.221886 fingerprint quality with 5.38% of the fingerprint assessments receiving a fingerprint quality of 5. For the samples that went through DNA analysis prior to fingerprint enhancements, the number of fingerprint assessments was 270. The mean was 1.666667±1.020241 fingerprint quality with 2.59% of the fingerprint assessments

receiving a fingerprint quality of 5. The ANOVA p-value was 0.000183. Fingerprint enhancement prior to DNA analysis provides significantly higher fingerprint quality than when DNA analysis is performed prior to fingerprint enhancement. There were two outliers for DNA analysis first (Figure 11). Outliers were determined by using excel® version 2310.

Table 22: Fingerprint ANOVA results for first analysis performed. Samples either went through fingerprint enhancement first or DNA analysis first.

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint	260	529	1.221886101	2.0346154		
DNA	270	450	1.020241243	1.6666667		
					0.000183	Significant

Table 23: Fingerprint quality results for first analysis performed. Samples either went through fingerprint enhancement first or DNA analysis first. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

First analysis	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
Fingerprint	121	63	36	26	14	260
DNA	164	61	23	15	7	270

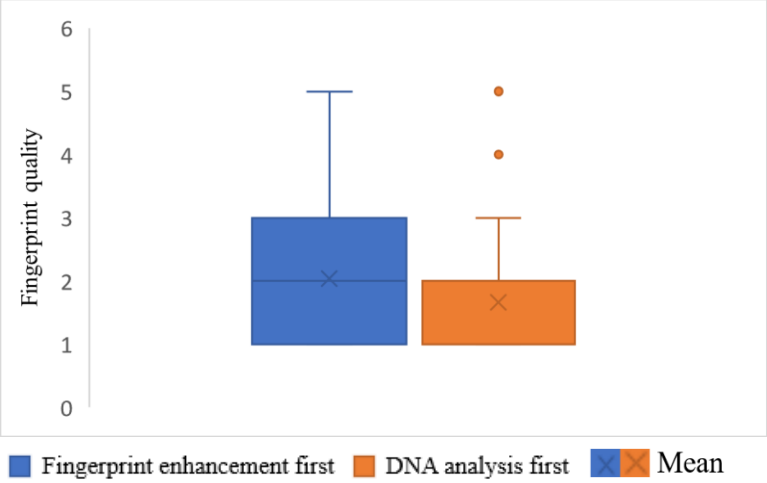


Figure 11: Fingerprint quality for first analysis performed. Samples either went through fingerprint enhancement first or DNA analysis first. Fingerprinting first had a total of 260 quality assessments while DNA first had 270 quality assessments.

4.2.2. Ninhydrin versus 1,2-Indaendione fingerprint enhancement methods

Five fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 108 samples. The examiners made their assessments from pictures of fingerprints on the paper substrates. Pictures that should have showcased the fingerprints present on one of the thermal paper samples that went through fingerprinting first with 1,2-indanedione and then DNA analysis were missed. As a result, the examiners were unable to assess the quality of this single sample. Therefore, the statistics for this single sample were omitted from the data. When fingerprinting with ninhydrin was performed after DNA analysis, there were 120 fingerprint quality assessments. The mean was 1.691667±0.994065 fingerprint quality with 2.50% of the fingerprint assessments resulting in a fingerprint quality of 5. When fingerprinting with 1,2-indanedione was performed after DNA analysis, there were 150 fingerprint quality assessments. The mean was 1.646667±1.043591 fingerprint quality with 2.67% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.719466. The differences in the fingerprint quality of samples that went through ninhydrin versus 1,2-indanedione fingerprint methods after DNA analysis was performed produced no significant result. There were two outliers for DNA analysis first and then fingerprint enhancement with ninhydrin, and two outliers for DNA analysis first and then fingerprint enhancement with 1,2-indanedione (Figure 12). Outliers were determined by using excel® version 2310.

Table 24: Fingerprint ANOVA results for fingerprint methods. Compared ninhydrin and 1,2-indanedione fingerprint methods when DNA analysis was performed prior to fingerprint enhancement.

Fingerprint method first analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
DNA						
Ninhydrin	120	203	0.994065021	1.6916667		
1,2-Indanedione	150	247	1.043591287	1.6466667		
					0.719466	Significant

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Table 25: Fingerprint quality for the fingerprint methods. Compared ninhydrin and 1,2-indanedione fingerprint methods when DNA analysis was performed prior to fingerprint enhancement. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Fingerprint method	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
Ninhydrin	68	33	10	6	3	120
1,2-Indanedione	96	28	13	9	4	150

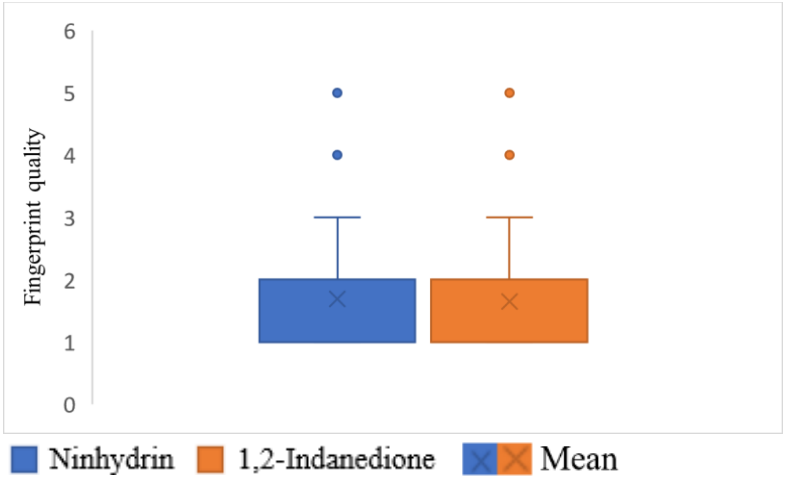


Figure 12: Fingerprint quality comparison of fingerprint methods. Compared ninhydrin and 1,2-indanedione fingerprint methods when DNA analysis was performed prior to fingerprint enhancement. The ninhydrin method had 120 quality assessments while 1,2-indanedione had 150 quality assessments.

4.2.3. Sequence of analysis when using ninhydrin method

Five fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 48 samples. Fingerprint quality significantly decreased when the samples went through DNA analysis first. Fingerprinting first with ninhydrin first had the highest mean of 2.116667±1.360816 fingerprint quality with 7.50% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA first then fingerprinting with ninhydrin had a mean of 1.691667±0.994065 fingerprint quality with 2.50% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.006182. When the samples went through fingerprinting first with ninhydrin, they had a significantly higher fingerprint quality than the samples that went through DNA analysis first and then fingerprinting with ninhydrin. There were two outliers for DNA analysis first and then fingerprint enhancement with ninhydrin (Figure 13). Outliers were determined by using excel® version 2310.

Table 26: Fingerprint ANOVA results for sequence of analysis with ninhydrin method. Compared fingerprint enhancement first with ninhydrin versus DNA analysis then fingerprint enhancement with ninhydrin.

First analysis with ninhydrin	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Ninhydrin						
Fingerprint	120	254	1.360816199	2.1166667		
DNA	120	203	0.994065021	1.6916667		
					0.006182	Significant

Table 27: Fingerprint quality results for sequence of analysis with ninhydrin method. Compared fingerprint enhancement first with ninhydrin versus DNA analysis then fingerprint enhancement with ninhydrin. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

First analysis with ninhydrin	**Fingerprint quality of 1**	**Fingerprint quality of 2**	**Fingerprint quality of 3**	**Fingerprint quality of 4**	**Fingerprint quality of 5**	**Total fingerprint assessments**
Ninhydrin						
Fingerprint	61	18	16	16	9	120
DNA	68	33	10	6	3	120

4.2.4. Sequence of analysis when using 1,2-Indaendione method

Five fingerprint examiners from Sacramento Sheriff's Office assessed the quality of the fingerprints for 54 samples. The examiners made their assessments from pictures of fingerprints. Pictures that should have showcased the fingerprints present on one of the thermal paper samples that went through fingerprinting first with 1,2-indanedione and then DNA analysis were missed. As a result, the examiners were unable to assess the quality of this single sample. Therefore, the statistics for this single sample were omitted from the data. The fingerprint quality significantly decreased when the samples went through DNA analysis first and then fingerprinting with 1,2-indanedione. Fingerprint enhancement first with 1,2-indanedione had the highest mean fingerprint quality of 1.964286±1.088929 fingerprint quality with 3.57% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA first then fingerprinting with 1,2-indanedione and a mean of 1.646667±1.043591 fingerprint quality with 2.67% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.011736. When the samples went through fingerprinting first with 1,2-indanedione, they had a significantly higher fingerprint quality than the samples that went through DNA analysis first and then fingerprinting with 1,2-indanedione. There were two outliers for DNA analysis first and then fingerprint with 1,2-indanedione (Figure 13). Outliers were determined by using excel® version 2310.

Table 28: Fingerprint ANOVA results for the sequence of analysis with 1,2-indanedione method. Compared fingerprint enhancement first with 1,2-indanedione versus DNA analysis then fingerprint enhancement with 1,2-indanedione.

First analysis with 1,2-indanedione	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
1,2-Indanedione						
Fingerprint	140	275	1.088928681	1.9642857		
DNA	150	247	1.043591287	1.6466667		
					0.011736	Significant

Table 29: Fingerprint quality results for the sequence of analysis with 1,2-Indanedione method. Comparison of fingerprint enhancement first with 1,2-indanedione versus DNA analysis then fingerprint enhancement with 1,2-indaendione. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

First analysis with 1,2-indanedione	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
1,2-Indanedione						
Fingerprint	60	45	20	10	5	140
DNA	96	28	13	9	4	150

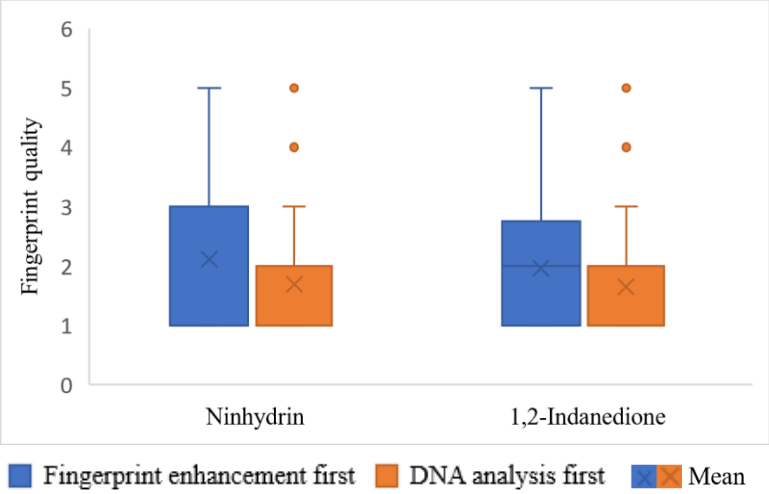


Figure 13: Fingerprint quality comparison for the fingerprint sequence of analysis**.** Both sequences involving ninhydrin had 120 fingerprint assessments samples. Fingerprinting first with 1,2-indanedione had 140 fingerprint assessments, while DNA analysis first and then fingerprinting with 1,2-indanedione had 140 assessments.

4.2.5. Intentional versus unintentional deposit method

Five fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 108 samples. The examiners made their assessments from pictures of fingerprints. Pictures that should have showcased the fingerprints present on one of the thermal paper samples that went through fingerprinting first with 1,2-indanedione and then DNA analysis were missed. As a result, the examiners were unable to assess the quality of this single sample. Therefore, the statistics for this single sample were omitted from the data. The quality of the fingerprint enhancement was compared between unintentional latent print deposit and intentional latent print deposit. Unintentional latent prints had a higher mean of 2.015094±1.209087 fingerprint quality with 4.91% of the fingerprint assessments resulting in a fingerprint quality of 5. Intentional fingerprints had a mean of 1.679245±1.036705 fingerprint quality with 3.02% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.000644. Unintentional latent fingerprint deposit had significantly higher fingerprint quality than intentional latent fingerprint deposit. There were two outliers for intentional latent fingerprint deposit (Figure 14A). Outliers were determined by using excel® version 2310.

The quality of fingerprint enhancement was compared between the following groups: 1) intentional latent print when fingerprinting enhancement was performed first, 2) intentional latent print when DNA analysis was performed first, 3) unintentional latent print when fingerprinting enhancement was performed first, and 4) unintentional latent print when DNA analysis was performed first. Unintentional latent print with fingerprint analysis performed first had the highest mean quality of 2.176923±1.254236 fingerprint quality with 6.15% of the fingerprint assessments resulting in a fingerprint quality of 5. The second highest was intentional latent print with fingerprint enhancements first with a mean quality of 1.892308±1.176291

fingerprint quality with 4.62% of the fingerprint assessments resulting in a fingerprint quality of 5. Unintentional latent print with DNA analysis performed first had the third highest mean quality of 1.859259±1.147112 fingerprint quality with 3.70% of the fingerprint assessments resulting in a fingerprint quality of 5. Finally, intentional latent print with DNA analysis performed first had the lowest mean quality of 1.474074±0.836032 fingerprint quality with 1.48% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was $8.74x10^{-06}$. There was a significant difference in the fingerprint qualities. The Tukey-Kramer's HSD test was performed. Any Q value above 3.92 was considered significant. The comparison of intentional latent print with fingerprint enhancement first compared to intentional latent print with DNA analysis performed first had a Q value of 4.325296. The difference in fingerprint quality was significantly higher in the first group. The comparison of intentional latent print with DNA analysis performed first and unintentional latent print with fingerprint enhancement performed first had a Q value of 7.268736. Unintentional latent fingerprint deposit with DNA analysis first had significantly higher fingerprint quality than intentional latent fingerprint deposit with DNA analysis first. There were two outliers for intentional latent print when DNA analysis was performed first, and two outliers for unintentional latent print when DNA analysis was performed first (Figure 14B). Outliers were determined by using excel® version 2310.

Table 30: Fingerprint ANOVA results for the method of depositing samples. Comparison of intentional versus unintentional fingerprint deposit method (A). Comparison of fingerprint deposit method and first analysis performed (B).

A)

Deposit method	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Intentional	265	445	1.036704878	1.6792453		
Unintentional	265	534	1.20908747	2.0150943		
					0.000644	Significant

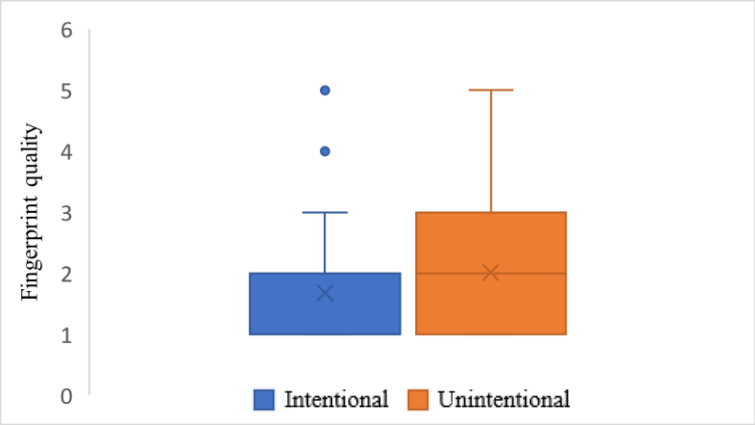
B)

Deposit method first analysis	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Intentional fingerprint	130	246	1.176291333	1.8923077		
Intentional DNA	135	199	0.836032114	1.4740741		
Unintentional fingerprint	130	283	1.254235519	2.1769231		
Unintentional DNA	135	251	1.147111642	1.8592593		
					8.74E-06	Significant

Table 31: Fingerprint quality results for the method of depositing samples. Samples were either deposited intentionally or unintentionally. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Deposit method	**Fingerprint quality of 1**	**Fingerprint quality of 2**	**Fingerprint quality of 3**	**Fingerprint quality of 4**	**Fingerprint quality of 5**	**Total fingerprint assessments**
Intentional	161	57	26	13	8	265
Unintentional	124	67	33	28	13	265

A)



B)

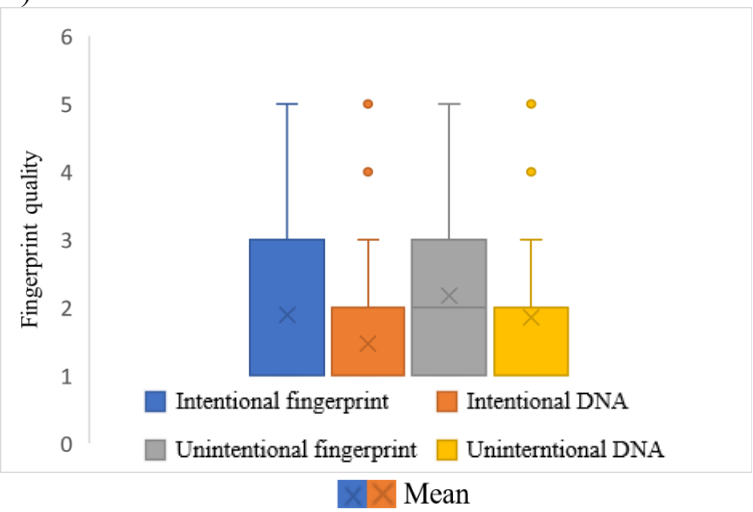


Figure 14: Fingerprint quality for the deposit method. Samples were either intentional latent prints or unintentional latent prints. Each category had 265 quality assessments (A). Samples went through the following four pathways: 1) intentional latent print when fingerprint enhancement was performed first, 2) intentional latent print when DNA analysis was performed first, 3) unintentional latent print when fingerprint enhancement was performed first, and 4) unintentional latent print when DNA analysis was performed first. Both categories that went through fingerprinting enhancement first had 130 quality assessments. Both categories that went through DNA analysis first had 135 quality assessments (B).

4.2.6. Sequence of analysis for money samples

The fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 24 samples. The fingerprint quality for the four different money categories were: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with

1,2-indanedione, 3) DNA analysis first then fingerprinting with ninhydrin, and 4) DNA analysis first then fingerprinting with 1,2-indanedione. Fingerprinting first with 1,2-indanedione had the highest mean of 1.633333±0.850287 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Fingerprinting first with ninhydrin had the next highest mean of 1.5±0.937715 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Next highest was DNA analysis first and then fingerprinting with 1,2-indanedione with a mean of 1.466667±0.776079 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Lastly, DNA analysis first then fingerprinting with ninhydrin had the lowest fingerprint quality of 1.4±0.498273 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.703006. The differences in the fingerprint qualities for each of these categories were not significant. There was one outlier for fingerprint enhancement first with ninhydrin, one outlier for fingerprint enhancement first with 1,2-indanedione, and one outlier for DNA analysis first and then fingerprint enhancement with 1,2-indanedione (Figure 15). Outliers were determined by using excel® version 2310.

Table 32: Fingerprint ANOVA results for money samples. The four different sequences of analysis for money were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first and then fingerprint enhancement with ninhydrin, and 4) DNA analysis first and then fingerprint enhancement with 1,2-indanedione.

Analysis sequence	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint ninhydrin	30	45	0.937715492	1.5		
Fingerprint 1,2-indanedione	30	49	0.850287308	1.6333333		
DNA ninhydrin	30	42	0.498272879	1.4		
DNA 1,2-indanedione	30	44	0.776079152	1.4666667		
					0.703006	Not significant

Table 33: Fingerprint quality results of money samples. The four different sequences of analysis for money were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first and then fingerprint enhancement with ninhydrin, and 4) DNA analysis first and then fingerprint enhancement with 1,2-indanedione. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Analysis Sequence	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
Fingerprint ninhydrin	22	3	3	2	0	30
Fingerprint 1,2-indanedione	17	8	4	1	0	30
DNA ninhydrin	18	12	0	0	0	30
DNA 1,2-indanedione	20	7	2	1	0	30

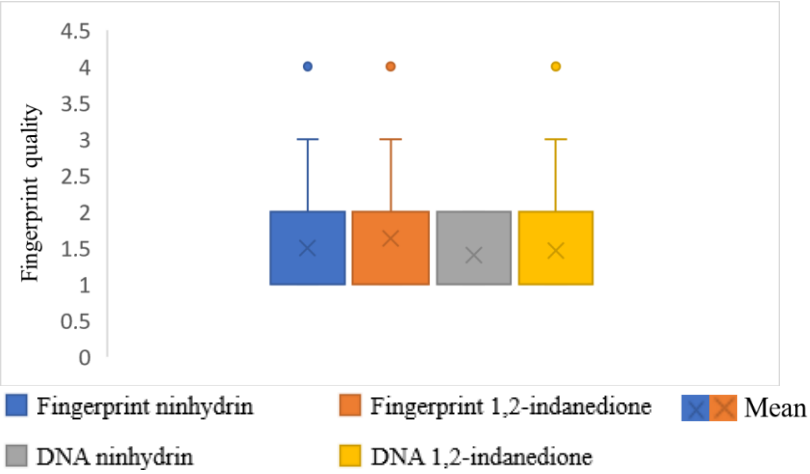


Figure 15: Fingerprint quality of money samples. The four different sequences of analysis for money were as follows: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. Each category had 30 quality assessments.

A)

B)

C)

D)

E)

Figure 16: The best fingerprint quality for money samples. Fingerprint enhancement first with ninhydrin and then DNA analysis (mean quality of 3.4) (A). Fingerprint enhancement first with 1,2-indanedione and then DNA analysis (mean quality of 2.8) (B). DNA analysis first then fingerprinting with ninhydrin (mean quality of 2) (C-D). DNA analysis first then fingerprinting with 1,2-indanedione (mean quality of 2.8) (E).

4.2.7. Sequence of analysis for copy paper samples

The fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 24 samples. The fingerprint quality for the four different copy paper categories were: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first then fingerprinting with ninhydrin, and 4) DNA analysis first then fingerprinting with 1,2-indanedione. Fingerprinting first with ninhydrin had the highest mean value of 2.633333±1.188547 fingerprint quality with 6.67% of the fingerprint assessments resulting in a fingerprint quality of 5. Fingerprinting first with 1,2-indanedione had the next highest mean fingerprint quality of 2.333333±0.922266 fingerprint quality with 3.33% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA analysis first then fingerprinting with 1,2-indanedione had the next highest fingerprint quality of 2±1.286535 fingerprint quality with 6.67% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA analysis first then fingerprinting with ninhydrin had the lowest fingerprint quality of 1.9±1.09387 fingerprint quality with 3.33% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.054438. The differences in the fingerprint quality for each of the copy paper categories were not significant. There was one outlier for fingerprint enhancement first with 1,2-indanedione, and one outlier for DNA analysis first and then fingerprint enhancement with ninhydrin (Figure 17). Outliers were determined by using excel® version 2310.

Table 34: Fingerprint ANOVA results for copy paper samples. The four different sequences of analysis for copy paper were as follows: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method.

Analysis sequence	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint ninhydrin	30	79	1.188546877	2.6333333		
Fingerprint 1,2-indanedione	30	70	0.922266075	2.3333333		
DNA ninhydrin	30	57	1.093870067	1.9		
DNA 1,2-indanedione	30	60	1.286535042	2		
					0.054438	Not significant

Table 35: Fingerprint quality results of copy paper samples. The four different sequences of analysis for copy paper were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first and then fingerprint enhancement with ninhydrin, and 4) DNA analysis first and then fingerprint enhancement with 1,2-indanedione. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Analysis sequence	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
Fingerprint ninhydrin	6	8	9	5	2	30
Fingerprint 1,2-indanedione	4	16	7	2	1	30
DNA ninhydrin	14	9	4	2	1	30
DNA 1,2-indanedione	16	4	6	2	2	30

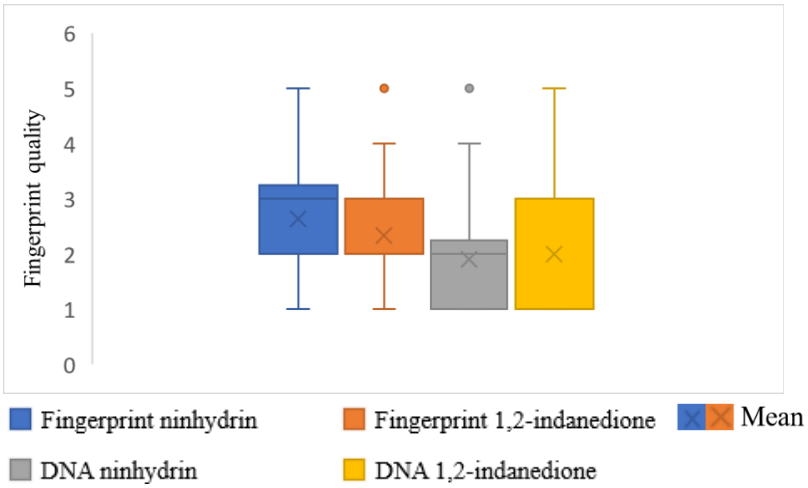


Figure 17: Fingerprint quality for copy paper samples**.** The four different sequences of analysis for copy paper were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. Each category had 30 quality assessments.

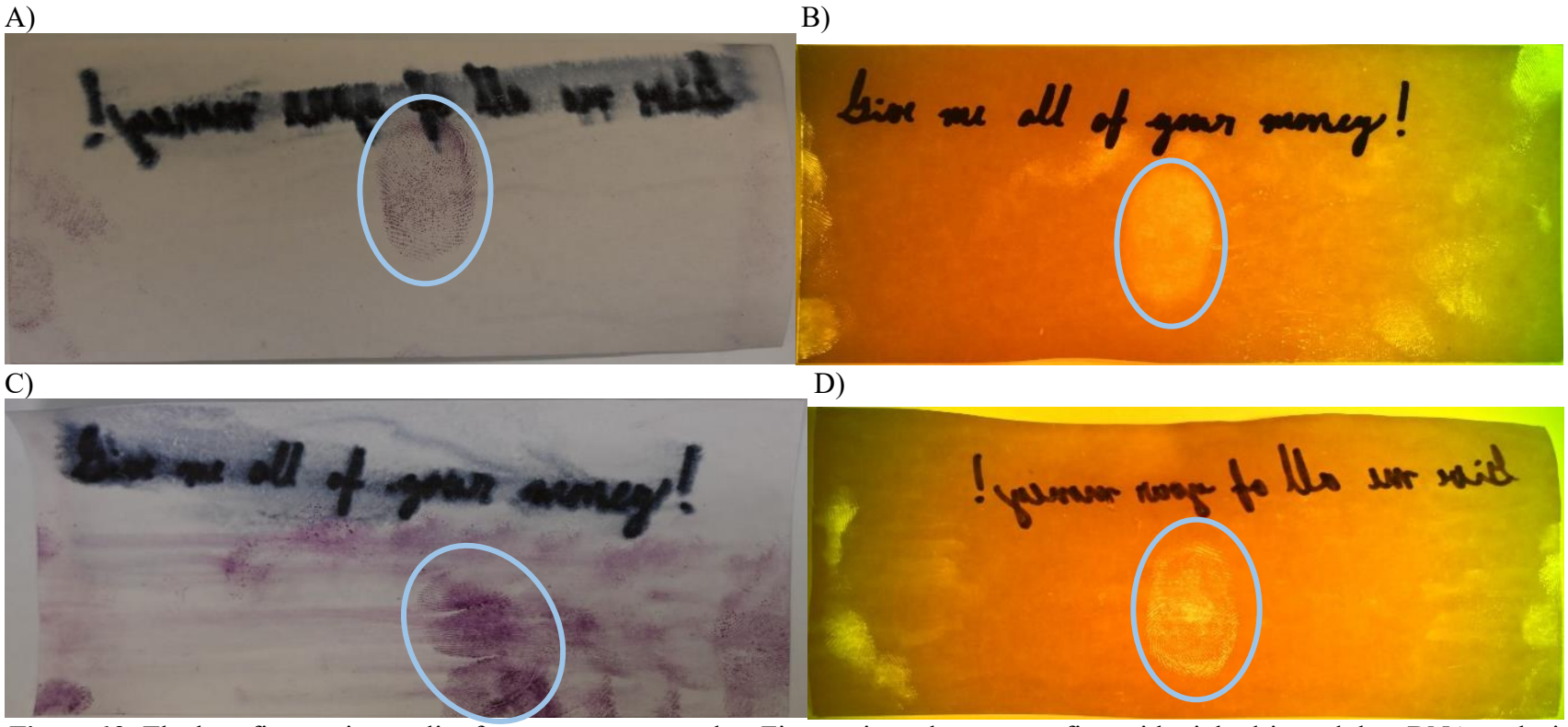


Figure 18: The best fingerprint quality for copy paper samples. Fingerprint enhancement first with ninhydrin and then DNA analysis (mean quality of 4.4) (A). Fingerprint enhancement first with 1,2-indanedione and then DNA analysis (mean quality of 3.8) (B). DNA analysis first then fingerprinting with ninhydrin (mean quality of 3.8) (C). DNA analysis first then fingerprinting with 1,2-indanedione (mean quality of 4) (D).

4.2.8. Sequence of analysis for cardboard samples

The fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 24 samples. The fingerprint quality for the four different cardboard categories were: 1) fingerprint enhancement first with ninhydrin, 2) fingerprinting first with 1,2-indanedione, 3) DNA analysis first then fingerprinting with ninhydrin, and 4) DNA analysis first then fingerprinting with 1,2-indanedione. Fingerprinting first with ninhydrin had the highest mean of 1.9±1.348051 fingerprint quality with 3.33% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA analysis first then fingerprinting with ninhydrin had the next highest mean quality of 1.7±1.149213 fingerprint quality with 3.33% of the fingerprint assessments resulting in a fingerprint quality of 5. Fingerprinting first with 1,2-indanedione had the next highest mean quality of 1.66667±0.379049 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Lastly, DNA analysis first then fingerprinting with 1,2-indanedione had a mean fingerprint quality of 1.0±0.0 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.000339. The difference of fingerprint quality in the four groups were significant. The Tukey-Kramer's HSD test was performed, and a Q value above 3.74 was considered significant. The comparison of fingerprinting first with ninhydrin versus fingerprint enhancement first with 1,2-indanedione had a Q value of 4.43454. The comparison of fingerprinting first with ninhydrin versus DNA analysis first then fingerprinting with 1,2-indanedione also had a Q value of 5.442391. Lastly, the comparison of DNA analysis first with ninhydrin versus DNA analysis with 1,2-indanedione had a Q value of 4.23297. Fingerprinting first with ninhydrin had a significantly higher fingerprint quality than both fingerprinting first with 1,2-indanedione and DNA analysis first then fingerprinting with 1,2-indanedione for cardboard samples. DNA

analysis first with ninhydrin had a significantly higher fingerprint quality than DNA analysis then fingerprinting with 1,2-indanedione for cardboard samples. There was one outlier for fingerprint enhancement first with 1,2-indanedione, and one outlier for DNA analysis first and then fingerprint enhancement with 1,2-indanedione (Figure 19). Outliers were determined by using excel® version 2310.

Table 36: Fingerprint ANOVA results for cardboard samples. The four different sequences of analysis for cardboard were as follows: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method.

Analysis sequence	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
Fingerprint ninhydrin	30	57	1.348050956	1.9		
Fingerprint 1,2-indanedione	30	35	0.379049022	1.1666667		
DNA ninhydrin	30	51	1.149212624	1.7		
DNA 1,2-indanedione	30	30	0	1		
					0.000339	Significant

Table 37: Fingerprint quality results for cardboard samples. The four different sequences of analysis for cardboard were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first and then fingerprint enhancement with ninhydrin, and 4) DNA analysis first and then fingerprint enhancement with 1,2-indanedione. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Analysis Sequence	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
Fingerprint ninhydrin	20	0	4	5	1	30
Fingerprint 1,2-indanedione	25	5	0	0	0	30
DNA ninhydrin	20	3	4	2	1	30
DNA 1,2-indanedione	30	0	0	0	0	30

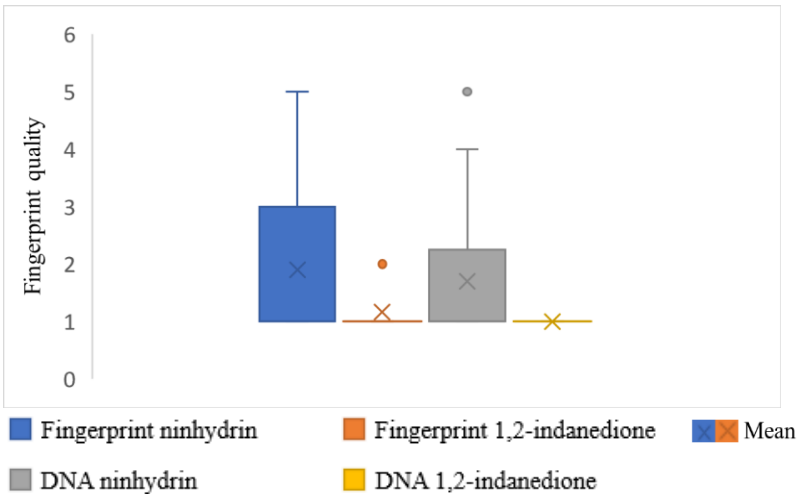


Figure 19: Fingerprint quality for cardboard samples**.** The four different sequences of analysis for cardboard were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. Each category had 30 quality assessments.

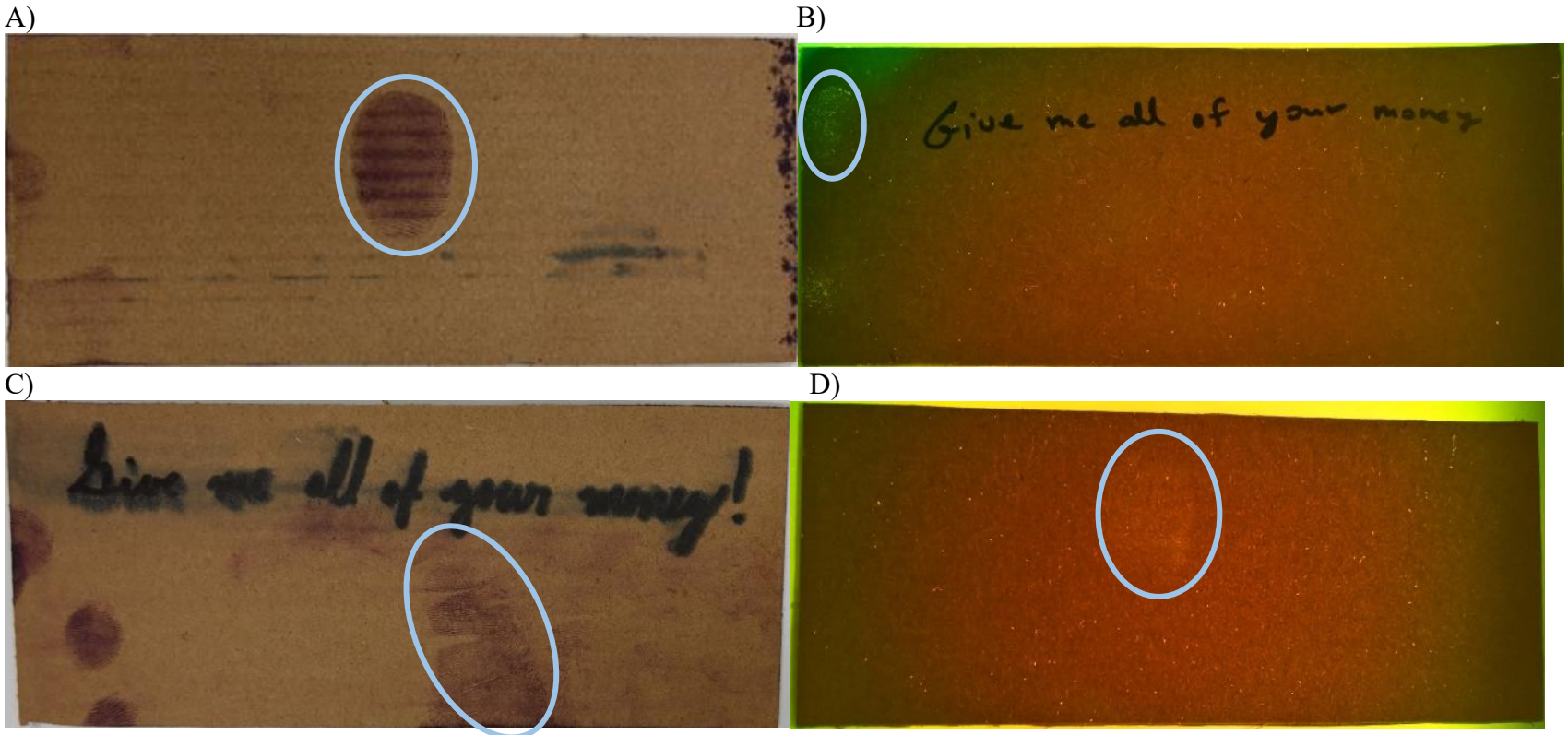


Figure 20: The best fingerprint quality for cardboard samples. Fingerprint enhancement first with ninhydrin and then DNA analysis (mean quality of 3.8) (A). Fingerprint enhancement first with 1,2-indanedione and then DNA analysis (mean quality of 2) (B). DNA analysis first then fingerprinting with ninhydrin (mean quality of 3.8) (C). DNA analysis first then fingerprinting with 1,2-indanedione (mean quality of 1) (D).

4.2.9. Sequence of analysis for cardstock samples

The fingerprint examiners from the Sacramento Sheriff's Office assessed the quality of the fingerprints for 24 samples. The fingerprint quality for the four different cardstock categories were: 1) fingerprinting enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first then fingerprinting with ninhydrin, and 4) DNA analysis first then fingerprinting with 1,2-indanedione. Fingerprint enhancement first with ninhydrin had the highest mean fingerprint quality of 2.433333±1.633345 fingerprint quality with 20.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Fingerprint enhancement first with 1,2-indanedione had the next highest mean of 2.366667±1.272612 fingerprint quality with 6.67% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA analysis first then fingerprinting with ninhydrin had the third highest mean fingerprint quality of 1.766667±1.072648 fingerprint quality with 3.33% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA analysis first then fingerprinting with 1,2-indanedione had the lowest mean fingerprint quality of 1.533333±0.973204 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.01366. The differences of fingerprint qualities for each of the categories were significant. The Tukey-Kramer's HSD test was performed to determine which categories had significant differences in fingerprint qualities. A Q value above 3.74 was considered significant. Only the comparison of fingerprinting with ninhydrin prior to DNA analysis versus DNA analysis first then fingerprinting with 1,2-indanedione had a significant Q value (3.901672). This means that for cardstock samples fingerprinting first with ninhydrin had significantly higher fingerprint qualities than DNA analysis first then fingerprint enhancement with 1,2-indanedione. There were two outliers for DNA analysis first and then fingerprint enhancement with ninhydrin, and one

outlier for DNA analysis and then fingerprint enhancement with 1,2-indanedione (Figure 21).

Outliers were determined by using excel® version 2310.

Table 38: Fingerprint ANOVA results for cardstock samples. The four different sequences of analysis for cardstock were as follows: 1) fingerprinting first with ninhydrin method, 2) fingerprinting first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method.

Analysis sequence	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Fingerprint ninhydrin	30	73	1.633345062	1.6333451		
Fingerprint 1,2-indanedione	30	71	1.272611579	1.2726116		
DNA ninhydrin	30	53	1.072648457	1.0726485		
DNA 1,2-indanedione	30	46	0.973204211	0.9732042		
					0.01366	Significant

Table 39: Fingerprint quality results of cardstock samples. The four different sequences of analysis for cardstock were as follows: 1) fingerprint enhancement first with ninhydrin, 2) fingerprint enhancement first with 1,2-indanedione, 3) DNA analysis first and then fingerprint enhancement with ninhydrin, and 4) DNA analysis first and then fingerprint enhancement with 1,2-indanedione. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Analysis sequence	**Fingerprint quality of 1**	**Fingerprint quality of 2**	**Fingerprint quality of 3**	**Fingerprint quality of 4**	**Fingerprint quality of 5**	**Total fingerprint assessments**
Fingerprint ninhydrin	13	7	0	4	6	30
Fingerprint 1,2-indanedione	9	10	4	5	2	30
DNA ninhydrin	16	9	2	2	1	30
DNA 1,2-indanedione	21	5	1	3	0	30

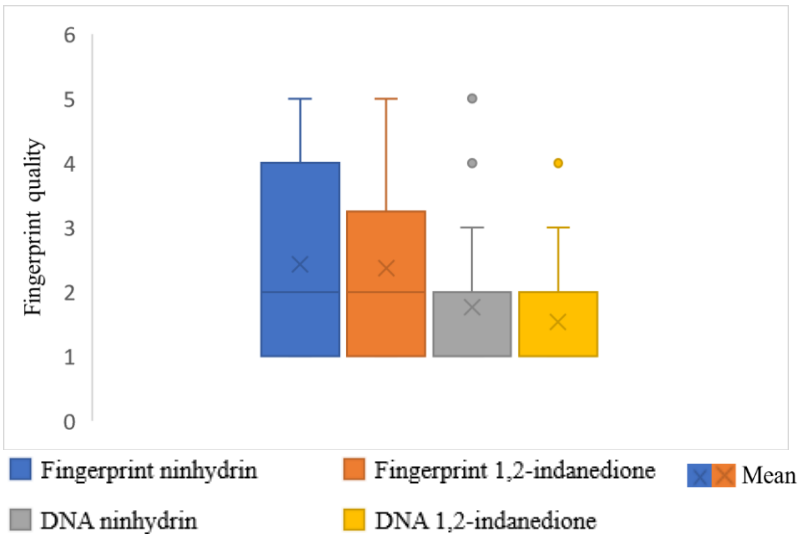


Figure 21: Fingerprint quality for cardstock samples. The four different sequences of analysis for cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. Each category had 30 quality assessments.

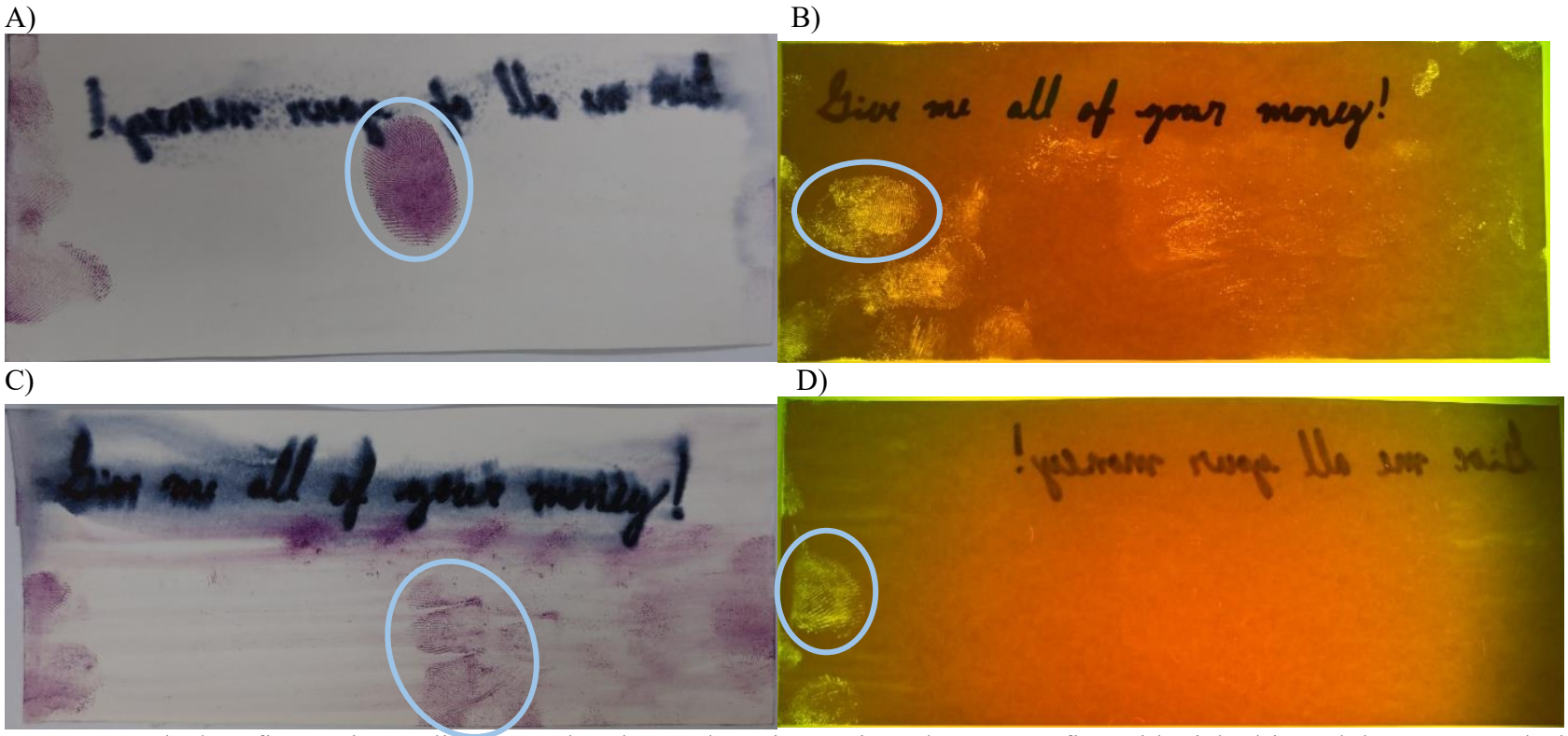


Figure 22: The best fingerprint quality for cardstock samples. Fingerprint enhancement first with ninhydrin and then DNA analysis (mean quality of 4.6) (A.). Fingerprint enhancement first with 1,2-indanedione and then DNA analysis (mean quality of 3.8) (B). DNA analysis first then fingerprinting with ninhydrin (mean quality of 3.6) (C). DNA analysis first then fingerprinting with 1,2-indanedione (mean quality of 3) (D).

4.2.10. Sequence of analysis for thermal paper samples

The fingerprint examiners from Sacramento Sheriff's Office assessed the quality of the fingerprints for 12 samples. The examiners made their assessments from pictures of fingerprints. Pictures that should have showcased the fingerprints present on one of the thermal paper samples that went through fingerprinting first with 1,2-indanedione and then DNA analysis were missed. As a result, the examiners were unable to assess the quality of this single sample. Therefore, the statistics for this single sample were omitted from the data. The fingerprint quality for the two different cardstock categories were: 1) fingerprinting first with 1,2-indanedione, and 2) DNA analysis first then fingerprinting with 1,2-indanedione. Fingerprinting with 1,2-indanedione first had a higher mean fingerprint quality of 2.5±1.277333 fingerprint quality with 10.00% of the fingerprint assessments resulting in a fingerprint quality of 5. DNA analysis first then fingerprinting with 1,2-indanedione had a mean fingerprint quality of 2.233333±1.194335 fingerprint quality of 6.67% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was 0.455524. There was no significant difference of fingerprint quality between the two groups for thermal paper.

Table 40: Fingerprint ANOVA results for thermal paper samples. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting first with 1,2-indanedione method, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method.

First analysis	Count	Sum	Standard deviation	Mean	P-value	ANOVA significance
1,2-Indanedione	20	50	1.277332747	2.5		
DNA	30	67	1.194335289	2.2333333		
					0.455524	Not significant

Table 41: Fingerprint quality results of thermal paper samples**.** The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting first with 1,2-indanedione method, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Analysis sequence	**Fingerprint quality of 1**	**Fingerprint quality of 2**	**Fingerprint quality of 3**	**Fingerprint quality of 4**	**Fingerprint quality of 5**	**Total fingerprint assessments**
Fingerprint 1,2-indanedione	5	6	5	2	2	20
DNA 1,2-indanedione	9	12	4	3	2	30

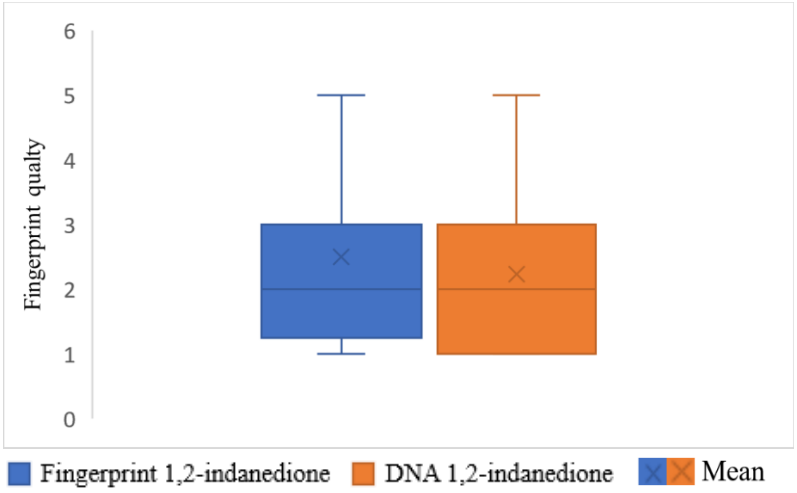


Figure 23: Fingerprint quality for thermal paper samples. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method. The first category had 20 quality assessments while the second category had 30 quality assessments.

A)

B)

Figure 24: The best fingerprint quality for thermal paper samples. Fingerprint enhancement first with 1,2-indanedione and then DNA analysis (mean quality of 4) (A). DNA analysis first then fingerprinting with 1,2-indanedione (mean quality of 4.2) (B).

4.2.11. Paper substrates

Five fingerprint examiners assessed the quality of the fingerprints for 108 samples. The examiners made their assessments from pictures of fingerprints. Pictures that should have showcased one of the fingerprints present on one of the thermal paper samples that went through fingerprinting first with 1,2-indanedione and then DNA analysis were missed. As a result, the examiners were unable to assess the quality of this single sample. Therefore, the statistics for this single sample were omitted from the data. Thermal paper had the highest fingerprint quality with a mean of 2.34±1.22241 fingerprint quality with 8.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Copy paper had the second highest mean value of 2.216667±1.153608 fingerprint quality with 5.00% of the fingerprint assessments resulting in a

fingerprint quality of 5. Next was cardstock with a mean of 2.025±1.305853 fingerprint quality with 7.50% of the fingerprint assessments resulting in a fingerprint quality of 5. Money had the fourth highest mean of 1.5±0.777844 fingerprint quality with 0.00% of the fingerprint assessments resulting in a fingerprint quality of 5. Cardboard had the lowest fingerprint quality with a mean of 1.441667±0.968372 fingerprint quality with 1.67% of the fingerprint assessments resulting in a fingerprint quality of 5. The ANOVA p-value was $5.9x10^{-11}$. The differences in the fingerprint qualities were significant. The Tukey-Kramer's HSD test was performed and any Q above 3.86 was considered significant. Money versus copy paper (Q value of 7.235307), money versus cardstock (Q value of 5.300283), and cardboard versus cardstock (Q value of 5.889203). Each of these values were above the 3.86 value. Copy paper and cardstock both had significantly higher fingerprint quality than money, and cardstock had significantly higher fingerprint quality than cardboard. There was one outlier for money, and four outliers for cardboard (Figure 25). Outliers were determined by using excel® version 2310.

Table 42: Fingerprint ANOVA results for the paper substrates. Comparison of the fingerprint quality assessment for the paper substrates.

Paper substrate	**Count**	**Sum**	**Standard deviation**	**Mean**	**P-value**	**ANOVA significance**
Money	120	180	0.777844468	1.5		
Copy paper	120	266	1.153608393	2.2166667		
Cardboard	120	173	0.968372396	1.4416667		
Cardstock	120	243	1.305853017	2.025		
Thermal paper	50	117	1.222409798	2.34		
					5.9E-11	Significant

Table 43: Fingerprint Tukey-Kramer's HSD test results for the paper substrates. Comparison of the fingerprint quality assessment for the paper substrates.

Paper substrate comparison	Absolute difference	Standard error	Q Tukey	Q critical	Significance
Money vs. copy paper	0.716666667	0.09905132	7.235307	3.86	Significant
Money vs. cardboard	0.058333333	0.09905132	0.58892	3.86	Not significant
Money vs. cardstock	0.525	0.09905132	5.300283	3.86	Significant
Money vs. thermal paper	0.84	5.425716326	0.154818	3.86	Not significant
Copy paper vs. cardboard	0.775	0.09905132	1.935024	3.86	Not significant
Copy paper vs. cardstock	0.191666667	0.09905132	1.935024	3.86	Not significant
Copy paper vs. thermal paper	0.84	5.425716326	0.154818	3.86	Not significant
Cardboard vs. cardstock	0.583333333	0.09905132	5.889203	3.86	Significant
Cardboard vs. thermal paper	0.898333333	5.425716326	0.16557	3.86	Not significant
Cardstock vs. thermal paper	0.315	5.425716326	0.058057	3.86	Not significant

Table 44: Fingerprint quality result for the paper substrates. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Paper substrate	Fingerprint quality of 1	Fingerprint quality of 2	Fingerprint quality of 3	Fingerprint quality of 4	Fingerprint quality of 5	Total fingerprint assessments
Money	77	30	9	4	0	120
Copy paper	40	37	26	11	6	120
Cardboard	95	8	8	7	2	120
Cardstock	59	31	7	14	9	120
Thermal paper	14	18	9	5	4	50

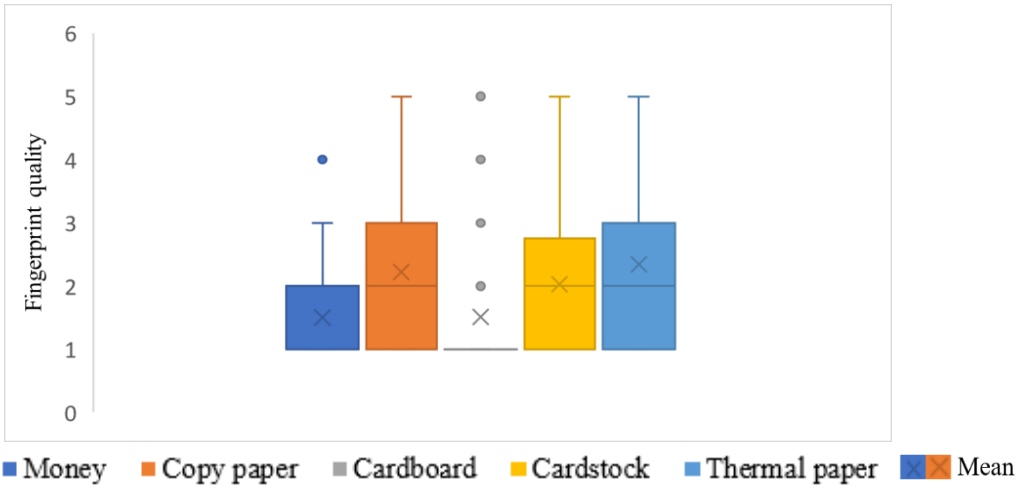


Figure 25: Fingerprint quality of the paper substrates. Money, copy paper, cardboard, and cardstock each had 120 quality assessments, while thermal paper had 50 quality assessments.

4.2.12. Each paper substrate and their sequence of analysis

When comparing the fingerprint quality for each of the paper substrates for fingerprinting first with ninhydrin, copy paper had the highest mean fingerprint quality followed by cardstock, cardboard, and lastly money. The ANOVA p-value was 0.003967. There is a significant difference in the fingerprint quality for the paper substrates. Tukey-Kramer's HSD test was used. A Q value above 3.74 was considered significant. The comparison for money versus copy paper (Q value of 4.76902), and money versus cardstock, (Q value of 3.927429) both had Q values above 3.74. When fingerprint enhancement was performed first with ninhydrin, both copy paper and cardstock had significantly higher fingerprint quality than money. The rest of the fingerprint comparisons for fingerprinting first with ninhydrin had a Q value below 3.74. There were no significant fingerprint quality differences for the rest of the paper substrates when fingerprinting with ninhydrin was performed first. There was one outlier for money (Figure 26). Outliers were determined by using excel® version 2310.

In the case of fingerprinting first with 1,2-indanedione, thermal paper had the highest mean fingerprint quality followed by cardstock, copy paper, money, and lastly cardboard. The

ANOVA p-value was $5.54x10^{-07}$. The difference in the fingerprint quality for the paper substrates was significant for fingerprinting first with 1,2-indanedione. Tukey-Kramer's HSD test was used. A Q value above 3.92 was considered significant. The comparisons of money versus copy paper (Q value of 3.941094), money versus cardstock (Q value of 4.128765), money versus thermal paper (Q value of 4.364312), copy paper versus cardboard (Q value of 6.56849), cardboard versus cardstock (Q value of 6.756161), and cardboard versus thermal paper (Q value of 6.714327) all had Q values above 3.92. Copy paper, cardstock, and thermal paper all had significantly higher fingerprint qualities than both money and cardboard. The rest of the comparisons did not have Q values above 3.92. There was no significant difference in the fingerprint qualities for the comparisons when fingerprint enhancement was performed first with 1,2-indanedione. There was one outlier for copy paper, and one outlier for cardboard (Figure 26). Outliers were determined by using excel® version 2310.

When comparing the fingerprint quality of the different paper substrates when they go through DNA analysis first and then fingerprint enhancement with ninhydrin, copy paper had the highest fingerprint quality followed by cardstock, cardboard, and lastly money. The ANOVA p-value was 0.255336. There was no significant difference in the fingerprint quality of the paper substrates when DNA analysis was performed first and then fingerprint enhancement with ninhydrin. There was one outlier for cardboard, and two outliers for cardstock (Figure 26). Outliers were determined by using excel® version 2310.

When comparing the fingerprint quality of the different paper substrates when they go through DNA analysis first and then fingerprint enhancement with 1,2-indanedione, thermal paper had the highest fingerprint quality followed by copy paper, cardstock, money, and lastly cardboard. The ANOVA p-value was $1.49x10^{-05}$. The Tukey-Kramer's HSD test was performed

and a Q value higher than 3.92 was considered significant. The following paper substrate comparisons had Q values above 3.92: copy paper versus cardboard (Q value of 5.090397), and cardboard versus thermal paper (Q value of 6.278156). Both copy paper and thermal paper had a significantly higher fingerprint quality than cardboard when comparing the samples that went through DNA analysis first and then fingerprint enhancement with 1,2-indanedione. There was one outlier for cardstock (Figure 26). Outliers were determined by using excel® version 2310.

Table 45: Fingerprint ANOVA results for paper substrates and their sequences of analysis. The four different sequences of analysis for money, copy paper, cardboard and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method.

Paper substrates, first analysis	**Count**	**Sum**	**Standard deviation**	**Average**	**P-value**	**ANOVA significance**
Ninhydrin						
Money	30	45	0.937715492	1.5		
Copy paper	30	79	1.188546877	2.6333333		
Cardboard	30	57	1.348050956	1.9		
Cardstock	30	73	1.633345062	2.4333333		
					0.003967	Significant
1,2-Indanedione						
Money	30	49	0.850287308	1.6333333		
Copy paper	30	70	0.922266075	2.3333333		
Cardboard	30	35	0.379049022	1.1666667		
Cardstock	30	71	1.272611579	2.3666667		
Thermal paper	20	50	1.277332747	2.5		
					5.54E-07	Significant
DNA Ninhydrin						
Money	30	42	0.498272879	1.4		
Copy paper	30	57	1.093870067	1.9		
Cardboard	30	51	1.149212624	1.7		
Cardstock	30	53	1.072648457	1.7666667		
					0.255336	Not significant
DNA 1,2-Indanedione						
Money	30	44	0.776079152	1.4666667		
Copy paper	30	60	1.286535042	2		
Cardboard	30	30	0	1		
Cardstock	30	46	0.973204211	1.5333333		
Thermal paper	30	67	1.194335289	2.2333333		
					1.49E-05	Significant

Table 46: Fingerprint Tukey-Kramer's HSD test results for each paper substrate and their sequence of analysis. The four different sequences of analysis for money, copy paper, cardboard, and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

Paper substrates, first analysis comparison	**Absolute difference**	**Standard error**	**Q Tukey**	**Q critical**	**Significance**
Ninhydrin					
Money vs. copy paper	1.133333	0.2376448	4.76902	3.74	Significant
Money vs. cardboard	0.4	0.2376448	1.683184	3.74	Not significant
Money vs. cardstock	0.933333	0.2376448	3.927429	3.74	Significant
Copy paper vs. cardboard	0.733333	0.2376448	3.085837	3.74	Not significant
Copy paper vs. cardstock	0.2	0.2376448	0.841592	3.74	Not significant
Cardboard vs. cardstock	0.533333	0.2376448	2.244245	3.74	Not significant
1,2-Indanedione					
Money vs. copy paper	0.7	0.1776156	3.941094	3.92	Significant
Money vs. cardboard	0.466667	0.1776156	2.627396	3.92	Not significant
Money vs. cardstock	0.733333	0.1776156	4.128765	3.92	Significant
Money vs. thermal paper	0.866667	0.198580	4.364312	3.92	Significant
Copy paper vs. cardboard	1.166667	0.1776156	6.56849	3.92	Significant
Copy paper vs. cardstock	0.033333	0.1776156	0.187671	3.92	Not significant
Copy paper vs. thermal paper	0.166667	0.19858035	0.839291	3.92	Not significant
Cardboard vs. cardstock	1.2	0.1776156	6.756161	3.92	Significant
Cardboard vs. thermal paper	1.333333	0.198580	6.714327	3.92	Significant
Cardstock vs. thermal paper	0.133333	0.198580	0.671433	3.92	Not significant
DNA then 1,2-indanedione					
Money vs. copy paper	0.533333	0.196448	2.714878	3.92	Not significant
Money vs. cardboard	0.466667	0.196448	2.375518	3.92	Not significant
Money vs. cardstock	0.066667	0.196448	0.33936	3.92	Not significant

Money vs. thermal paper	0.766667	0.196448	3.902637	3.92	Not significant
Copy paper vs. cardboard	1	0.196448	5.090397	3.92	Significant
Copy paper vs. cardstock	0.466667	0.196448	2.375518	3.92	Not significant
Copy paper vs. thermal paper	0.233333	0.196448	1.187759	3.92	Not significant
Cardboard vs. cardstock	0.533333	0.196448	2.714878	3.92	Not significant
Cardboard vs. thermal paper	1.233333	0.196448	6.278156	3.92	Significant
Cardstock vs. thermal paper	0.7	0.196448	3.563278	3.92	Not significant

Table 47: Fingerprint quality results for each paper substrate and their sequences of analysis. The four different sequences of analysis for money, copy paper, cardboard, and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method. Five fingerprint examiners assigned each sample a fingerprint quality of 1-5 with 5 being the highest quality.

First analysis on paper substrates	**Fingerprint Quality of 1**	**Fingerprint Quality of 2**	**Fingerprint Quality of 3**	**Fingerprint Quality of 4**	**Fingerprint Quality of 5**	**Total Fingerprint Assessments**
Ninhydrin						
Money	22	3	3	2	0	30
Copy paper	6	8	9	5	2	30
Cardboard	20	0	4	5	1	30
Cardstock	13	7	0	4	6	30
1,2-Indanedione						
Money	17	8	4	1	0	30
Copy paper	4	16	7	2	1	30
Cardboard	25	5	0	0	0	30
Cardstock	9	10	4	5	2	30
Thermal paper	5	6	5	2	2	20
DNA ninhydrin	18	12	0	0	0	30
Money	14	9	4	2	1	30
Copy paper	20	3	4	2	1	30
Cardboard	16	9	2	2	1	30
Cardstock	16	9	2	2	1	30
DNA 1,2-indanedione						
Money	20	7	2	1		
Copy paper	16	4	6	2	2	30
Cardboard	30	0	0	0	0	30
Cardstock	21	5	1	3	0	30
Thermal paper	9	12	4	3	2	30

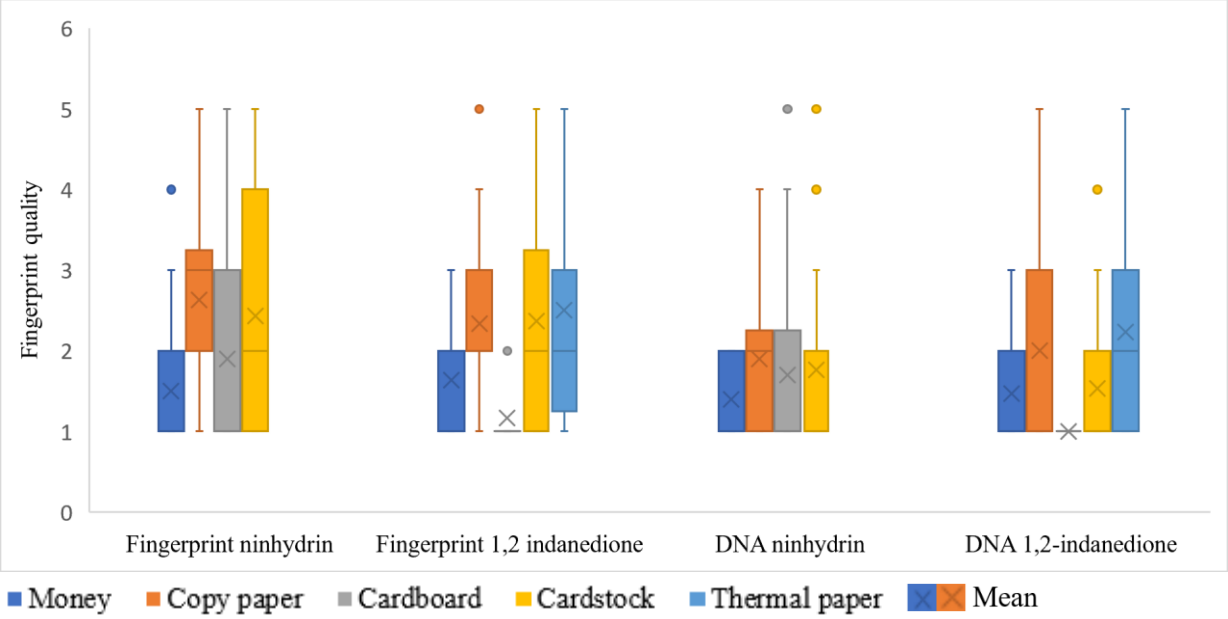


Figure 26: Fingerprint quality for each paper substrate and their sequence of analysis. The four different sequences of analysis for money, copy paper, cardboard, and cardstock were as follows: 1) fingerprint enhancement first with ninhydrin method, 2) fingerprint enhancement first with 1,2-indanedione method, 3) DNA analysis first then fingerprinting with ninhydrin method, and 4) DNA analysis first then fingerprinting with 1,2-indanedione method. The two different sequences of analysis for thermal paper were as follows: 1) fingerprinting with 1,2-indanedione method first, and 2) DNA analysis first then fingerprinting with 1,2-indanedione method. Each ninhydrin method had 30 quality assessments for money, copy paper, cardboard, and cardstock. Fingerprint enhancement first with 1,2-indanedione had 30 quality assessments for money, copy paper, cardboard, and cardstock, but only 20 quality assessments for thermal paper. DNA analysis first then fingerprint enhancement with 1,2-indanedion had 30 quality assessments for each of the paper substrates.

5. Conclusions

When analyzing the quality and quantity of the DNA for samples that went through fingerprinting first and then DNA, and samples that went through DNA analysis first and then fingerprinting enhancement, there was no significant difference in the quality and quantity of the two groups except for money. Fingerprint enhancement with ninhydrin on money significantly decreased the number of loci that had alleles above the analytical threshold compared to samples that went through DNA analysis first. When examining the quality of fingerprints for both categories, and when DNA was analyzed prior to fingerprinting, the fingerprint quality

significantly decreased. Further, when comparing the quality of fingerprints for money, the difference in quality in the sequence of analysis was not significant. These results suggest that DNA analysis affects fingerprint quality more than fingerprint enhancement affects DNA quality and quantity for copy paper, cardboard, cardstock, and thermal paper. Thus, for the best results, fingerprint enhancement should be performed prior to DNA analysis for these four paper substrates. Since fingerprint enhancement significantly decreased the DNA quality and quantity for money samples, DNA analysis should be performed prior to fingerprint enhancement. The difference between the types of paper substrates could be attributed to the amount of cellulose in each of the paper substrates. The amino acids from the fingerprint absorb better with high cellulose surfaces (Almog, 2001), (Speaks, 1970), (Champod et al., 2004). There is little documentation on the cellulose content in each of the paper substrates, which could be subject for future studies to determine if the higher cellulose content of the substrate is more suitable for a specific fingerprint method when DNA analysis is also utilized.

The DNA results showed that money had a significantly higher mean DNA concentration than the other paper substrates, but both cardboard and cardstock had significantly more interpretable DNA profiles than money. These results might be due to money being a paper substrate that has been touched by many individuals who have deposited their DNA. Despite efforts to decontaminate the money before sampling, DNA could still have been present. This could have caused money samples to have more DNA and mixtures in the DNA profiles leading them to be uninterpretable. Further, copy paper, cardstock, and thermal paper each had significantly higher fingerprint quality results than money. These results could also be because money is handled by many people and thus the oils were deposited all over the paper limiting the amount of clear fingerprints. Future studies could use brand new money and clean it with 10%

bleach and then 70% ethanol to ensure there is no DNA or fingerprints on the money prior to fingerprint and DNA deposition on the sample.

Unintentional fingerprint deposit had significantly higher fingerprint quality than intentional fingerprint deposit. This could be because when the volunteers were unintentionally depositing their fingerprint, they were handling the substrate more allowing for more fingerprints to be deposited and providing a greater chance of depositing a better quality of fingerprint than when the volunteer only provided a single, intentional fingerprint.

Like money, cardboard also had significantly less fingerprint quality than copy paper, cardstock, and thermal paper. When comparing the cardboard fingerprint pathways, the fingerprint quality was significantly higher when ninhydrin method was used compared to 1,2-indanedione. This result is supported by Sirchie's product information (Sirchie, 2013). 1,2-Indanedione does not work as well on poor quality porous surfaces such as cardboard. These results suggests that the best sequence of analysis for cardboard samples is fingerprint enhancement with 1,2-indanedione first and then DNA analysis.

While previous studies focused on how the fingerprint enhancement process affects the DNA analysis, this study went a step further, to find the best sequence of analysis for using both identification methods. Studies found that ninhydrin decreases DNA quality and quantity on paper substrates, while this study found that ninhydrin only decreased DNA quality and quantity for money samples (Sewell et al., 2004; Carlin et al., 2023). Other studies found that fingerprint enhancement significantly decreases the DNA recovery on common office paper such as copy paper (Sewell et al., 2004; Zaghoul et al., 2019). This study did not find a significant decrease in the DNA concentration or quality when either ninhydrin or 1,2-indanedione enhancement was performed prior to DNA analysis. Further, this study confirmed findings that fingerprint

enhancement with 1,2-indanedione prior to DNA analysis does not significantly reduce DNA concentration and quality (Azoury et al., 2002; Tsai et al., 2016). Further, when previous studies compared two or more fingerprint methods and their effect on DNA quantity and quantity, there was a significant difference between the methods (Tsai et al., 2016; Bathrick et al., 2021). Through the various enhancement methods researched, one study found that DFO and physical developer were the most detrimental to DNA, while 1,2-indanedione-zinc (IND-Zn) and 1,2-indanedione-zinc/laser had the lowest impact on DNA quality and quantity (Bathrick et al., 2021). Another study found that both iodine fuming and 1,2-indanedione with ethyl acetate did not show an effect on DNA, while silver nitrate and 1,2-Indaendione with acetic acid reduced the quantity and quality of DNA (Tsai et al., 2016). In this study, when comparing the different fingerprint enhancement methods, the DNA quality and quantity were not significantly different, with the exception for money. When fingerprint enhancement was performed first, the number of loci with called alleles were significantly higher when 1,2-indanedione was used rather than ninhydrin.

This study aimed to identify the sequence of analysis that provides the best results for both fingerprint enhancement and DNA analysis on five different paper substrates (money, copy paper, cardboard, cardstock, and thermal paper). Based on this study's results, the best sequence of analysis on these five paper substrates is as follows:

- Money: DNA analysis first and then fingerprint enhancement with either ninhydrin or 1,2-indanedione.
    - If fingerprint enhancement is performed prior to DNA analysis, then 1,2-Indaendione fingerprint enhancement method should be used.

- Copy paper: fingerprint enhancement with either ninhydrin or 1,2-indanedione first, and then DNA analysis.
- Cardboard: fingerprint enhancement with ninhydrin first, and then DNA analysis.
- Cardstock: fingerprint enhancement with either ninhydrin or 1,2-indanedione first, and then DNA analysis.
- Thermal paper: fingerprint enhancement with 1,2-Indaendione first, and then DNA analysis.

Trace DNA typically contains low concentrations of DNA (Tang et al., 2020). The present study showed limited quality and quantity of DNA in the results. The limited quantity of DNA could be attributed to the collection stage of the study. The volunteers did not wash their hands for an hour prior to depositing their DNA and fingerprint(s). The short time interval between handling the different paper types and washing their hands limited the amount of DNA and oils that could have been deposited on the paper. Further, by not including a step where volunteers touch body areas such as the scalp line, the DNA present was likely limited. Future studies should have the volunteers refrain from washing their hands and should touch their scalp line prior to depositing their samples. The additional step of touching one's scalp will gather DNA and oil onto the volunteer's fingers which will then leave a better latent print and more trace DNA. This could also make the DNA results more reliable and diminish the number of outliers, such as the ones found in this study, since trace DNA is dependent on different variables. The most important factors of trace DNA deposition are friction and pressure, but other variables include the number of times fingers have touched an object previously and the moisture of the fingerprint. Touching the scalp line could decrease the chances that these variables affect the DNA results. Further, this study had small population sizes for each of the categories which

could have caused more outliers than if larger population sizes were used. Future studies should expand the population size for each of the different categories by performing 20 replicates and thus increase the reliability of the results. Further, this study only used ninhydrin and 1,2-Indaendione fingerprint enhancement methods because these methods are used at the Sacramento Sheriff's office. Future studies should broaden the number of enhancement methods to include more methods such as 1,8-Diazafluoren-9-one (DFO), 5-Methylthioninhydrin (5-MTN), vacuum metal deposition, silver nitrate, or silver physical developer.

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