Evaluation and Comparative Analysis of Rapid DNA Analysis and Direct Amplification

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Abstract

After this presentation, attendees will gain a better understanding of the differences between Rapid DNA Analysis and Direct Amplification for the swift development of investigative leads for law enforcement.

This presentation will impact the forensic science community by providing forensic laboratories with the knowledge to determine if Rapid DNA Analysis and/or Direct Amplification may be suitable for the swift development of investigate leads for law enforcement through an evaluation of the performance, efficiency, and cost of both systems.

Traditional forensic DNA analysis involves multiple steps and can be time-consuming. In certain situations, it may be beneficial to speed up this process and obtain an interpretable DNA profile within a few hours. Rapid DNA workflows are optimized to produce DNA profiles in a relativity short amount of time, albeit from high-level, single-source DNA samples, such as buccal swabs. The application of these rapid DNA workflows for the processing of casework samples, which do not always offer a high quality and/or quantity of DNA, is of great interest to the forensic community as well as law enforcement.

Commercially available rapid DNA instruments have been marketed toward law enforcement agencies for the analysis of casework samples to generate investigative leads quickly. To provide law enforcement with options for more rapid and efficient processing techniques that lead to real-time investigative leads, the Houston Forensic Science Center launched a pilot project to potentially determine the most suitable rapid DNA workflow to integrate into the accredited Forensic Biology Section and undergo a more significant validation. To aid in this determination, this study evaluated the performance, efficiency, and cost of a single Rapid DNA Analysis system and several direct amplification kits.

Samples (n = 450) of various types and concentrations, deposited on various substrates were processed using the Applied Biosystems™ RapidHit™ ID system (RHID) utilizing INTEL cartridges and the following direct amplification kits: Applied Biosystems™ GlobalFiler™ Express PCR Amplification Kit lysed in Prep-n-Go™ Buffer, Promega PowerPlex® Fusion 6C System lysed with Casework Direct System, Qiagen Investigator 24plex QS and Qiagen Investigator 26plex QS Kits lysed with Investigator Casework GO! Kit.

The success rate of each rapid DNA system was measured by the percentage of complete and concordant genotypes generated at the 20 CODIS core loci when compared to genotypes obtained from the traditional laboratory workflow. For GlobalFiler™ Express, the success rate of obtaining a complete DNA profile was 95% for blood and 85% for semen. For PowerPlex® Fusion 6C, the success rate was 80% for blood and 95% for semen. For Investigator 24plex QS, the success rate was 95% for blood and 95% for semen. For the Investigator 26plex QS, the success rate was 100% for blood and 90% for semen. For RapidHit™ ID, the success rate was 100% for blood and 85% for semen. A failure rate of 3.3% due to instrument errors was observed with the RHID system. None of the systems produced full profiles with saliva or miscellaneous samples containing 1ng or less DNA. Concordance between rapid DNA systems was observed for 98.83% of the STR alleles compared. Five samples exhibited either a single drop-in event or multiple alleles suggesting the presence of a contaminant.

The average analysis time for any of the direct amplification kits is approximately 3 to 3.5 hours regardless of the number of samples. The average analysis time for RHID analysis is approximately 1.5 hours per sample. The cost of the RHID system can range from $220/sample down to $90/sample. Considering only the cost of the chemistry and no overhead, supplemental reagents, or consumables, GlobalFiler™ Express is ~$18/sample, PowerPlex® Fusion 6C is ~$13/sample, Investigator 24plex QS is ~$23/sample, and Investigator 26plex QS is ~$22/sample.

Overall, both the RapidHit™ ID system and the direct amplification kits performed similarly. Both performed well with blood and semen samples, but variable results were obtained from saliva and miscellaneous samples containing ≤1ng DNA. Both workflows have benefits and drawbacks that should be considered. While the RHID instrument can be mobile, the direct amplification workflow is restricted to the laboratory, but has the added benefit of processing multiple samples simultaneously. Reprocessing is also possible with both workflows. Both workflows coupled with an internal database can be powerful resources for developing timely investigative leads for law enforcement.

Introduction

- Forensic DNA analysis traditionally involves a multi-step workflow.
- Recent advancements in DNA technology have led to a demand for the implementation of more rapid and efficient processing techniques that further enhance the capabilities of forensic laboratories in providing law enforcement with real-time investigative leads.
- The speeding up of the investigative process leads to potential benefits such as increased public confidence in the investigative process, reduced crime by catching offenders earlier, eliminating potential suspects, exonerating the innocent, and overall reduction in cost related to man-hours.
- However, the adoption of any new method or technology requires careful consideration. Decreasing analysis time is only beneficial if the quality of the data is consistent with current methods.
- To provide law enforcement with options for the swift development of investigative leads in criminal investigations, the Houston Forensic Science Center launched a pilot project to determine the most suitable rapid DNA workflow to integrate into the accredited Forensic Biology Section and to undergo a more significant validation.
- To aid in this determination, the study was designed to evaluate the performance, efficiency, and cost of a single Rapid DNA Analysis System as well as several direct PCR amplification kits.
- This study compared DNA profiles obtained during the traditional casework process to DNA profiles generated from a single rapid DNA analysis system, the Applied Biosystems™ RapidHIT™ ID System, and four direct PCR amplification chemistries manufactured by three different companies: Applied Biosystems™ GlobalFiler™ Express PCR Amplification Kit, Promega PowerPlex® Fusion 6C System, and QIAGEN® Investigator 24plex QS and Investigator 26plex QS Kits.
- In this study, the Houston Forensic Science Center (HFSC) reports the ability of direct PCR amplification and Rapid DNA Analysis to generate DNA profiles from samples of various concentrations that have been deposited on various substrate types.

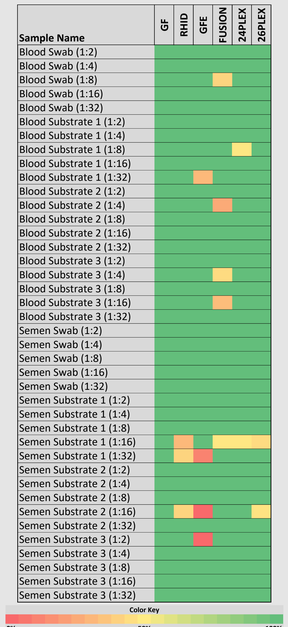

Figure 2. Heat map of % complete profiles for all samples at the 20 CODIS core loci. 50 RFU was used as the analytical threshold.

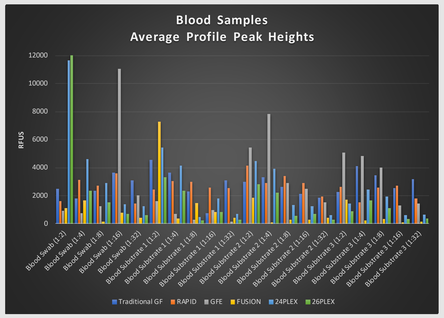

Figure 3. Average Profile Peak Heights for Blood Samples.
The average profile peak heights across the 20 core loci from each blood sample amplified with each rapid workflow and traditional casework workflow.

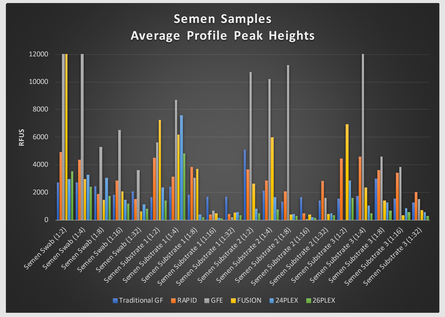

Figure 4. Average Profile Peak Heights for Semen Samples.
The average profile peak heights across the 20 core loci from each semen sample amplified with each rapid workflow and traditional casework workflow.

Materials & Methods

- Anonymous whole-blood, semen, saliva, urine, and fecal samples were purchased from Lee BioSolutions (Maryland Heights, MO).
- Contact and miscellaneous samples were collected from laboratory personnel and anonymized.
- Samples of various types and concentrations, deposited on various substrates were processed as depicted in Figure 1.
- Samples were amplified on a ProFlex™ PCR System (ThermoFisher Scientific, Waltham, MA).
- Capillary electrophoresis was performed on a 3500 Series Genetic Analyzer with Data Collection Software v3.0 (ThermoFisher Scientific, Waltham, MA).
- GMID-X v1.4 (ThermoFisher Scientific, Waltham, MA) was utilized for data analysis.

Figure 1. Sample Processing Schematic.

Results & Conclusions

- Overall, the RapidHit™ ID system and the direct amplification kits performed similarly.
  - All systems performed well with blood and semen samples (See Figures 2 – 4).
  - Success rate was measured by the percentage of complete and concordant genotypes generated at the 20 CODIS core loci (See Table 1).
  - Concordance between rapid DNA systems was observed for 98.83% of the STR alleles compared.
    - Five samples exhibited either a single drop-in event or multiple alleles suggesting the presence of a contaminant.
  - A failure rate of 3.3% due to instrument errors was observed with the RHID system.
  - Data from saliva and miscellaneous samples containing ≤1ng DNA were ultimately not used in the decision-making process because of the variable results obtained.
  - Refer to Table 2 for a basic cost and time analysis.
- Both workflows have benefits and drawbacks that should be considered.
  - While the RHID instrument can be mobile, the direct amplification workflow is restricted to the laboratory, but has the added benefit of processing multiple samples simultaneously.
  - Reprocessing is possible with both workflows.
- Both workflows coupled with an internal database can be powerful resources for developing timely investigative leads for law enforcement.

Rapid Workflow	Blood	Semen
RapidHit™ ID	100%	85%
GlobalFiler™ Express	95%	85%
PowerPlex® Fusion 6C	80%	95%
Investigator 24plex QS	95%	95%
Investigator 26plex QS	100%	90%

Table 1. Success rates for each Rapid DNA Workflow.

Rapid Workflow	Cost/Sample†	Analysis Time
RapidHit™ ID	$220 - $90	1.5 hours
GlobalFiler™ Express	~$18	3 to 3.5 hours‡
PowerPlex® Fusion 6C	~$13	3 to 3.5 hours‡
Investigator 24plex QS	~$23	3 to 3.5 hours‡
Investigator 26plex QS	~$22	3 to 3.5 hours‡

Table 2. Cost and Time Analysis.
†Considering only the cost of the chemistry with no overhead, supplemental reagents, or consumables.
‡Analysis time is not dependent on number of samples.

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