Moussa, Ola, ParaDNA®: A Novel Instrument for Presumptive DNA Analysis. Master's of Forensic Genetics, May, 2018, 36 pp, 13 tables, 17 figures, references, 12 titles

Current methods for human identification are time consuming and can take weeks to complete, leading to a backlog of evidence needing to be processed and a slowdown in investigations. The ParaDNA® Instrument is designed to address this issue using the Screening and Intelligence Systems which can detect the relative amount of DNA in an evidence item, and analyze 5 short tandem repeats (STRs) and Amelogenin, respectively [3]. The Instrument uses HyBeacons® which target specific STRs to identify the presence of DNA, and detect STR alleles. This is a validation of the ParaDNA® Screening and Intelligence Systems using saliva and blood samples to assess the sensitivity and reliability of the instrument. The data collected using the ParaDNA® Instrument show that it can reliably identify the relative amount of DNA in a sample, and display useful STR profiles that are 99% concordant with Qiagen® Investigator 24Plex QS STR Kits.

ParaDNA®: A NOVEL INSTRUMENT FOR PRESUMPTIVE DNA ANALYSIS

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ParaDNA®: A NOVEL INSTRUMENT FOR PRESUMPTIVE DNA ANALYSIS

THESIS

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CHAPTER I

INTRODUCTION

Human identification through short tandem repeats (STRs) is the most commonly used method in forensic laboratories worldwide. STRs are DNA regions with repeat units that are 2 to 7 base pairs in length and are highly variable among individuals, which makes them effective for human identification purposes [4]. STR analysis involves extraction, quantification, amplification, and allele separation through capillary electrophoresis which can be laborious and costly, subsequently leading to a backlog of evidence samples that need to be processed. Currently, there are various chemical tests that can presumptively detect the presence of DNA through biological stains, however, the process remains mostly subjective, and results in many submitted samples that do not provide an informative profile, or samples not being submitted for thorough analysis at all even though DNA is present [5].

The Plano Police Department (Plano PD) is a law enforcement agency that regularly processes evidence from crime scenes. The current protocol of the Crime Scene Investigation Unit involves sending biological fluids collected to outside forensic laboratories for serological screening and DNA testing. Currently, there is no direct method for confidently prioritizing samples collected to assess the likelihood that they contain pertinent biological evidence relevant in aiding an investigation. This uncertainty can significantly slow the progress of an investigation, and is fiscally and materially wasteful. This is not only a problem for the Plano PD, but also for many other forensic laboratories that are currently running a backlog of cases.

This issue may be alleviated by screening evidence items before running full STR analysis to prioritize samples that would be most likely to yield informative results.

The ParaDNA® Instrument developed by Laboratory of the Government Chemist (LGC) (Middlesex, UK), is a bench top device that is designed to address this issue by screening samples for the presence of DNA in 75 minutes using melt-curve analysis. The instrument is comprised of two systems that were tested in this project, as follows:

- The Screening System which identifies the presence and relative amount of DNA on an evidence sample, and gives a gender call result [5]. This kit is most useful for initially screening and prioritizing samples which should be sent for analysis by first assessing the amount of DNA present.
- The Intelligence System which analyzes 5-STRs plus Amelogenin to provide a DNA profile enabling investigators to gain rapid investigative leads and sample prioritization for further human identity applications [3]. This kit has the capacity to rapidly and directly compare evidence and reference samples, thus quickly identifying potential suspects, while also excluding non-suspects [6].

The ParaDNA® instrument is supplied with a disposable plastic device called a Sample Collector (Figure 1). The device has four nibs which are used in a similar manner to a swab. DNA is recovered using the Sample Collector either directly from the evidence sample, or indirectly (secondhand) from a swab (used previously to recover DNA from the sample evidence).

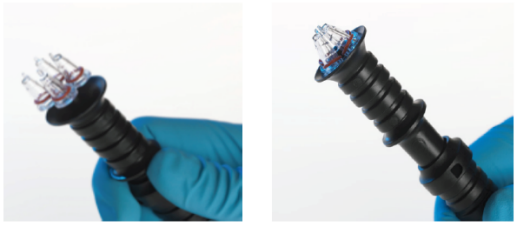

Figure 1: ParaDNA Sample Collector 4-nib (LGC, Middlesex, UK), [6].

The sample collector nibs fit into the ParaDNA® PCR reaction plates which contain four independent PCR reaction tubes pre-loaded with reagents needed for DNA amplification and detection. The reaction plates are then loaded into the ParaDNA® Instrument for analysis (Figures 2, 3).

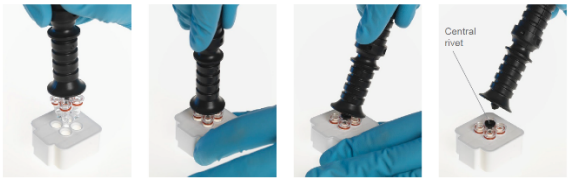

Figure 2: Loading the 4-nibs of the Sample Collector device into a test plate, and snapping off the ParaDNA® Sample Collector handle (LGC, Middlesex, UK), [6].

Figure 3: ParaDNA® Instrument (LGC, Middlesex, UK), [6].

The ParaDNA® Instrument uses HyBeacon® fluorescent probes which target specific STRs. Asymmetric PCR is used to create copies of the target DNA, after which the reaction mixture is heated to denature the double stranded DNA molecule (Figure 4). The reaction mixture is then cooled to 20°C, allowing a blocker molecule to anneal to the repeat sequence, followed by a fluorescent dye. Once bound to complementary DNA, the fluorescence from the probe is

increased (Figure 5). The temperature is then elevated and the probe will melt off, causing a decrease in fluorescence [6] (Figure 6). The melt temperature is specific to the number of repeat probes that are bound to the DNA and the amplified product is characterized by the associated measured change in fluorescence. In this way the ParaDNA® system determines allele designations for the target loci [6]. Shorter alleles have a lower melting temperature, while longer alleles will have a higher melting temperature, due to their increased affinity for the probe.

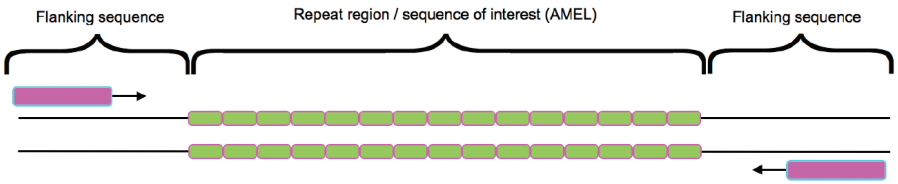

Figure 4: PCR amplification of STR region. Reaction mix is heated to denature the DNA. [6]

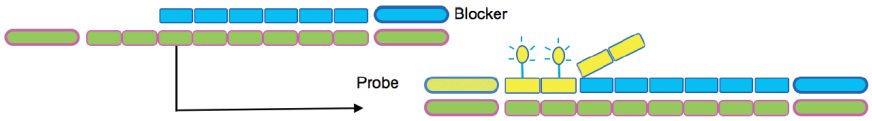

Figure 5: HyBeacon® fluorescent probe technology [6]. Reaction mix is cooled allowing blocker and probe to anneal, followed by a fluorescent dye. [6]

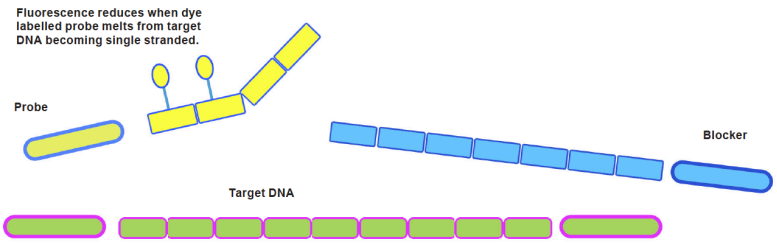

Figure 6: HyBeacon® fluorescence stops when probe melts away from target DNA [6]. The temperature is elevated again, allowing probe to fall off, causing a decrease in fluorescence. [6]

The Screening Kit identifies alleles at the loci: D16S539 (D16), TH01, and Amelogenin (AMEL). All loci for the Screening System are amplified across 4 independent PCR reactions within the four reaction plates (Figure 7). A percent (%) score is reported that

represents a relative quantitative assessment by combining allele calls across ranges. This is designed to assist in determining which samples should be chosen for DNA analysis [6]. A higher % score indicates a higher amount of DNA present.

Fluorescent Channel	Tube			
	A	B	C	D
1	**D16** (8-12+)	**D16** (12-15+)	**THO1** (5-9.3+)	**AMEL** (XY)

Figure 7: Screening assay design. Loci are amplified across 4 independent PCR reactions. A % score is reported that represents the relative amount of DNA by combining allele calls across ranges. [7]

The Intelligence System identifies the same loci as the Screening System, but also with an additional three loci: D18S1358 (D18), D3S1358 (D3), and D8S119 (D8). The Intelligence System results display the allele calls as well as a % score. All loci for the Intelligence System are amplified across 12 independent PCR reactions within the four reaction plates.

Fluorescent Channel	Tube			
	A	B	C	D
1	**D16** (8-12+)	**D16** (12-15+)	**D18** (10-14+)	**THO1** (5-9.3+)
2	**D8** (8-11+)	**D8** (11-14+)	**D8** (14-17+)	**AMEL** (XY)
3	**D18** (14-17+)	**D18** (17-21+)	**D3** (13-16+)	**D3** (16-19+)

Figure 8: Intelligence assay design [7]. Loci are amplified across 12 independent PCR reactions within the 4 reaction plates.

The "+" next to the alleles describes alleles that are rare or uncommon in the population. These can be micro variants, or larger alleles that have too small of a difference in melting temperature to accurately determine allele length. This is not an issue for the Screening System since regardless it will still detect DNA.

This is a pilot validation project for the ParaDNA® Instrument that was run specifically on behalf of the Plano PD. Developmental validations have been carried out for both the Screening and Intelligence systems by the manufacturing company, LGC. According to these validations, both the Screening and Intelligence Systems are human and

non-human primate specific [3]. Sensitivity studies showed that the Screening System could detect DNA down to 62.5 pg total, while the Intelligence System provided a usable profile at 250 pg total (62.5 pg per well) [3]. According to LGC, the Intelligence System provides a robust and sensitive means of generating STR profiles rapidly, and highlights the limits under which reliable results can be obtained [3]. In addition, the Screening System validation demonstrated that the instrument can be used to preferentially select items to submit for STR analysis [5]. This leads to backlog reduction and cost savings. However, impressive though these initial validations are, individual laboratories proposing to use the ParaDNA® Instrument should perform their own internal validation studies, prior to incorporating the instrument into their regular laboratory practice.

This project sought to validate the ParaDNA® instrument owned by the Plano PD. It is hoped that the instrument can subsequently be used in the future to determine which samples should be sent to an outside lab for analysis. The specific aims of this validation are outlined below:

1. Determine the sensitivity of both the Intelligence and Screening Systems
2. Determine the concordance level of allele calls made by the Intelligence System to those made by full STR analysis using the Qiagen® Investigator 24plex QS and Go! STR kits.
3. Create an elimination database that can be used to assess possible contamination within the Plano PD.
4. Create submission guidelines for the Plano PD that will help determine which samples should be sent for further DNA analysis.

CHAPTER II

MATERIALS AND METHODS

Sample Collection

Sensitivity of the ParaDNA® Intelligence and Screening systems was tested by utilizing human saliva and blood samples, appropriately diluted, to evaluate upper and lower limits of DNA detection. Saliva and blood samples were provided by the Plano PD in accordance with IRB # 2017-164. Biological triplicate samples were prepared and tested to assess the sensitivity and reproducibility of the system according to the LGC ParaDNA® sampling guidelines for swabs. A total of four swabs with sterile water (per system) with no DNA were used as negative controls. The negative controls were run normally in the instrument on each system to insure no contamination had occurred. Neat samples served as positive controls. These samples contain a known DNA profile that had previously been run through full STR analysis to insure that all reagents were working correctly. The required volume was pipetted onto glass slides, and allowed to dry:

Table 1: Description of sample collection method for sensitivity study. Neat stains of 10μL and 1μL of saliva and blood were pipetted onto microscope slides and allowed to dry. Dilutions of blood and saliva were prepared in triplicate for each system (Intelligence or Screening) and 50μL were pipetted onto microscope slides and allowed to dry. The samples were then collected using a wet swab and sampled using the ParaDNA Sample Collector and then run on both the Intelligence and Screening Systems.

	Intelligence		Screening	
Sample Type	Saliva on Cotton swab-Donor 1	Blood on Cotton swab- Donor 2	Saliva on Cotton swab-Donor 1	Blood on Cotton swab- Donor 2
Neat (10 μL)	3	3	3	3
Neat (1μL)	3	3	3	3
1/2 Dilution (50μL)	3	3	3	3
1/5 Dilution (50 μL)	3	3	3	3
1/10 Dilution (50μL)	3	3	3	3
1/100 Dilution (50μL)	3	3	3	3

ParaDNA® Sampling Technique and Testing

The samples were tested using the indirect collection method because this is the technique that the Plano PD hopes to utilize. This method involves swabbing evidence material first using a conventional swab, and then collecting the cellular material from the swab by using the Sample Collector since this is the method that will be used by the Plano PD in real casework scenarios. This will be done by rotating the Sample Collector with one hand while rotating the swab with the other hand to insure that all four nibs come in contact with the swab head for one minute, after which the nibs will be inserted into the 4-well PCR plate to be run on the instrument [6].

STR Analysis

After each sample was run for each corresponding system, DNA was extracted using the Qiagen® EZ1® (Qiagen Inc., Germantown, MD) automated extraction instrument, and the EZ1® Investigator Kit, an instrument that performs automated nucleic acid purification, and assessed by using the Qiagen® Investigator Quantiplex Pro Kit and an Applied Biosystems® (Thermo Fisher Scientific, Waltham, MA) 7500 Real-Time system to determine the quantity of DNA in each dilution set. Extracted samples were then amplified using the Qiagen® Investigator 24Plex QS STR kit and run on an Applied Biosystems® 3500 Genetic Analyzer, respectively [12]. The 3500 is a genetic analyzer used for fragment analysis and rapid sequencing applications. Samples were analyzed as a DNA sample would be in a forensic DNA laboratory to observe the quality of STR profiles obtained from each dilution. The 18 samples used for the elimination database and concordance study were processed using the Qiagen® Investigator 24Plex GO! Kit, and run on the genetic analyzer as described above [11].

% Score and Allele Call Comparisons

The average % score given by the ParaDNA® Screening system and alleles called were compared to the average number of alleles called by the ParaDNA® Intelligence system, as well as to the type of profile obtained (full or partial) from full DNA analysis. From this, we assessed and compared the lower limits of DNA detection by each system. We then created a submission guideline for the Plano PD based on the % score given from the instrument.

To evaluate the concordance level of the Intelligence system, previously collected triplicate saliva and blood samples (dried on glass slides and swabbed as described before), as well as 18 additional buccal swabs from Plano PD personnel were run on the ParaDNA®

Intelligence system. Allele calls made on the ParaDNA® Intelligence instrument for the five loci and Amelogenin were compared to the alleles called on the same loci in GeneMapper® IDX (Thermo Fisher Scientific, Waltham, MA)., a data analysis software designed for STR DNA analysis for forensic samples.

Statistical Analysis

The results of the Screening sensitivity study were compared using a linear regression graph to determine if there is a statistical relationship between the quantification value of the dilutions and the % score yielded. This was not done for the Intelligence Sensitivity because the main focus was to know at which point (DNA quantification value) the Intelligence System would stop calling alleles, and generating a usable profile, as opposed to the relationship between the quantification value and the number of alleles called.

CHAPTER III

RESULTS

Screening % score vs quantification value

The % scores for the dilution sets were categorized into five groups, 0%, 1-25%, 26-50%, 51-75%, and 76-100%. The distribution is shown in figure 9.

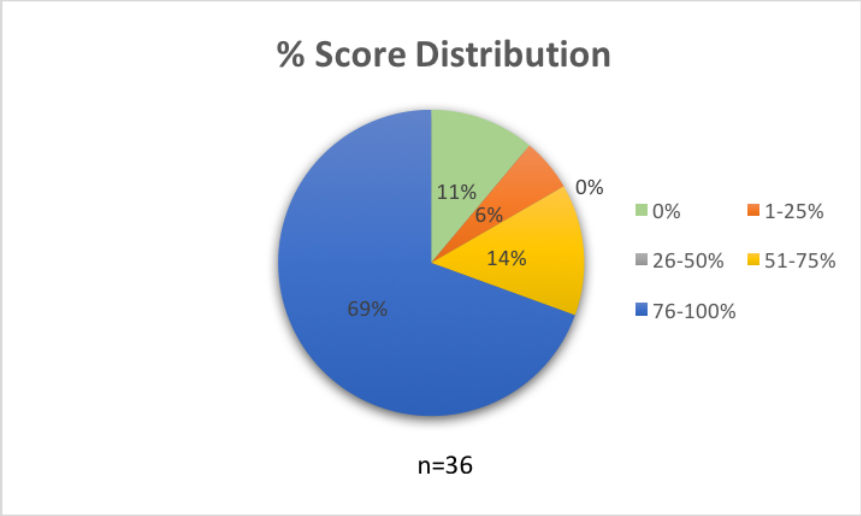

Figure 9: % Score distributions given by ParaDNA® Screening System. Saliva and blood dilutions(n=36) were run on the ParaDNA® Screening System and the % scores given were categorized into five ranges to look at the distribution of % scores.

Of the 36 samples, 4 yielded a score of 0%, 2 samples yielded a score between 1-25%, none yielded a score between 26-50%, 5 samples yielded a score between 51-75% and 25 yielded a score between 76-100%.

The 36 dilution samples of saliva and blood were extracted and quantified to determine the exact amount of DNA found in each sample. The quantification values were then compared

to their corresponding % score given by the ParaDNA® Screening System. The results are shown in Figures 10 and 11, respectively. All of the negative controls yielded a 0% score, and no quantification value.

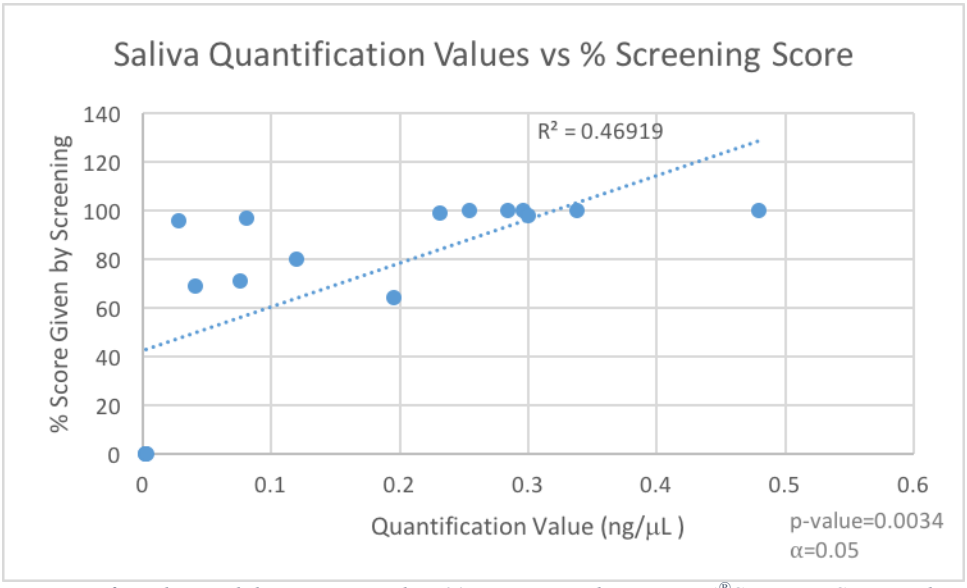

*Figure 10: Dilutions sets for saliva and their corresponding % scores given by ParaDNA®Screening System. The saliva dilutions were prepared in triplicate, run on the ParaDNA®Screening System, and then extracted and quantified. The quantification values in nanograms per microliter are shown in the X-axis. The corresponding % score given for each sample is shown in the Y-axis. *Two outliers were removed. Both were high quantification values with 100% Screening score.*

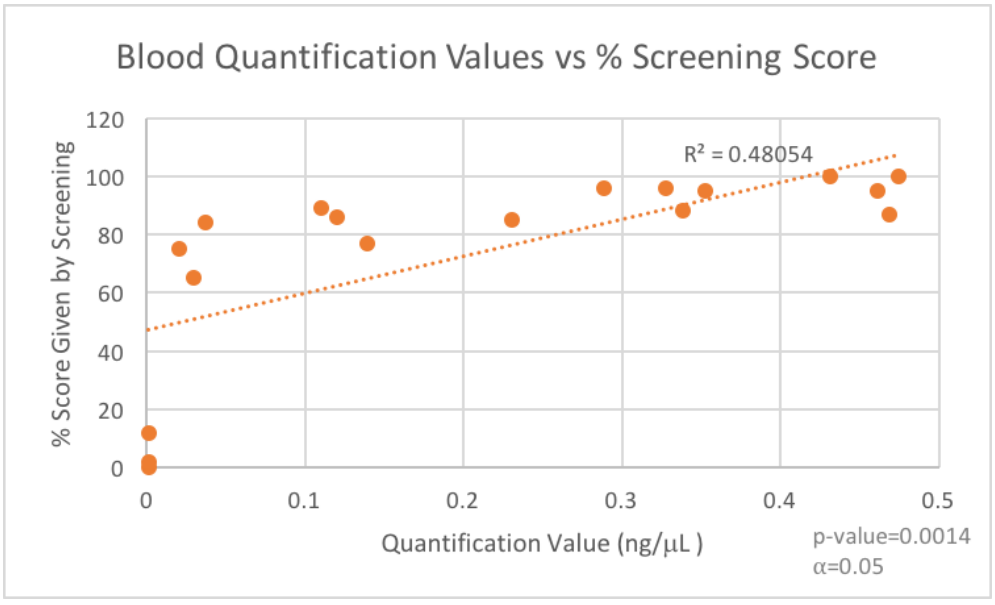

Figure 11: Dilution sets for blood and their corresponding % scores given by the ParaDNA®Screening System. The blood dilutions were prepared in triplicate, run on the ParaDNA®Screening System, and then extracted and quantified. The quantification values in nanograms per microliter are shown in the X-axis. The corresponding % score given for each sample is shown in the Y-axis.

Both the saliva and blood dilutions show more variability in % scores at lower amounts of DNA, and begin to even out at 0.2ng/µL, with more consistent and higher % scores. The Screening System did detect DNA in blood down to 0.001ng/µL (1pg/µL), while the lowest quantity of DNA detected in the saliva was 0.02ng/µL (20pg/µL).

The % score given by the Screening System was compared to the quantification value obtained for both saliva and blood. There is a significant positive correlation between the % score and the quantification value (Figure 12) of R = 0.46919 (saliva) and R = 0.48054 (blood).

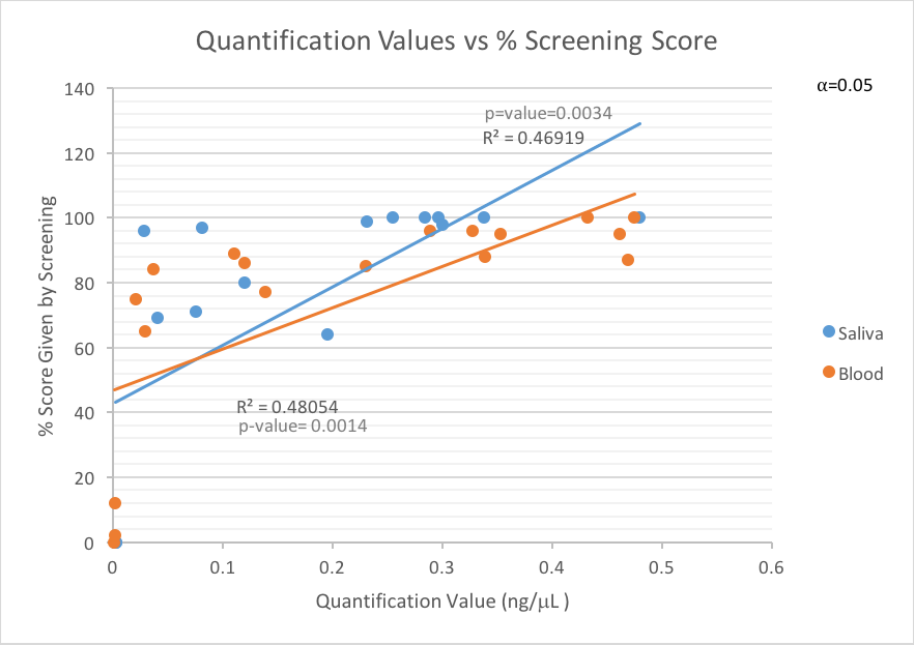

Figure 12: Linear regression showing the relationship between quantification values of both the blood (orange) and saliva(blue) samples and their corresponding % scores given by the ParaDNA® Screening System.

**One sample was removed as an outlier*

The Screening System failed to detect DNA around 0.002ng/µL (2pg/µL) for both the saliva and blood samples. At 0.02ng/µL (20pg/µL) and up, we begin to see significantly higher % scores, with all being 65% or higher.

Three samples were re-melted to see if there would be any change in % score. Re-melting can be run to re-analyze a sample that has already been through the standard protocol [6]. The results of the re-melts are shown in table 2.

Table 2: Original % scores and re-melt % scores on Screening System. Three samples were re-melted to observe if re-melting of samples will change the result.

Sample	Fluid	Type	%Score	Quant Value (ng/μL)
(1) Neat 10μL	Saliva	Original	71	0.075
		Re-melt	61	
(1) 1:10	Saliva	Original	69	0.041
		Re-melt	63	
(3) Neat 1μL	Blood	Original	65	0.029
		Re-melt	59	

The re-melt % scores were not much different than the original % scores, but were always slightly lower. The greatest difference is seen in sample (1) Neat 10μL, from 71% to 61%.

Intelligence allele calls vs quantification value

The 36 dilution samples of saliva and blood were extracted and quantified to determine the exact amount of DNA found in each sample. The number of confident allele calls of the 36 dilution samples of saliva and blood (not including negative controls) were recorded and the results are shown in figures 13 and 14, respectively. Confident allele calls are ones that were called in "green" on the ParaDNA® Instrument software. None of the negative controls yielded any confident allele calls.

Saliva Quant Value (ng/µL)	Number of Confident Allele Calls
0.000929*	0
0.001688*	0
0.002031*	0
0.033536	3
0.064856*	4
0.090109	6
0.13204	6
0.17526	8
0.193645*	4
0.278585	7
0.295698*	6
0.355115	9
0.3667*	6
0.562926*	7
0.74719*	7
0.792009	11
0.989482*	10
1.277184	9

Figure 13: Heat map showing the relationship between quantification values (ng/µL) of saliva DNA extracts and the number of confident allele calls made by the ParaDNA® Intelligence System. The saliva dilutions were prepared in triplicate, run on the ParaDNA® Intelligence System, and then extracted and quantified. Green represents higher number of allele calls, while red represents lower number of allele calls Samples run on software v1.6*

Number of confident allele calls	Blood Quant Value (ng/µL)
0	0.000897
0	0.001269
0	0.001631
3	0.014243
6	0.025524
5	0.02963
6	0.107539*
9	0.114638
6	0.128033
8	0.2377
7	0.242791*
11	0.254633
10	0.276115
10	0.319757
9	0.398043*
7	0.417747
11	0.42051
11	1.15928

*Figure 14: Heat map showing the relationship between quantification values (ng/µL) of blood and the number of confident allele calls made by the ParaDNA® Intelligence System. The blood dilutions were prepared in triplicate, run on the ParaDNA® Intelligence System, and then extracted and quantified. Green represents higher number of allele calls, while red represents lower number of allele call. *Samples run on software v1.6*

The Saliva dilution data suggests that the ParaDNA® Intelligence System can confidently detect DNA down to 0.03ng/µL (30pg/µL), while the blood dilutions show that the system can go as low as 0.01ng/µL (10pg/µL).

A heat map was generated to look at the relationship between quantification values for both saliva and blood to the number of confident allele calls made (figure 15).

Saliva Quant Value (ng/µL)	Number of confident allele calls	Number of confident allele calls	Blood Quant Value (ng/µL)
0.000929*	0	0	0.000897
0.001688*	0	0	0.001269
0.002031*	0	0	0.001631
0.033536	3	3	0.014243
0.064856*	4	6	0.025524
0.090109	6	5	0.02963
0.13204	6	6	0.107539*
0.17526	8	9	0.114638
0.193645*	4	6	0.128033
0.278585	7	8	0.2377
0.295698*	6	7	0.242791*
0.355115	9	11	0.254633
0.3667*	6	10	0.276115
0.562926*	7	10	0.319757
0.74719*	7	9	0.398043*
0.792009	11	7	0.417747
0.989482*	10	11	0.42051
1.277184	9	11	1.15928

*Figure 15: Heat map showing the relationship between quantification values (ng/µL) of both saliva and blood and the number of confident allele calls made by the ParaDNA® Intelligence System.*Samples run on software v1.6*

Neither saliva nor blood generated a full ParaDNA® Intelligence System profile with all 12 alleles confidently called.

The Intelligence System calls any profile with 7+ confident allele calls a usable profile. This data can be used for triage or comparison purposes [1]. The blood and saliva samples were then categorized into six different groups according to their quantification value. Within each group, there were 6 samples. The percent of these samples within each group that resulted in a usable ParaDNA® profile were calculated and shown in figure 16.

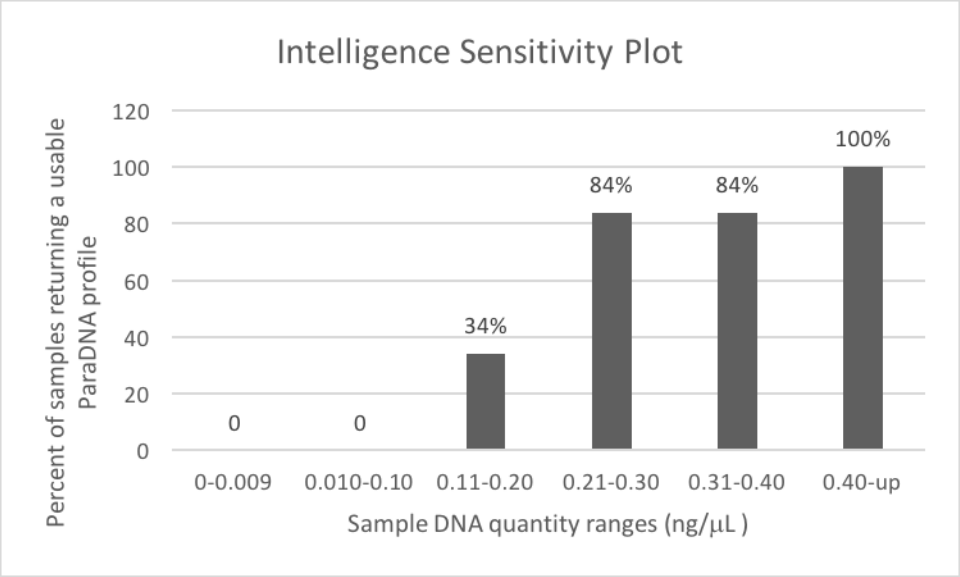

Figure 16: Sensitivity plot of the ParaDNA® Intelligence test with sample quantities of dilution sets of human saliva and blood. The dilutions were prepared in triplicate, run on the ParaDNA® Intelligence System, and then extracted and quantified. The samples were then grouped into six different DNA quantity ranges shown in the X-axis (n=6 per group). Percent of samples giving usable (≥7alleles) shown in the Y-axis.

The blood and saliva dilution data suggest that the ParaDNA® Intelligence System can produce usable profiles down to samples with a quantity of 0.11-0.20 ng/µL. Within this group, 34% of samples gave ≥7allele calls on the Intelligence System. As the DNA quantity increases, the percent of samples that produce usable profiles also increases, rounding out at 100% of the samples producing usable profiles at 0.4 ng/µL.

Concordance between ParaDNA® Intelligence and STR Analysis

Confident allele calls made by the ParaDNA® Intelligence System and alleles called from STR analysis using the Qiagen® Investigator 24Plex QS STR kit were compared for concordance. A confident allele call from Intelligence is one that was called and marked green. The dilution samples were used for these comparisons, as well as 18 other buccal swabs taken from various Plano PD personnel. The results for the dilutions are shown in table 3. The results

for the buccal swabs showed on average, 10 alleles were confidently called, all of which were concordant with their respective STR profile (appendix table 13).

Table 3: Concordance of allele calls on ParaDNA® Intelligence to STR analysis. The average number of alleles confidently called by the Intelligence System and the average number of those alleles that differed from STR analysis. (S) refers to saliva samples, (B) refers to blood samples.

Sample	Average Number of Alleles Called by Intelligence	Average Number of Confident Allele Calls that Differed from Allele STR Analysis (Intelligence)
Neat 10µL(S)*	8	0
Neat 1µL (S)*	6	0
1:2 (S)*	9	0
1:5 (S)*	5	1
1:10 (S)*	7	0
1:100 (S)*	0	0
Neat 10µL(B)	10	0
Neat 1µL (B)	5	0
1:2 (B)	10	0
1:5 (B)*	7	0
1:10 (B)	8	0
1:100 (B)	0	0

** Samples run with new software update v1.6*

Two of the samples in the blood dilution yielded incorrect confident allele calls, calling a heterozygous alleles as homozygous. The data was sent to Foster +Freeman (Sterling, VA) where they looked at the melt curve data and recommended updating the ParaDNA® software, as it was incorrectly calling homozygote peaks. Samples that are starred (*) in table 4 are samples

that were analyzed on the new software version. After the software update, none of the samples yielded an incorrect allele call except for saliva sample 1:5 (2) as seen in table 12 in the appendix (results of samples run on Intelligence v1.6).

The concordance level of each locus was also looked at individually for all samples run on the ParaDNA® Intelligence System (n=54), and the percent of concordant confident allele calls was calculated.

Table 4: Percent concordance of each locus looked at in the Intelligence System compared to the Qiagen® Investigator 24Plex Kits n=54

Locus	% concordant with Qiagen (Intelligence)	% not concordant (Intelligence)
D16S539	100%	0%
D18S51	100%	0%
TH01	100%	0%
D8S1179	100%	0%
D3S1358	100%	0%
Amelogenin	98.15%	1.85%

% Score and allele calls vs profile obtained

The full STR profiles generated from the dilution sets were then analyzed to determine a correlation between the % score given on the Screening System and the number of confident allele calls on the Intelligence System to the type of profile that would be obtained. The optimal amount of DNA recommended for the Qiagen® Investigator 24Plex QS STR kit is 0.0335ng/µL

(0.25 ng) for a half reaction when 7.5µL of DNA is inputted into a total of 12.5µL reaction. Any quantification value that was higher than the optimal amount was normalized to 0.033ng/µL before amplifying. The quantification value for each sample can be found in the appendix (Tables 11 and 12).

Table 5: Average % score given by ParaDNA® Screening and average number of confident allele calls made by ParaDNA® Intelligence and quantification value for each dilution compared to the type of STR profile produced. (S) refers to saliva samples while (B) refers to blood samples.

Sample	Average % Score given by Screening	Average Number of confident allele calls by Intelligence	Average quantification value (ng/µL)	Average quantification value (ng/µL) after normalization	Profile obtained
Neat 10µL(S)	90	8	0.522	0.033	Full
Neat 1µL (S)	80	6	0.109	0.033	Full
1:2 (S)	99	9	0.625	0.033	Full
1:5 (S)	100	5	0.271	0.033	Full
1:10 (S)	88	7	0.404	0.033	Full
1:100 (S)	0	0	0.0020	0.0024	Partial
Neat 10µL(B)	97	10	0.327	0.033	Full
Neat 1µL (B)	75	5	0.026	0.029	Full
1:2 (B)	94	10	0.567	0.033	Full
1:5 (B)	90	7	0.289	0.033	Full
1:10 (B)	84	8	0.120	0.033	Full
1:100 (B)	5	0	0.0013	0.0013	Partial

All of the dilutions above 1:100 generated full and usable profiles with all peaks above 100 RFU. The 1:100 dilutions for both the blood and saliva generated partial profiles with dropout and peaks below 100 RFU. On average, the partial profiles for the 1:100 saliva samples only produced 3-4 allele calls that were above 100 RFU. The 1:100 blood samples, on average, only produced 2 alleles above 100 RFU, although the Screening System did give a % score higher

than the saliva. All of the dilutions that called at least 5 alleles on the Intelligence System resulted in a full profile in STR analysis.

CHAPTER IV

DISCUSSION

Screening % score vs quantification value

A correlation can be seen when comparing the Screening % score to the quantity of DNA present in the sample. All samples with a % score of $\geq 61\%$ had a DNA quantification amount that was greater than the target amplification amount (0.033ng/μL) needed for half reactions run on Qiagen® Investigator 24Plex QS STR Kits.

There was a significant decrease in the % scores from the 1:10 dilutions to the 1:100 dilutions, where all of the % scores previously were $\geq 61\%$, but then a decrease to 0% in all saliva 1:100 samples and 0-12% in the 1:100 blood samples. This is to be expected, but because of this, there were not any % scores in between 12-60. More of these type of %'s can be seen in more mock case touch- type samples, such as those looked at in Whitney West's validation project for the Plano PD. None the swabs used as negative controls gave a % score nor generated a STR profile.

Intelligence allele calls vs. quantification value

Overall, as the quantification value of the sample increased, the number of confident allele calls made by the ParaDNA® Intelligence System also increased, although there were a few exceptions. A few of the higher quantity samples displayed less allele calls than lower quantity samples. This can partly be explained by the new software update, as it seemed to be

more conservative in the allele calls it yielded. Below is an example of alleles called from software version 1.1 versus the same sample with results on version 1.6 (Table 6).

Table 6: Allele calls made for the first saliva dilution 1:5 on software v1.1 and v1.6. Green alleles are confident allele calls, yellow are uncertain, and gray squares represents not enough information to show a result.

		D16	D16	D18	D18	TH01	TH01	D8	D8	AME	AME	D3	D3
Saliva 1:5(1)	**v1.1**	11	11	17	17	9	9	13	15	X	X	16	18
	v1.6	11	-	17	-	9	-	13	15	X	-	16	18

Version 1.1 confidently called all alleles except the 13 repeat allele at D8, while v1.6 was unsure about alleles at D16, D18, TH01, and AMEL. This accounts for most of the confident allele call variability in the saliva samples seen in the heat map in figure 13. Although there were lower amounts of confident allele calls made on the new version, a correlation between quantification value and the number of alleles called on the ParaDNA® Intelligence System was seen (Figure 16). The greater the amount of DNA, the more confident allele calls made, with 100% of samples generating a usable profile at 0.4 ng/µL and up.

Concordance between STR analysis and Intelligence allele calls

The first set of samples run on the Intelligence System called confident alleles that were not concordant with the sample's STR profile. One example is the first 1:10 blood dilution, calling a homozygote 12,12 at locus D16 instead of 12,13. Although the second 12 allele was confidently called, a small peak at allele 13 can be seen in the ParaDNA® analysis software (Figure 17).

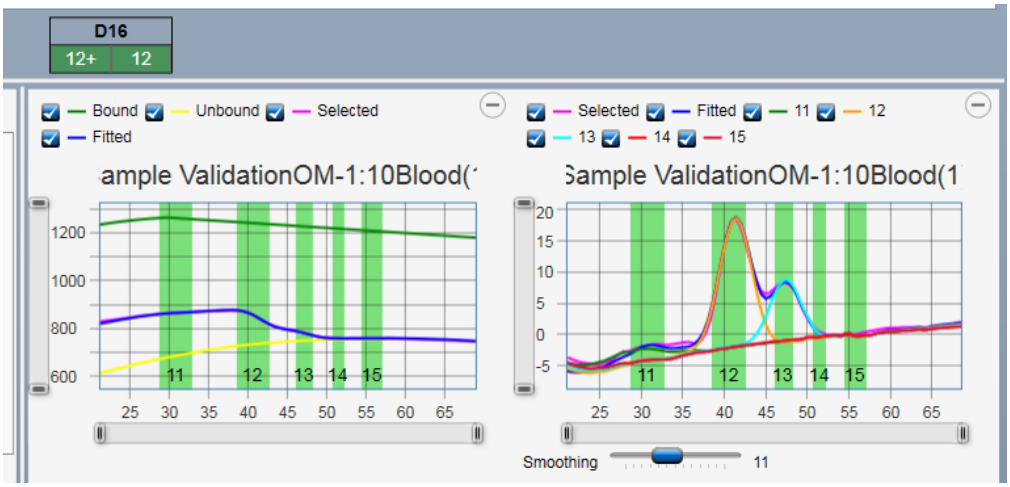

Figure 17: ParaDNA® Data Analysis software v1.1 showing a small peak at allele 13 for locus D16 for blood dilution sample, 1:10(1)

The software most likely saw this as a stutter peak, therefore calling this sample a homozygote at D16. When reanalyzed by Foster + Freeman on v1.6, there was not a confident allele call for the second allele, rather a gray box appeared indicating there is not enough data to give a confident call (table 7).

Table 7: Allele calls made for the first blood dilution 1:10 on software v1.1 and v1.6. Green alleles are confident allele calls, yellow are uncertain, and gray squares represents not enough information to show a result.

		D16	D16	D18	D18	TH01	TH01	D8	D8	AME	AME	D3	D3
Blood 1:10(1)	**v1.1**	12	12	15	17	-	-	9	10	X	Y	16	18
	v1.6	12	-	15	17	-	-	9	10	X	Y	16	18

Of the 54 samples (36 dilutions and 18 buccal swabs), only one sample confidently called an incorrect allele. The sample that gave a nonconcordant allele call was the second saliva dilution 1:5. The ParaDNA® Intelligence System confidently called a Y chromosome at Amelogenin although this donor was a female Unlike the last example, this sample was reanalyzed on the 1.6 software, but still incorrectly called the Y chromosome (table 8).

Table 8: Allele calls made for the second saliva dilution 1:5 on software v1.1 and v1.6. Green alleles are confident allele calls, yellow are uncertain, and gray squares represents not enough information to show a result.

		D16	D16	D18	D18	TH01	TH01	D8	D8	AME	AME	D3	D3
Saliva 1:5(2)	**v1.1**	11	11	17	17	8	9	13	14+	X	Y	-	-
	v1.6	11	-	17	-	8	9	13	14+	X	Y	-	-
	STR	11	11	17	17	9	9	13	15	X	X	16	18

This sample was also flagged as a possible mixture. The results for this sample were also sent to Foster + Freeman where they determined the sample looked messy, but were unable to determine what could have caused this. There was no detection of a mixture or a Y chromosome in the STR profile. Other than the two samples described above, the rest of the confident allele calls for the dilution samples and buccal swabs were all concordant with Qiagen® Investigator 24Plex QS STR and Qiagen® Investigator 24Plex GO! Kits.

Not only were most of the confident allele calls concordant with the STR profiles, but the questionable calls in yellow (only samples run on v1.6) were also 100% concordant with the Qiagen® Investigator 24Plex STR Kits, excluding the messy sample described above in table 7.

Additionally, although none of the 4 negative (with sterile water) swabs yielded a confident allele call, the Intelligence System did call a Y chromosome in negative (1) and an X chromosome and a Y chromosome at Amelogenin in negative (2). These samples did not generate any sort of peaks in the STR profile, and can be attributed to low level noise in the system. If this sample were to be seen in a real life case scenario, it is highly unlikely that the results would be used anyway since the alleles were not confidently called, and no other alleles at other loci were present.

The buccal swabs run on the Intelligence System are those of Plano PD personnel and the STR profiles obtained will be used for the elimination database generated to assess any possible contamination for future use [2].

Screening % Score and Intelligence allele calls vs profile obtained

Samples that received a score ≥ 61% on the ParaDNA® Screening System returned a full profile in STR analysis. The samples with a % score ≤ 12% only returned a partial profile with peaks below 100RFU, which would not be used in analysis in most crime labs. There were no samples that received a score in between 12 and 61%, so it is difficult of make conclusions as to the type of profile that would be obtained from these types of samples. Overall, there is a correlation between the ParaDNA® Screening System % score and the type of STR profile obtained. The higher the % score, the more likely it is to obtain a quality STR profile. Lower % scores indicate a chance of getting uninformative STR profiles. From these results, and results obtained from Whitney West's validation on touch samples, submission guidelines for samples run on the ParaDNA® Screening System were made:

Table 9: Submission guidelines made for samples run on the ParaDNA® Screening System for the Plano Police Department. Samples falling within these specific % ranges.

ParaDNA % score	Comments/what to expect	Send to lab?
61-100%	• High quality and quantity DNA detected • All peaks above 100RFU	• High priority: Send for further analysis first
39-60%	• Good Quality and quantity DNA detected • Likely to produce peaks above 100RFU	• High/medium priority: Send for further analysis • Test if needed
20-38%	• Low to medium quality and quantity DNA detected • Some peaks below 100RFU	• Low priority • Test if no other samples available
≤20%	• No DNA or only trace amounts of DNA Detected • All peaks below 100RFU	• Unlikely to give interpretable results

Samples that receive a score of 61-100% are expected to yield high quality and quantity DNA and generate full STR profiles with peaks above 100RFU. These samples should be the highest priority and sent for further analysis. Samples with scores in between 39 and 60% are expected to have good quality and quantity of DNA and are likely to yield full STR profiles. Score between 20-38% are expected to have low to medium amounts of DNA and may yield a partial STR profile with peaks below 100 RFU. These samples are low priority and should be sent for testing if no other samples are available. Samples below 20% are not expected to have DNA, or only trace amounts of DNA. They are unlikely to give interpretable results in an STR profile.

CHAPTER V

CONCLUSIONS

This validation study describes how the ParaDNA® Instrument has proved to be capable of reliably predicting the relative amount of DNA found in a sample using the Screening Kit, and is able to produce reliable allele calls from 5 different loci, and Amelogenin, that are concordant with Qiagen® Investigator 24Plex QS and GO! STR Kits using the Intelligence System.

The Screening System can be used reliably as a presumptive test for the presence of DNA to allow the Plano PD to preferentially select items to submit for STR analyses, and thereby increase profiling success rates, and reduce backlogs [5]. The system can detect DNA at concentrations as low as 0.001ng/µL (1pg/µL) and yields an accurate indication of the amount of DNA present in an item. Samples with higher % scores can be prioritized for further STR testing to ensure more interpretable results are obtained, while lower score <20% indicate an item may only contain trace amounts of DNA and is unlikely to yield interpretable STR results.

The Intelligence System directly analyzes 5 STR loci plus Amelogenin to generate a profile that can be used by the Plano PD to gain rapid investigative leads. This study shows that the Intelligence System allele calls are 99% concordant with Qiagen® Investigator 24Plex QS and GO! STR Kits when tested with blood and saliva when looking at 54 samples. The Intelligence System was able to generate a usable DNA profile at amounts of DNA at

concentrations as low as 0.1ng/µL, and could detect DNA at concentrations as low as 0.01ng/µL (10pg/µL).

The ParaDNA® Instrument with the Screening and Intelligence Systems offers an effective methodology for presumptive screening of DNA and will be useful to the Plano PD for prioritizing samples that should be sent for STR analysis, as well as allow them to gain quicker investigative leads. Further studies should be validated on this instrument to look at the reliability of using other types of biological fluids, such as semen or urine, as well as assess the robustness of the mixture detection on the Intelligence System.

APPENDIX

Table 10: Blood and saliva dilutions and their Screening % score, quantification value and the type of STR profile obtained.

Sample Name	***Fluid***	***% Score***	***Quant Value (ng/μL)***	***Profile Obtained***
(1)Neat 10μL	Saliva	71	0.075333	Full
(2)Neat 10μL	Saliva	100	1.7675908	Full
(3)Neat 10μL	Saliva	100	0.4797006	Full
(1) Neat 1μL	Saliva	64	0.1953475	Full
(2) Neat 1μL	Saliva	80	0.1192623	Full
(3) Neat 1μL	Saliva	97	0.0808598	Full
(1)1:2	Saliva	98	0.3001171	Full
(2) 1:2	Saliva	100	0.2962701	Full
(3) 1:2	Saliva	100	0.3375711	Full
(1) 1:5	Saliva	99	0.2307042	Full
(2) 1:5	Saliva	100	0.2843850	Full
(3) 1:5	Saliva	100	0.2545585	Full
(1) 1:10	Saliva	69	0.04065711	Full
(2) 1:10	Saliva	96	0.02768133	Full
(3) 1:10	Saliva	100	0.74011486	Full
(1) 1:100	Saliva	0	0.00295568	Partial
(2) 1:100	Saliva	0	0.00211152	Partial
(3) 1:100	Saliva	0	0.00215050	Partial
(1)Neat 10μL	Blood	96	0.32727181	Full
(2)Neat 10μL	Blood	100	0.43178480	Full
(3)Neat 10μL	Blood	95	0.35245281	Full
(1)Neat 1μL	Blood	84	0.03705340	Full
(2)Neat 1μL	Blood	75	0.02052355	Full
(3)Neat 1μL	Blood	65	0.02940163	Full
(1)1:2	Blood	87	0.4687268	Full
(2) 1:2	Blood	95	0.4612871	Full
(3) 1:2	Blood	100	0.4749349	Full
(1) 1:5	Blood	88	0.3386735	Full
(2) 1:5	Blood	96	0.2882618	Full
(3) 1:5	Blood	85	0.2306385	Full
(1) 1:10	Blood	77	0.1389988	Full
(2) 1:10	Blood	89	0.1104560	Full
(3) 1:10	Blood	86	0.1197842	Full
(1) 1:100	Blood	12	0.0013276	Partial
(2) 1:100	Blood	2	0.0013852	Partial
(3) 1:100	Blood	0	0.0010658	Partial
(1) Negative Control	Sterile Water	0	0	None
(2) Negative Control	Sterile Water	0	0	None
(3) Negative Control	Sterile Water	0	0	None
(4) Negative Control	Sterile Water	0	0	None

Table 11: Blood and saliva dilutions run on the Intelligence System v1.1. Their corresponding % score, quant value, and alleles called are shown. The STR results for the donors are shown at the top for comparison. Green are confident calls, grey is no call because of poor signal, and yellow are uncertain calls.

Sample Name	***Type***	***% Score***	***Quant Value(ng/µL)***	***D16***	***D16***	***D18***	***D18***	***TH01***	***TH01***	***D8***	***D8***	***AME***	***AME***	***D3***	***D3***
Saliva donor STR results	-	-	-	11	11	17	17	9	9	13	15	X	X	16	18
Blood donor STR results	-	-	-	12	13	15	17	6	8	9	10	X	Y	16	18
(1) Neat 10µL	Saliva	56	0.1752	11	-	17	-	9	-	14	15	X	X	16	18
(2) Neat 10µL	Saliva	39	0.2785	11	-	17	-	9	-	13	15	X	Y	16	18
3 Neat 10µL	Saliva	54	0.3551	11	-	17	17	9	-	15	-	X	X	16	18
(1) Neat 1µL	Saliva	18	0.033536	11	-	14+	-	9	-	13	15	X	Y	16 +	-
(2) Neat 1µL	Saliva	50	0.090109	11	11	17	-	9	-	13	15	X	-	16	18
(3) Neat 1µL	Saliva	40	0.13204	11	-	17	17	-	-	13	15	Y	-	16	-
(1)1:2	Saliva	69	0.792009	11	11	17	17	9	9	13	15	X	X	16	18
(2) 1:2	Saliva	70	1.277184	11	12+	14+	17	9	-	13	15	X	X	16	18
(3) 1:2	Saliva	45	0.74719	11	-	14+	17	9	9	13	15	X	X	16	18
(1) 1:5	Saliva	51	0.3667	11	11	17	17	9	9	13	15	X	X	16	18
(2) 1:5	Saliva	43	0.193645	11	11	17	17	8	9	13	14 +	X	Y	-	-
(3) 1:5	Saliva	35	0.295698	11	11	17+	-	9	9	13	14 +	X	X	16	18
(1) 1:10	Saliva	24	0.064856	-	-	14+	17	9	9	15	-	X	X	16	16
(2) 1:10	Saliva	60	0.562926	11	11	17	-	9	9	13	15	X	X	16	18
(3) 1:10	Saliva	62	0.989482	11	11	17	17	9	9	13	15	X	X	16	18
1 Neat 10µL	Blood	100	0.254633	12	13	15	17	6	8	9	10	X	Y	16	18
2 Neat 10µL	Blood	83	0.319757	12	13	15	17	6	8	9	10	X	Y	16	18
3 Neat 10µL	Blood	95	0.276115	12	13	15	17	6	8	9	10	X	Y	16	18
1 Neat 1µL	Blood	27	0.014243	-	-	17+	-	-	-	9	10	-	-	18	-
2 Neat 1µL	Blood	60	0.02963	12	-	17	-	-	-	9	9	X	-	18	-
3 Neat 1µL	Blood	36	0.025524	12	13	15	17+	6	8	-	-	X	Y	16	-
(1)1:2	Blood	78	0.417747	12	13	15	17	6	8	9	17	X	Y	16	18
(2) 1:2	Blood	80	0.42051	12	13	15	17	6	8	9	10	X	Y	16	18
(3) 1:2	Blood	100	1.15928	12	13	15	17	6	8	9	10	X	Y	16	18
(1) 1:5	Blood	37	0.2377	12	13	15	17	-	-	9	14 +	X	Y	16	18
(2) 1:5	Blood	85	0.398043	13	13	15	17+	6	8	9	10	X	Y	16	18
(3) 1:5	Blood	89	0.242791	12	12	15	17	-	-	9	10	X	Y	18	-
(1) 1:10	Blood	68	0.107539	12	12	15	17	-	-	9	10	X	Y	16	18
(2) 1:10	Blood	56	0.114638	12	13	17	-	6	8	9	10	X	Y	16	18
(3) 1:10	Blood	66	0.128033	13	-	15	17	6	-	9	10	X	Y	18	-
(1) 1:100	Blood	0	0.000897	-	-	-	-	-	-	-	-	-	-	-	-
(2) 1:100	Blood	0	0.001631	-	-	14+	-	-	-	-	-	-	-	-	-
(3) 1:100	Blood	0	0.001269	-	-	-	-	-	-	-	-	-	Y	-	-

Table 12: Blood and saliva dilutions ran on the Intelligence System v1.6. Their corresponding % score, quant value, and alleles called are shown. Green are confident calls, grey is no call because of poor signal, and yellow are uncertain calls.

Sample Name	***Type***	***% Score***	***Quant Value(ng/ µL)***	***D16***	***D16***	***D18***	***D18***	***TH01***	***TH01***	***D8***	***D8***	***AME***	***AME***	***D3***	***D3***
(3) 1:2	Saliva	45	0.74719	11	-	14+	17	9	9	13	15	X	-	16	18
(1) 1:5	Saliva	51	0.3667	11	-	17	-	9	-	13	15	X	-	16	18
(2) 1:5	Saliva	43	0.193645	11	-	17	-	8	9	13	14 +	X	Y	-	-
(3) 1:5	Saliva	35	0.295698	11	-	17+	-	9	-	13	14 +	X	-	16	18
(1) 1:10	Saliva	24	0.064856	-	-	14+	17	9	-	15	-	X	-	16	-
(2) 1:10	Saliva	60	0.562926	11	-	17	-	9	9	13	15	X	-	16	18
(3) 1:10	Saliva	62	0.989482	11	-	17	17	9	9	13	15	X	X	16	18
(1) 1:100	Saliva	0	0.001688	-	-	-	-	-	-	-	-	-	-	-	-
(2) 1:100	Saliva	0	0.002031	-	-	-	-	-	-	-	-	-	-	-	-
(3) 1:100	Saliva	1	0.000929	-	-	-	-	-	-	-	-	-	Y	16 +	-
(2) 1:5	Blood	71	0.398043	13	-	15	17	6	-	9	10	X	Y	16	18
(3) 1:5	Blood	70	0.242791	12	-	15	17	-	-	9	10	X	Y	18	-
(1) 1:10	Blood	55	0.2377	12	-	15	17	-	-	9	10	X	Y	16	18
Neat 1µL (1)Remelt	Saliva	12	N/A	11	-	17+	-	9	9	13	15	X	X	16 +	-
Negative (1)	Sterile Water	1	0	-	-	-	-	-	-	-	-	Y	-	-	-
Negative (2)	Sterile Water	6	0	-	-	-	-	-	-	-	-	X	Y	-	-
Negative (3)	Sterile Water	0	0	-	-	-	-	-	-	11 +	-	-	-	-	-
Negative (4)	Sterile Water	0	0	-	-	-	-	-	-		-	-	-	-	-

Table 13: Buccal swabs from Plano PD personnel that were run on the Intelligence System v1.6. Their corresponding % score, quant value, and alleles called are shown. Below each Intelligence allele calls are the corresponding STR allele calls. Green are confident calls, grey is no call because of poor signal, and yellow are uncertain calls.

Sample Name	***Type***	***% Score***	***Quant Value(ng/µL)***	***D16***	***D16***	***D18***	***D18***	***TH01***	***TH01***	***D8***	***D8***	***AME***	***AME***	***D3***	***D3***
Donor 1	Intel	91	-	10	11	13	14	8	9	15	-	X	Y	16	18
	STR		-	10	11	13	14	8	9	12	15	X	Y	16	18
Donor 2	Intel	76	-	9	12	16	18	6	7	12	16	X	X	16	17
	STR		-	9	12	16	18	6	7	12	16	X	X	16	17
Donor 3	Intel	77	-	9	11	16	17	6	7	14	-	X	X	16	17
	STR		-	9	11	16	17	6	7	12	14	X	X	16	17
Donor 4	Intel	99	-	11	12	13	15	6	9	13	14	X	Y	16	16
	STR		-	11	12	13	15	6	9	13	14	X	Y	16	16
Donor 5	Buccal	83	-	9	14	12	18	6	7	12	14	X	Y	15	15
	STR		-	9	14	12	18	6	7	12	14	X	Y	15	15
Donor 6	Buccal	45	-	11	-	14+	-	6	6	12	13	X	-	17	18
	STR		-	11	11	14	16	6	6	12	14	X	X	17	18
Donor 7	Buccal	83	-	11	13	19	20	8	9	12	13	X	Y	14	15
	STR		-	11	13	19	20	8	9	12	13	X	Y	14	15
Donor 8	Buccal	77	-	11	11	12	12	9	9.3+	13	14	X	Y	14	16
	STR		-	11	11	12	12	9	9.3	13	14	X	Y	14	16
Donor 9	Buccal	65	-	11	13	15	18	7	9	13	-	X	-	15	16
	STR		-	11	13	15	18	7	9	13	13	X	X	15	16
Donor 10	Buccal	96	-	11	13	12	13	8	9.3+	13	14	X	Y	14	16
	STR		-	11	13	12	13	8	9.3	13	14	X	Y	14	16
Donor 11	Buccal	65	-	13	13	15	15	6	9.3+	13	17	X	-	16	17
	STR		-	13	13	15	15	6	9.3	13	17	X	X	16	17
Donor 12	Buccal	88	-	9	12	12	16	6	7	13	14	X	X	16	17
	STR		-	9	12	12	16	6	7	13	14	X	X	16	17
Donor 13	Buccal	92	-	11	13	13	16	9	9.3+	8	10	X	Y	14	16
	STR		-	11	13	13	16	9	9.3	8	10	X	Y	14	16
Donor 14	Buccal	77	-	11	13	12	15	7	8	12	15	X	X	15	18
	STR		-	11	13	12	15	7	8	12	15	X	X	15	18
Donor 15	Buccal	81	-	11	12	12	17	7	7	12	14	X	Y	15	15
	STR		-	11	12	12	17	7	7	12	14	X	Y	15	15
Donor 16	Buccal	64	-	10	11	14	20	6	8	11	12	X	Y	17	17
	STR		-	10	11	14	20	6	8	11	12	X	Y	17	17
Donor 17	Buccal	77	-	10	10	13	18	7	8	12	13	X	X	16	18
	STR		-	10	10	13	18	7	8	12	13	X	X	16	18
Donor18	Buccal		-	12	13	15	17	6	8	9	10	X	Y	16	18
	STR		-	12	13	15	17	6	8	9	10	X	Y	16	18

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