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Short tandem repeat, or STR, analysis is expensive and often creates a waiting game for law enforcement agencies to receive these results due to high demand and the current backlog at forensic laboratories. ParaDNA® by LGC utilizes HyBeacon Probe technology to rapidly analyze DNA and provide a percentage value of the amount of DNA present, as well as 2-5 loci, depending on whether the Screening System or Intelligence System is being used. Currently, this technology is novel and is intended for sample screening and prioritizing purposes for the Plano Police Department. This validation study has shown that the ParaDNA® Screening System can obtain genetic data from touch DNA and trace DNA samples by using both direct and indirect sampling methods. The results from this validation have further indicated that if the Screening System provides a score higher than 60%, these samples should be prioritized and sent to forensic laboratories for full STR analysis.

VALIDATION OF THE PARADNA® SCREENING SYSTEM

WITH TOUCH DNA USING MOCK

EVIDENCE SAMPLES

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THESIS

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By

Whitney R. West, B.S.

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INTRODUCTION

Plano Police Department

The city of Plano, Texas is one of the largest communities in the Dallas metropolitan area. With a population of over 271,000 individuals, the Plano Police Department is the largest law enforcement agency in Collin County in northern Texas [3]. In a report completed at the end of 2016, the Plano Police Department Crime Analysis unit reported 393 violent crimes, and 5,486 property crimes alone [4]. DNA, or deoxyribonucleic acid, is often collected at these scenes to identify suspects and assist in criminal investigations. Currently, the Plano Police Department (PD) does not have an accredited DNA laboratory and therefore sends their evidence collections to a forensic laboratory without any knowledge of the actual DNA content present. Due to this uncertainty, time and resources are wasted, and a backlog is created in the forensic laboratories where the collections are sent. The Plano Police Department Crime Scene Investigation Unit recently acquired a new presumptive screening instrument for DNA, ParaDNA® (LGC, Teddington, Middlesex, UK), to assist in resolving the forensic evidence backlog, save time and resources, and provide clues to potential suspects for investigators. The current study aimed to validate ParaDNA® using various mock evidence samples chosen to reflect what the Plano Police Department Crime Laboratory commonly tests for touch DNA. Three aims were addressed in this study: 1) to determine whether the ParaDNA® Screening System can obtain genetic material and generate data from touch DNA on various substrates, 2) evaluate direct and indirect swabbing methods, and 3) assess instrument efficiency by comparing the percentage values from ParaDNA® to standard full STR analysis.

DNA Analysis

Short tandem repeats, or STRs, are repeating patterns of 2 to 7 base pairs in length present in different regions of DNA. Although STRs can be relatively small in length, they are highly variable between individuals and are used as the polymorphism of choice for forensic analysis [5, 6].

DNA analysis begins with the process of extraction where the cells are lysed, and the DNA is released. To confirm that the DNA being analyzed is human, and not from a source such as bacteria, a process called quantification is conducted. The quantification process allows analysts to not only confirm that the DNA is human but also reveals how much human DNA is present in a sample. Knowing the amount and concentration of DNA present in a sample is crucial for the next step in the process, polymerase chain reaction (PCR). PCR is a concentration-sensitive process that amplifies specific targeted regions of the DNA into millions of copies. The copies created in PCR contain the targeted regions of the DNA where the variable STRs of choice are found. Using capillary electrophoresis, these STRs are separated based on size and charge in a step also known as genetic analysis. An electropherogram, or DNA profile, is produced and analysts can then visualize the individualizing patterns and compare the profiles from evidence samples to known reference samples [5].

ParaDNA® by LGC

ParaDNA®, by LGC (Teddington, Middlesex, UK), is a novel screening instrument designed for consumers to determine what samples are suitable for DNA analysis. The instrument utilizes HyBeacon probe technology, developed by LGC. HyBeacon probes are fluorescent oligonucleotides that fluoresce when hybridized to target complementary DNA sequences. By utilizing melting curve analysis, these probes are able to determine alleles at

different target STR loci. With longer STR alleles, complications arise with this method due to the close melting temperatures of the repeat units. To differentiate these longer repeats, a non-fluorescent blocker oligonucleotide is used to reduce the length of the target sequence available to the probe in order to increase the melting temperature difference. Anchors are present on both the HyBeacon probes and the blocker oligonucleotide to flank the target sequences to prevent slippage and noise. The process can be visualized in Figure 1 [1, 7].

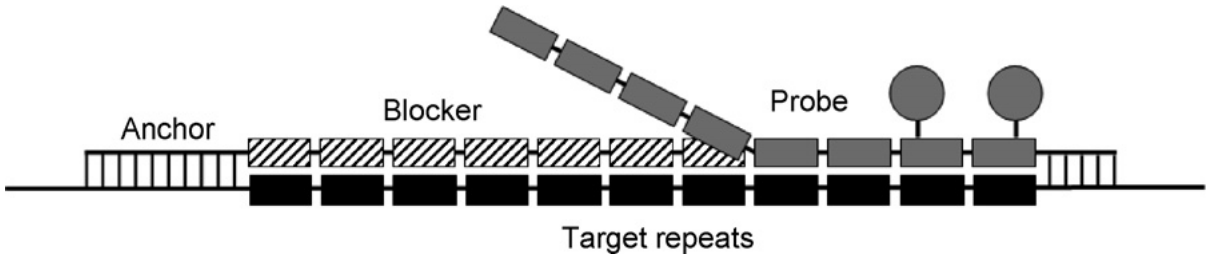

Figure 1 - HyBeacon Probe Process (adapted from French et al. 2008 [1])

By utilizing HyBeacon technology, ParaDNA® performs rapid STR analysis in approximately 75 minutes facilitating the selection of samples suitable for production of useful STR profiles. By using ParaDNA®, investigators are able to observe genetic data from evidence samples such as blood, saliva, and even touch DNA, where small amounts are known to exist. This genetic data can then be compared to a full STR profile once available [2].

The ParaDNA® Screening System provides a basic insight into the amount of DNA on evidence samples. The test identifies two STR loci (D16S539, THO1) and amelogenin, a common sex-identifying marker, along with a quantitative value of the DNA that is present (Figure 2).

Figure 2 - Report generated upon completion of the ParaDNA® Screening System [2]

After analysis, the results immediately provide a green indication detailing the quantity of DNA, or a red indication representing the absence of DNA. If enough DNA is present, the readout provides the sex of the contributor to the sample. The Screening System would give the Plano PD the ability to create a systematic protocol to determine what quantitative range of DNA from the ParaDNA® instrument is necessary to yield the most effective STR profiles, while avoiding costs associated with processing poor samples likely to fail subsequent testing [2].

Touch DNA

Touch DNA, also referred to as trace DNA, is defined as "DNA that is left behind from the skin cells when a person touches or comes into contact with an item." [8]. Trace DNA is an umbrella term that explains DNA "that cannot be attributed to an identifiable body fluid" [9]. It is commonly tested in forensic DNA analysis, but being invisible to the naked eye, it is difficult to know where and how much sample is being collected. Touch DNA could provide crucial information to a case, and to investigators if a profile is generated from the sample [10].

Direct and indirect transfer of DNA have been explained where direct transfer occurs while speaking or coughing; whereas, indirect transfer occurs through an intermediate such as from one individual to another individual to a substrate [9]. For example, when two individuals shake hands, the idea behind indirect transfer is that DNA is transferred between those individuals and then to secondary substrates that they touch or come into contact with

afterwards. Factors affecting the transfer of touch DNA to substrates have been briefly described by Daly et al. (2012), whereby it is suggested that different substrates such as wood, fabric, and glass can affect how the DNA is transferred, possibly due to the porous and non-porous nature of these surfaces [11]. It has also been suggested that the amount of DNA left behind in touch DNA could be donor dependent, implying that a "good" shedder would leave behind more DNA versus a "bad" shedder leaving behind less DNA [12]. LGC has reported validation results for touch DNA with varying substrates for the Screening System. In this study, common evidentiary substrates (mobile phones, tools, and latent prints) were analyzed with ParaDNA®. The results indicated the presence of small, unknown quantities involved with touch DNA [13].

By validating ParaDNA®, Plano PD hopes to determine the efficacy of touch DNA in investigations, determine a protocol to properly handle touch DNA, and gain insight into the viability of a sample before sending it off for STR analysis.

MATERIALS AND METHODS

Evaluation of touch DNA using the ParaDNA® Screening System (to be completed at the Plano Police Department):

The samples to be evaluated in this study were approved by the Institutional Review Board of the University of North Texas Health Science Center and collected in accordance with IRB #2017-164. These samples were collected by Dr. Rick Staub of the Plano PD. Eight substrates were used to collect touch DNA: a metal gun handle, glove, cell phone, latent print on a pane of glass, horn plate on a steering wheel, and articles of clothing including a beanie-style knit hat, cotton T-shirt, and eyeglasses. These substrates were selected by the Plano PD criminalists based on what is most commonly sampled for touch DNA, as well as current literature describing how different substrates and factors might affect the transfer of touch DNA [10-12]. Three donors were used: myself, an officer from the Plano PD that handled the gun during testing, and a UNTHSC classmate. The donor held or wore the substrate; immediately following, direct sampling was completed by the nib collector from the ParaDNA® instrument, seen in Figure 3, and loaded onto the ParaDNA® instrument.

Figure 3 – ParaDNA® Nib Collector [2]

In order to evaluate the differences in sampling methods (i.e. direct vs. indirect) after the substrate was swabbed with the nib collector for each kit, the substrate was then swabbed with a

half wet, half dry cotton swab. The nib collector was then used to collect the DNA indirectly from the cotton swab and loaded on the ParaDNA® instrument. For statistical purposes, the substrates were swabbed three times per sampling method (direct and indirect). The schema can be seen in Table 1 below:

Substrates	Samples
Gun Handle	Screening – Direct x3, Indirect x3
Glove (3 will be used for maximum surface area for swabbing)	Screening – Direct x3, Indirect x3
Cell phone	Screening – Direct x3, Indirect x3
Pane of Glass (Latent print – Palm, thumb, pinky)	Screening – Direct x3, Indirect x3
Horn Plate on steering wheel	Screening – Direct x3, Indirect x3
Wearer DNA on beanie-style knit hat	Screening – Direct x3, Indirect x3
Wearer DNA on T-shirt	Screening – Direct x3, Indirect x3
Eyeglasses (nose pads and side arms)	Screening – Direct x3, Indirect x3

Table 1 - Sampling Schema

STR analysis of cotton swabs with EZ1® Investigator Kit and Qiagen® 24Plex QS Kit:

Analysis of the cotton swabs was performed using the extraction protocol laid out by Qiagen® for the EZ1® (Qiagen Inc., Germantown, MD) automated extraction instrument, and the EZ1® Investigator Kit [14]. Quantification was completed using the Qiagen® Investigator Quantiplex Pro Kit and an Applied Biosystems® (Thermo Fisher Scientific, Waltham, MA) 7500 Real-Time PCR system. Following quantification, PCR (polymerase chain reaction) and genetic analysis were performed on an Applied Biosystems® GeneAmp™ PCR System 9700 and an Applied Biosystems® 3500 Genetic Analyzer, respectively. The conditions for PCR and genetic

analysis were set on these instruments according to the protocol for the Qiagen® 24Plex QS kit [15]. After genetic analysis, the DNA profiles generated were visualized with GeneMapper® IDX (Thermo Fisher Scientific, Waltham, MA). This software allows for comparison of allele calls between both the ParaDNA® instrument and DNA profile. Allele call percentages were calculated by taking the 21 loci tested in the Qiagen® 24Plex QS kit and determining a possible 42 alleles available to be called within that kit. Each replicate profile was then evaluated for the number of alleles called out of the possible 42, and a percentage of allele calls was developed.

RESULTS

Obtaining genetic material and data from touch DNA:

The ParaDNA® Screening System was able to detect touch DNA from the various substrates in the project; however, there were some outliers. Percent values from the ParaDNA® ranged from 0 to 95%, with the most 0 calls being from the latent print, and the 95% coming from the horn plate on a steering wheel. The percent values are shown in Figure 4 for all eight substrates, and for both the direct and indirect swabbing methods.

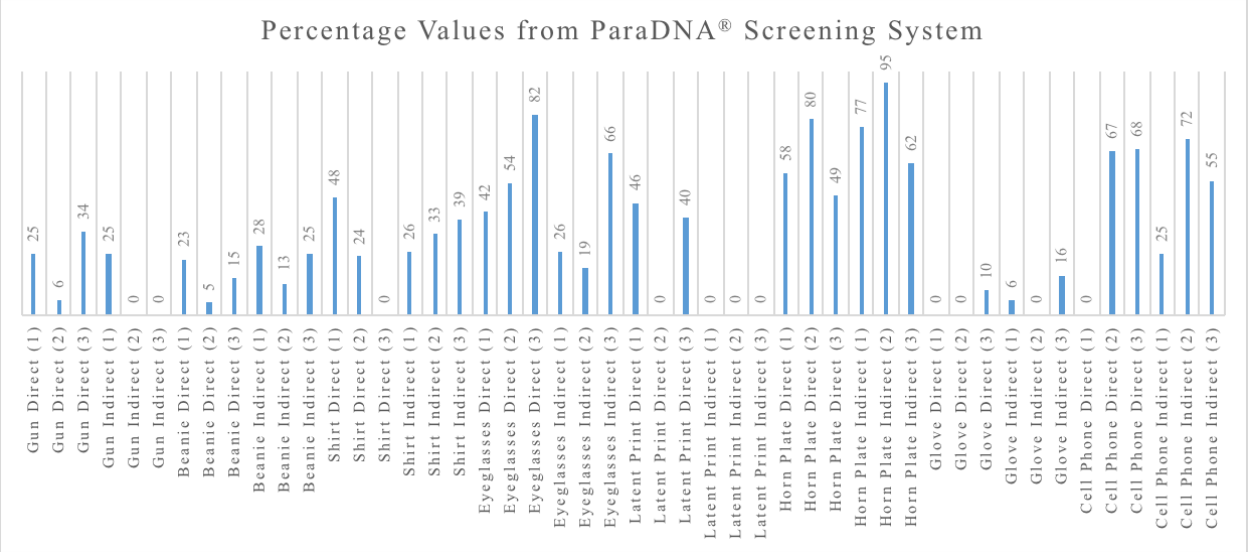

Figure 4 - Percent Values from ParaDNA® Screening System

Gender calls were also made in some of the substrates. Out of 48 possible calls, 3 male and 9 female calls were observed (25% of sample tests), as seen in Figure 5.

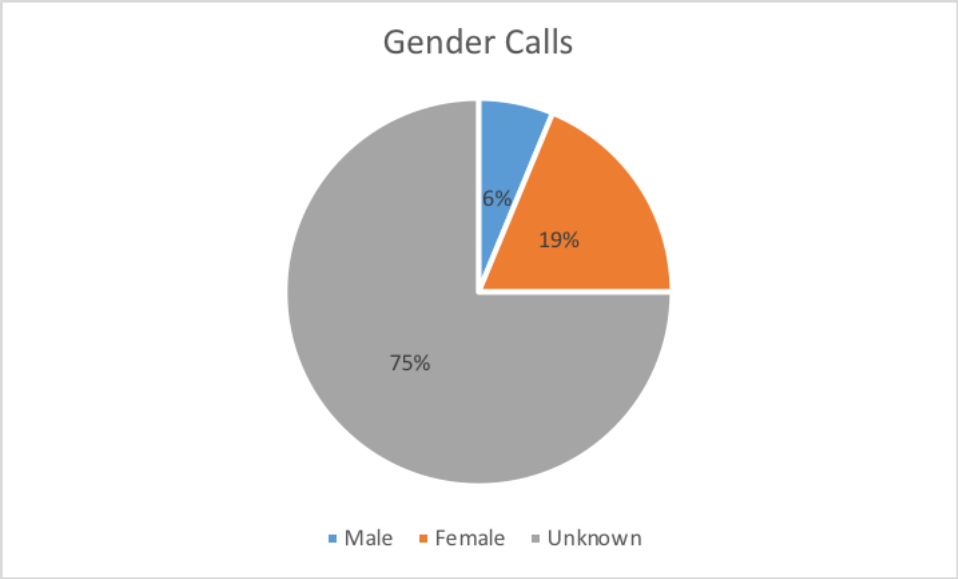

Figure 5 - Gender calls from the ParaDNA® Screening System

In various substrates, alleles were called at the D16 and TH01 STR positions. A table outlining the allele calls for the two loci represented in the Screening System can be found in the appendix as Appendix 1. Based on the table, the general trend appears that the higher the percentage value determined on the ParaDNA®, the more alleles are called in the system. Although the substrates used were touch DNA or trace DNA, the instrument was sensitive enough to pick up the low levels associated with these samples and accurately call alleles (See Appendix 1).

Evaluation of direct and indirect sampling methods:

The difference between direct and indirect sampling methods was observed for each substrate. The resultant percentage values from the ParaDNA® are shown in Figure 6.

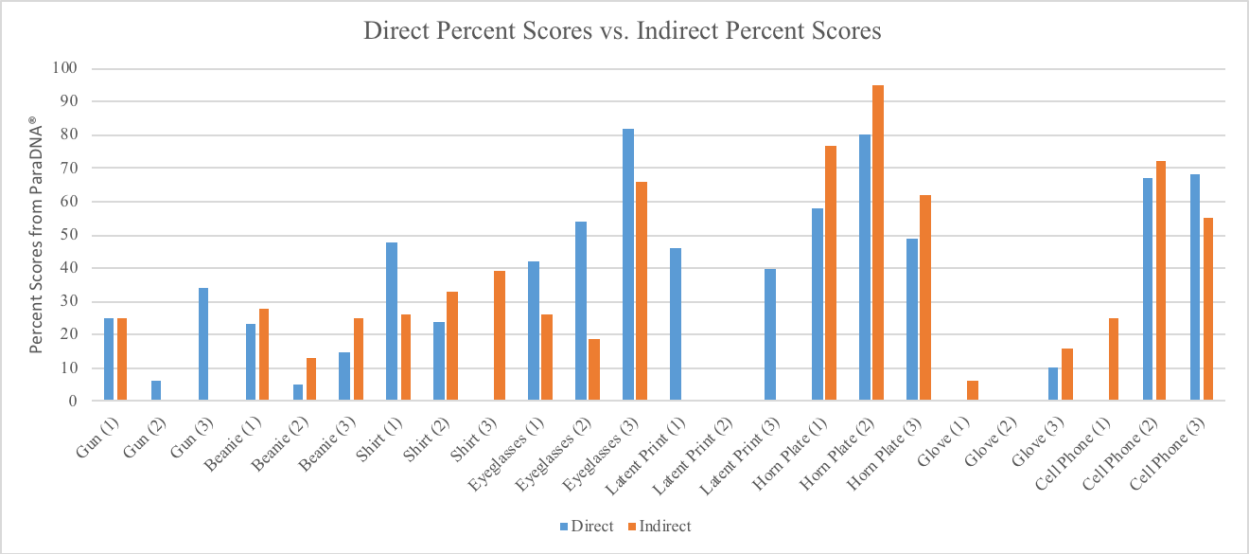

Figure 6 - ParaDNA® Screening System results for each swabbing method

The results indicate that there could be differences between direct and indirect sampling of certain substrates, and that one method may be better for a particular surface compared to the other.

Porous substrates

The horn plate represented the highest percentage values compared to all other substrates with over 49% in both the direct and indirect samplings. The wearer DNA samples (i.e. beanie style knit hat, T-shirt) were fairly consistent between the sampling results with the indirect sampling giving the most consistency for both substrates. The glove sample had low results for both direct and indirect with no readings above 16%.

Non-porous substrates

The latent print gave direct results of 46% and 40% but gave 0% for all three indirect samplings, giving the lowest indirect scores of all the substrates. The gun showed similar results with the best values coming from the direct samplings with the highest score of 34% and two indirect scores of 0% for replicates 2 and 3. Lastly, the eyeglasses and cell phone again had high direct

sampling scores, but for all of their replicates also had indirect scores called with the lowest indirect score being 19% on replicate 2 of the eyeglasses.

Efficiency of the ParaDNA® Screening System compared to full STR analysis:

As seen in Figure 4, the ParaDNA® Screening System was able to obtain data from the low-level samples present on most of the substrates. The indirect swabs were quantified following their ParaDNA® testing. Results from the quantification are shown in Table 2.

Substrate	Quant (ng/µL)
Gun (1)	0.011848
Gun (2)	0.005466
Gun (3)	0.004342
Beanie (1)	0.001607
Beanie (2)	0.019712
Beanie (3)	0.002209
Shirt (1)	0.006665
Shirt (2)	0.014963
Shirt (3)	0.007149
Eyeglasses (1)	0.005905
Eyeglasses (2)	0.008926
Eyeglasses (3)	0.008466
Latent Print (1)	0.003771
Latent Print (2)	0.001342
Latent Print (3)	0.001147
Horn Plate (1)	0.103743
Horn Plate (2)	0.592208
Horn Plate (3)	0.06689
Glove (1)	0.014286
Glove (2)	0.006917
Glove (3)	0.004553
Cell Phone (1)	0.015058
Cell Phone (2)	0.002705
Cell Phone (3)	0.012655

Table 2 - Quantification values from the AB® Real-Time 7500

The quantification values ranged from 0.001147 ng/µL to 0.592208 ng/µL. The latent print resulted in the lowest concentration and the horn plate resulted in the highest concentration, respectively. The values contained a lot of variability between them. The eyeglasses and the latent print values remained fairly uniform across the three replicates, but the wearer DNA substrates (i.e. beanie style knit hat and T-shirt) varied greatly between the replicates. These values were compared against the percentage values from the ParaDNA® Screening System

(Figure 7).

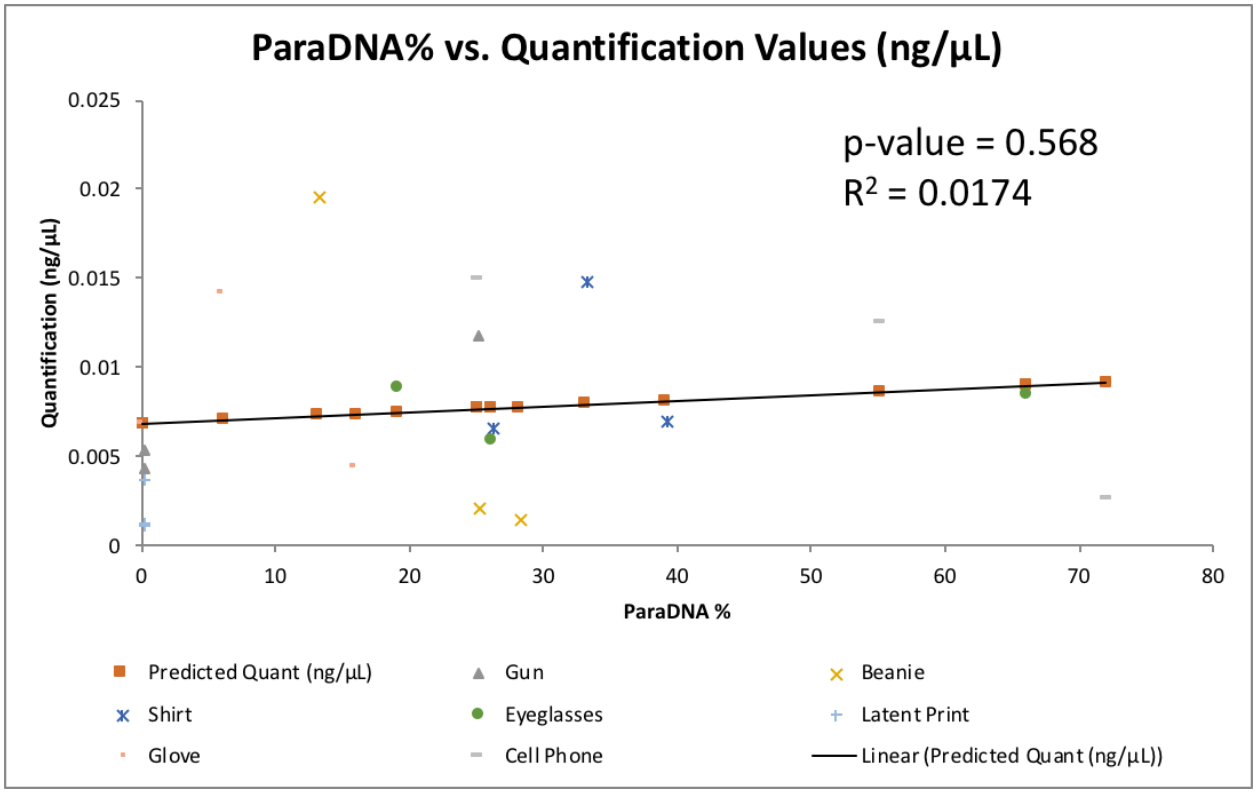

Figure 7 - ParaDNA® percentage and quantification (ng/µL) values and comparison. The quantification replicates from the horn plate are not shown due to being an outlier outside 1.5 times above the interquartile range of quantification values.

An outlier was defined as data points that fall outside 1.5 times below or above the interquartile range of the data. The horn plate was identified as an outlier and was removed from Figure 7 as it fell outside the upper bound of the quantification data set.

Comparing these two sets of data reveal that the horn plate suggested a direct relationship between percentage to concentration of DNA (quantification values can be found in Figure 6) contained in the indirect sample. However, in the case of the cell phone, the percentage values ranged from 25-72%, but the quantification resulted in low concentration levels at 0.002705-0.015058 ng/µL with the lowest concentration observed resulting from the 72% score, and the highest concentration observed resulting from the 25% score. The gun substrate specifically would be a common substrate tested in casework, however, in this experiment had low

quantification values and only received a high score of 25% in one of the replicates tested. The overall p-value, at 95% confidence, from comparing these data sets indicate that the values are not significant, and the R^2 value was low giving a low to no correlation between these values. The percent values from the ParaDNA® were then compared with the allele call percentages. This comparison can be seen in Figure 8.

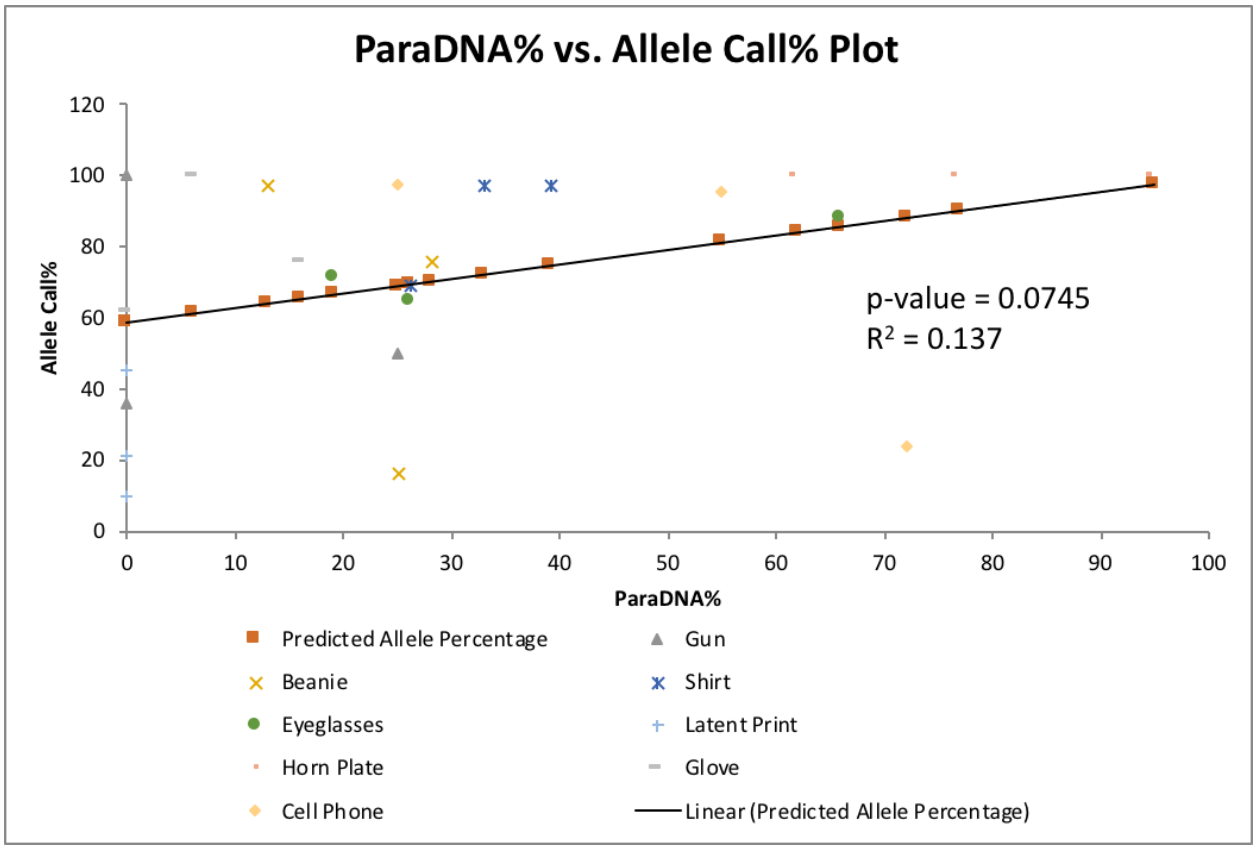

Figure 8 - Indirect ParaDNA® Screening System percentage values with the allele call percentages

As indicated in Figure 8, the values do not appear to be significant as the p-value was greater than 0.05. The R^2 value was 0.137 indicating very low correlation between these two sets of data. The eyeglasses suggest the best relationship between the two data sets as the values fall along the predicted allele percentage line. However, again in the case of the latent print, there appears to be no suggested relationship.

DISCUSSION

Overall, the ParaDNA® Screening System was able to obtain data from most of the low-level touch DNA samples; however, there are apparent factors that might affect the percentage value and how the system provides this value. One factor that should be examined is the type of substrate that is being sampled. In this validation, it appears that substrates that are more porous, such as the horn plate or steering wheel of a car, might provide better results than those that are less porous such as the case of a latent print on glass. This factor can be related back to the findings of Daly et al. 2012, where again more porous substrates such as wood were able to effectively collect more DNA more than non-porous substrates such as glass [11]. In the case of the gun, one thing to consider would be the metals and how they could affect the analysis as a possible PCR inhibitor.

A second factor to evaluate is the direct and indirect sampling methods and how sampling may affect results from the ParaDNA® instrument. It appears that this could be driven by the type of substrate as well as the type of swab used to collect the sample. Results of the latent print triplicate samples, and other non-porous substrates, in this indicated that the direct method is more effective than the indirect method. One possible reason behind these results is that since the nib collector utilizes static to pull the sample from the substrate, the cotton swabs used in the indirect samplings failed to release the DNA to the nib collector. Another factor to be considered is how much DNA the nib collector pulled from the direct samplings. If the latent print contained trace amounts of DNA, the direct sampling with the nib collector before the indirect swabbing could have taken away a large enough portion of the sample to affect the indirect results.

Finally, a third factor to consider is the low-level amounts of DNA involved with touch DNA and how that could have affected the results. In some cases, the ParaDNA® gave a low

percentage score, whereas the allele call percentages were high. In other cases, the reverse was true, where the ParaDNA® score was high, but the allele call percentage was low. A possible explanation for this could be the sensitivity of the instrument, as well as how the instrument evaluates the sample and creates the percentage value. The Screening System evaluates two loci at D16 and TH01. If the touch DNA sample is too small and these loci are not called, this could be a possibility for the low score, as well as if the gender marker amelogenin is not called. In observing the quantification values, it does appear that there is evidence of a direct relationship between the substrates ParaDNA® scores and the quantification values. In the STR profiles, the values for the allele call percentages were seemingly high in comparison to the ParaDNA® percentages. In forensic laboratories, the PCR kits are validated in-house to determine proper thresholds for casework samples that allow analysts to know whether a peak is a true peak or background noise. A common threshold for laboratories is 100 RFUs, or relative fluorescence units. The threshold for this project was set at 50 RFUs, and in many of the profiles obtained the peaks did not reach 100 RFUs; in other words, although they were called in this instance, many of them would not have been called in a real casework setting, and will be taken into consideration in developing the protocol for at what ParaDNA® reading the Plano Police Department should consider before sending samples to forensic laboratories for DNA analysis.

CONCLUSION

The ParaDNA® Screening System has displayed the ability to obtain data for touch DNA samples, confirming the alternative hypothesis. Based on Table 3, the Plano Police Department will have the ability to triage evidence samples and only send the most viable samples for STR analysis. Having this ability should save the department time and resources.

ParaDNA® Percentage Value	Comments/what to expect	Send to lab or no?
≥ 60%	• All peaks above 100 RFU	• Interpretable results • High priority, send first
20% - 55%	• Most peaks above 100 RFU	• Could give interpretable results in cases • Send if there are no samples higher than these
≤ 18%	• Most peaks below 100 RFU	• Not likely to give any interpretable results

Table 3 - Protocol for how to triage touch DNA samples after ParaDNA® Screening System

Based upon these results, the sampling method for different substrates should be determined on a case by case basis for different substrate types. Generally, we conclude that non-porous substrates tended to have better results with direct sampling. More porous substrates, by contrast, performed similarly with either direct or indirect sampling. Using the results obtained in this study, recommended guidelines for consideration in prioritization of forensic samples to be submitted for full STR analyses were developed based upon results from the ParaDNA®

Screening System. These guidelines are shown in Table 3. These guidelines indicate that if the ParaDNA® Screening System provides a percentage score above 60%, these samples should be prioritized and sent for full STR analysis. Samples that obtain scores between approximately 20% to 55% could provide identifying STR information if sent for analysis; however, if there are samples with higher ParaDNA® scores, these should be prioritized second. Lastly, scores ≤19% should be sent if there are no other samples in the higher percentages. These samples present low RFU values in STR analysis and would, in most cases, not provide valuable information for casework.

APPENDIX

Sample Name	% Score	Gender Call	Quant Value (ng/uL)	Date ran on ParaDNA	Head #	D16(L)	D16(H)	THO(L)	THO(H)	AMEL(L)	AMEL(H)
Gun Direct (1) Lower Handle	25	Male	-	3/8/18	1	11	-	-	-	Y	-
Gun Direct (2) Upper Handle	6	Male	-	3/8/18	2	-	-	-	-	Y	-
Gun Direct (3) Slide & Trigger	34	Unknown	-	3/8/18	3	11	-	9	9	-	-
Gun Indirect (1) Lower Handle	25	Male	0.011848	3/8/18	4	11	-	-	-	Y	-
Gun Indirect (2) Upper Handle	0	unknown	0.005466	3/8/18	1	-	-	-	-	X	-
Gun Indirect (3) Slide & Trigger	0	unknown	0.004342	3/8/18	2	-	-	-	-	-	-
Beanie Direct (1)	23	unknown	-	3/8/18	3	9	-	7	-	X	Y
Beanie Direct (2)	5	unknown	-	3/8/18	4	12	-	-	-	-	-
Beanie Direct (3)	15	unknown	-	3/8/18	1	-	-	9	-	X	-
Beanie Indirect (1)	28	unknown	0.001607	3/8/18	2	11	12+	9	-	-	-
Beanie Indirect (2)	13	unknown	0.019712	3/8/18	3	11	-	-	-	-	-
Beanie Indirect (3)	25	Female	0.002209	3/8/18	4	11	12+	-	-	X	X
Shirt Direct (1)	48	Male	-	3/8/18	1	11	-	6	6	Y	-
Shirt Direct (2)	24	unknown	-	3/8/18	2	11	12+	-	-	X	-
Shirt Direct (3)	0	unknown	-	3/8/18	3	9	-	-	-	-	-
Shirt Indirect (1)	26	unknown	0.006665	3/8/18	4	11	-	-	-	X	-
Shirt Indirect (2)	33	unknown	0.014963	3/8/18	1	11	-	6	-	X	-
Shirt Indirect (3)	39	Female	0.007149	3/8/18	2	11	-	-	-	X	X
Eyeglasses Left Arm Direct (1)	42	unknown	-	3/9/18	1	11	-	6	7	X	-
Eyeglasses Nose Direct (2)	54	Female	-	3/9/18	2	11	12	-	-	X	X
Eyeglasses Right Arm Direct (3)	82	Female	-	3/9/18	3	11	-	6	6	X	X
Eyeglasses Left Arm Indirect (1)	26	unknown	0.005905	3/9/18	4	11	-	6	-	-	-
Eyeglasses Nose Indirect (2)	19	unknown	0.008926	3/9/18	1	-	-	6	6	X	-
Eyeglasses Right Arm Indirect (3)	66	unknown	0.008466	3/9/18	2	11	-	6	6	X	-
Latent Print (Palm) Direct (1)	46	Female	-	3/9/18	3	11	-	6	6	X	X
Latent Print (thumb) Direct (2)	0	unknown	-	3/9/18	4	-	-	-	-	-	-
Latent Print (pinky) Direct (3)	40	unknown	-	3/9/18	1	11	-	6	6	-	-
Latent Print (palm) Indirect (1)	0	unknown	0.003771	3/9/18	2	-	-	-	-	-	-
Latent Print (thumb) Indirect (2)	0	unknown	0.001342	3/9/18	3	-	-	-	-	-	-
Latent Print (pinky) Indirect (3)	0	unknown	0.001147	3/9/18	4	-	-	-	-	-	-
Horn Plate (top of wheel) Direct (1)	58	unknown	-	3/9/18	1	11	-	6	-	X	-
Horn Plate (horn plate) Direct (2)	80	Female	-	3/9/18	2	11	11	6	6	X	X
Horn Plate (bottom of wheel) Direct (3)	49	unknown	-	3/9/18	3	11	-	-	-	X	-
Horn Plate (top of wheel) Indirect (1)	77	Female	0.103743	3/9/18	4	11	11	6	6	X	X
Horn Plate (horn plate) Indirect (2)	95	Female	0.592208	3/9/18	1	11	11	6	9.3+	X	X
Horn Plate (bottom of wheel) Indirect (3)	62	unknown	0.06689	3/9/18	2	11	12+	6	6	X	-
Glove Direct (1)	0	unknown	-	3/9/18	3	-	-	-	-	-	-
Glove Direct (2)	0	unknown	-	3/9/18	4	-	-	-	-	-	-
Glove Direct (3)	10	unknown	-	3/9/18	1	11	-	-	-	-	-
Glove Indirect (1)	6	unknown	0.014286	3/9/18	2	11	12+	-	-	-	-
Glove Indirect (2)	0	unknown	0.006917	3/9/18	3	-	-	-	-	-	-
Glove Indirect (3)	16	unknown	0.004553	3/9/18	4	11	11	-	-	-	-
Cell Phone (screen) Direct (1)	0	unknown	-	3/9/18	1	-	-	-	-	Y	-
Cell Phone (Side) Direct (2)	67	unknown	-	3/9/18	2	11	11	6	6	X	-
Cell Phone (back) Direct (3)	68	unknown	-	3/9/18	3	11	-	6	6	X	Y
Cell Phone (screen) Indirect (1)	25	unknown	0.015058	3/9/18	4	11	11	-	-	X	-
Cell Phone (side) Indirect (2)	72	Female	0.002705	3/9/18	1	11	11	6	9.3+	X	X
Cell Phone (back) Indirect (3)	55	unknown	0.012655	3/9/18	2	11	-	6	6	X	-

Appendix 1 - List of data from project (Green - confident call, yellow - not confident call, gray – no call)

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