Predicting the Quality of DNA Profiles through the Evaluation of the ParaDNA® Screening Instrument

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Abstract

In order to determine if the Palm Beach County Sheriff's Office (PBSO) Forensic Biology Unit could limit the number of low quality samples processed in the laboratory, the LGC Forensics ParaDNA® Screening Instrument with the Screening Kit was evaluated. The ParaDNA® Instrument processes evidentiary samples in 75 minutes, reporting a percent score indicative of whether the sample will yield a positive result from STR analysis and whether the DNA originates from a male or female. In order to determine if the ParaDNA® Instrument could be implemented at PBSO, 82 samples were analyzed on the ParaDNA® Instrument and a portion of the same sample processed through current PBSO protocols. The samples were tested with the ParaDNA® Screening Kit, which amplifies 3 loci (TH01, D16S539, and Amelogenin) and detects the presence of DNA through a HyBeacon™ Assay. At the end of a test, the instrument software reports a percent score, which relates to the quality of DNA present, and a gender call. The ParaDNA® percent score of each sample was compared to the quantification value, the profile obtained, and the average RFU value for each dye channel. These comparisons were used to determine the correlation between the ParaDNA® percent score and the STR profile result. The results of this evaluation indicated that the ParaDNA® Screening Instrument with the Screening Kit may serve as a useful tool to prioritize evidentiary samples to be processed for STR analysis by helping to determine which samples may yield the most interpretable DNA profiles.

Figure 1: *ParaDNA® Screening Kit Cartridge, Screening Instrument, and Sample Collector*

Project Goals

- Test the usability of the ParaDNA® instrument and sample collector
- Test the efficiency of the sample collector to recover DNA from various types of evidence, including blood, saliva, mixed/differential samples, and touch samples
- Determine the correlation of the results obtained with the ParaDNA® instrument to the results obtained through traditional STR testing using current PBSO analysis methods
- Define the routes of implementation of the ParaDNA® instrument in the PBSO laboratory

Materials and Methods

Sample Collection
A total of 82 samples were prepared for this study. Samples were identified as blood (15), mixed/differential (10), saliva (34), and touch (23). All samples were prepared at the PBSO laboratory. Samples run on the ParaDNA® Instrument were collected either directly from the sample or indirectly from a swab of the sample.

ParaDNA® Testing
All 82 samples were tested on the ParaDNA® Instrument using the Screening Kit. The percent score and gender call was recorded for each sample.

STR Analysis
STR analysis was performed on 92 samples (10 samples required differential extraction), following current PBSO validated procedures. Extraction was performed on an EZ1 Advanced XL using the DNA Investigator Kit. Quantification was performed using Plexor® HY and an AB 7500 Real-Time PCR System. Amplification was performed using PowerPlex® 16 and a GeneAmp® PCR System 9700. Capillary electrophoresis was performed on a 3130*xl* Genetic Analyzer. Profiles were analyzed using the GeneMapper® ID-*X* Software version 1.3.

Comparisons
Comparisons were made between the results of the ParaDNA® testing and the results of STR analysis to determine the correlations between the results. The ParaDNA® percent score for each sample was compared to the quantification value, the profile obtained (full, partial, mixture, none), and the average relative fluorescence across each dye channel.

Results

ParaDNA® Percent Scores
The percent scores reported by the ParaDNA® Instrument were categorized into four groups: 75-100%, 25-74%, 1-24%, and 0%. Figure 2 shows the percentage of samples in each category. Table 1 shows the percent scores assigned by the instrument per sample type.

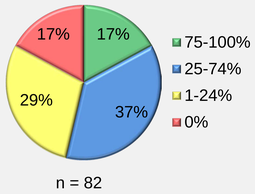

Figure 2: *Percent scores assigned by the ParaDNA® Instrument*

Table 1: *Percent scores assigned by ParaDNA® Instrument by sample category*

	75-100%	25-74%	1-24%	0%
Blood	6	4	3	2
Mixed	8	2	0	0
Saliva	0	18	14	2
Touch	0	6	7	10

ParaDNA® Percent Scores vs. Quantification Values
The ParaDNA® percent scores were compared to the quantification values for each sample to determine the correlation between the percent score and the amount of DNA present. Table 2 shows the average quantification values and percent of samples above the PBSO target amplification value of 0.8 ng before and after normalization.

Table 2: *Average quantification values and percent of samples above target (0.8 ng) before and after normalization*

ParaDNA® Percent Scores	Number of Samples	Average Quantification Value (ng/µL)	Percent Above Target	Percent Above Target After Normalization
75-100%	22	22.12	100	100
25-74%	32	5.96	34	63
1-24%	24	0.08	4	25
0%	14	0.04	0	21

ParaDNA® Percent Scores vs. Profiles Obtained
After STR analysis was complete, the profiles obtained were classified into one of five categories: full, partial, mixture – full, mixture – partial, or none. Figure 3 shows the percentage of profile types that were obtained for each ParaDNA® percent score category.

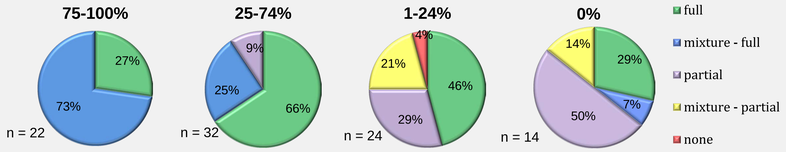

Figure 3: *Percentage of profile types obtained for each ParaDNA® percent score category*

ParaDNA® Percent Scores vs. Stochastic Threshold
The average RFU was calculated, by dye channel, for each sample in order to determine how many samples were above the PBSO stochastic threshold of 208 RFU in each ParaDNA® percent score range. This was used to determine the correlation between the ParaDNA® percent score and the quality of the profile obtained. Figure 4 shows the number of profiles above and below the stochastic threshold for each ParaDNA® percent score category.

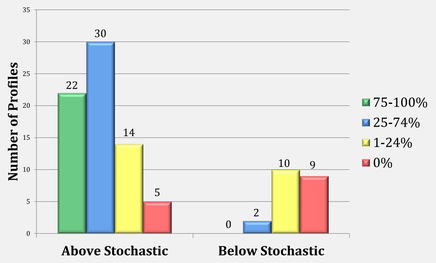

Figure 4: *Number of profiles above and below stochastic threshold by ParaDNA® percent score category*

Conclusions

What to expect:

75-100%
- Full profiles
- Above stochastic threshold
- Balanced heterozygote peaks

25-74%
- Mostly full profiles
- Above stochastic threshold
- Fairly balanced heterozygote peaks

1-24%
- Partial or full profiles
- Below stochastic threshold
- Imbalanced heterozygote peaks

0%
- Partial or full profiles
- Below stochastic threshold
- Imbalanced heterozygote peaks

How ParaDNA® can help:

- Reduce laboratory backlog by:
  - Decreasing total number of samples tested
  - Eliminating samples with little to no DNA present
  - Increasing laboratory efficiency
- Save laboratory resources by:
  - Allowing for testing of high quality samples
  - Reducing wasted time and money on samples with no DNA

Recommendations

Table 3: *Prioritization of samples based on ParaDNA® percent scores*

ParaDNA® Percent Score	Priority	Comments
75-100	High	Test first; Likely to obtain interpretable results
25-74	High/Medium	Test if needed; Likely to obtain interpretable results
1-24	Low	Test if needed; May or may not obtain interpretable results
0	Low	Save for later testing; Not likely to obtain interpretable results

Table 3 summarizes the recommendations for the implementation of the ParaDNA® Screening Instrument in the PBSO laboratory. This study can be repeated with the ParaDNA® Intelligence Kit, when available. The Intelligence Kit tests the same three loci as the Screening Kit, as well as three additional loci. The results of the Intelligence Kit are reported as allele calls, which may provide an investigative lead in casework scenarios.

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