Assessment and Initial Impressions of the New Applied Biosystems RapidINTEL Plus Sample Cartridge

Jennifer C. Cihlar[1,2], Sarah Schmitz[3], Kirsten Naughton[3], Dane Plaza[3], Robert O'Brien[4], Peterjon K. McAnany[5], Robert Lagacé[5]

[1] Center for Human Identification, University of North Texas Health Science Center; 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[2] Department of Microbiology, Immunology, and Genetics, University of North Texas Health Science Center; 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[3] Bode Technology, 10430 Furnace Road, Suite 107, Lorton, VA 22079, USA
[4] Florida International University, Global Forensic and Justice Center, 8285 Bryan Dairy Road, Suite 125, Largo, FL 33777, USA
[5] Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080, USA

Introduction

Rapid DNA technologies can generate a short tandem repeat (STR) profile in approximately ninety minutes with minimal human intervention. Use of Rapid DNA technologies has continued to grow, fueled by the potential impact on turn-around times and the opportunity to include non-traditional operators and place the instrument in non-traditional locations. While the initial focus has been on reference buccal swabs, recent developments are aimed at improving Rapid DNA instruments' ability to process more challenging samples (i.e., samples of lower DNA quality and/or quantity). The Applied Biosystems RapidINTEL Plus Sample Cartridge (in development by Thermo Fisher Scientific) is one example of recent developments for the RapidHIT ID instrument. This sample cartridge now includes internal quality control and quantitation markers to aid in identifying inhibited, degraded, or low-quantity DNA samples, and the user can select from two different instrument protocols once the cartridge is loaded into the instrument.

Materials and Methods

Forty samples were run on the RapidHIT ID instrument with the RapidINTEL Plus Sample Cartridge at three different sites (University of North Texas Health Science Center (UNTHSC), Bode Technology (BODE), and Global Forensic and Justice Center (GFJC); N=120 in total). These samples included a sensitivity series with known volumes of blood ranging from 0.1 µL to 20 µL and mock casework samples (e.g., touch, blood, and saliva samples).

Figure 1. RapidHIT ID Instrument.

Metrics such as profile completeness, peak height balance, locus balance, and number of flagged alleles were evaluated to assess performance. Sample collection details (including type of swab), processing (where applicable), and instrument protocol selections were well-documented for cross-site comparison and assessment of potential impact on results.

Figure 2. A Puritan Cotton Swab (A) was used when selecting the "General" protocol on the RapidHIT ID instrument, and the Puritan PurFlock Micro Ultrafine Flock Swab (B) was used when selecting the "Specialized" protocol on the RapidHIT ID instrument.

Results

Sensitivity Study

Data from the sensitivity series were used to assess the newly added quantitation markers. The sensitivity series samples were prepared with varying amounts of blood (0.1 – 20 µL) added to cotton and Micro Flock swabs. Figure 3 illustrates that the peak heights from both the small and large quantitation markers increased as the amount of blood (and, thus, DNA) on the swab increased.

Data from the sensitivity series were also used to assess the new RapidINTEL Plus Sample Cartridge's ability to evaluate varying sample inputs (Figure 4). Genotypes from each of the profiles produced were categorized by concordance to known profiles and the presence of any quality flags. Results for the 0.1 µL and 0.5 µL blood input amounts were compared across both the Specialized and General protocols available for selection with the RapidINTEL Plus Sample Cartridge (Figure 5).

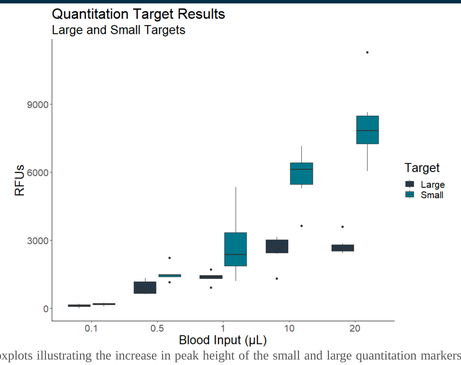

Figure 3. Boxplots illustrating the increase in peak height of the small and large quantitation markers as the amount of blood included in the sample cartridge is increased. The horizontal bar within the box represents the median for the associated data. The "whiskers" or vertical bars on the boxplot represent 1.5 * interquartile range. The individually drawn points (black dots) represent points that extend beyond 1.5*interquartile range.

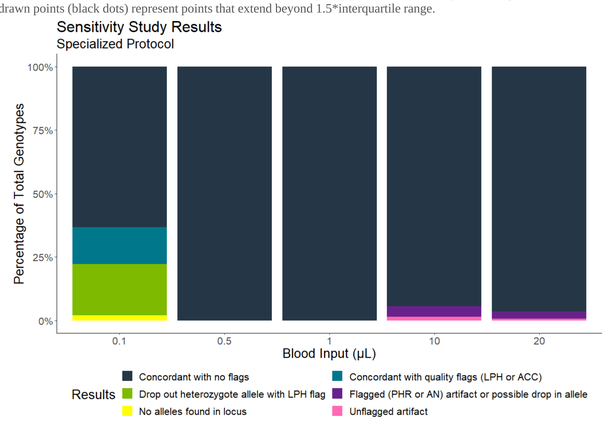

Figure 4. Bar graphs illustrating the automated genotyping results for each of the varying sample input amounts. 100% of the genotypes at 0.5 µL and 1 µL of blood were concordant with no quality flags. At lower amounts of blood, more drop out or quality flags can be seen. At higher amounts of blood, more artifacts can be seen. Each allele in the "Unflagged artifact" category was a high stutter peak of a homozygous 15 allele in the D19S433 locus.

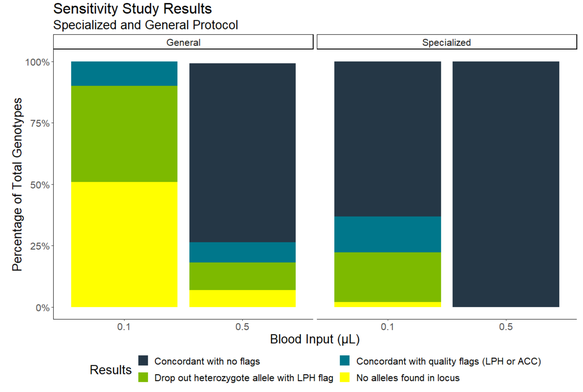

Figure 5. Bar graphs illustrating the automated genotyping results for the 0.1 µL and 0.5 µL blood input amounts using both the General (n=5 for 0.1 µL; n=6 for 0.5 µL) and Specialized (n=6 for 0.1 µL; n=5 for 0.5 µL) protocol. The higher percentage of genotypes that were concordant with no quality flags using the Specialized protocol illustrates the increase in sensitivity provided by the Specialized protocol.

Mock Casework Study

Mock casework samples, including blood on cloth, blood on cotton swab, blood on denim, blood on tile, buccal swab, cigarette, cup, face mask, gum, straw, and FTA card, were prepared and sent to all three sites (UNTHSC, BODE, and GFJC) to process on the RapidHIT ID instrument with the RapidINTEL Plus Sample Cartridge. Data from the mock casework samples further illustrate the sensitivity and robustness of the RapidINTEL Plus Sample cartridge on a variety of sample types. Figures 6-8 illustrate that protocol and processing (e.g., sub-sampling, cutting, etc.) options can result in varying workflows across laboratories/agencies. Further, these differences potentially can impact the results that are generated.

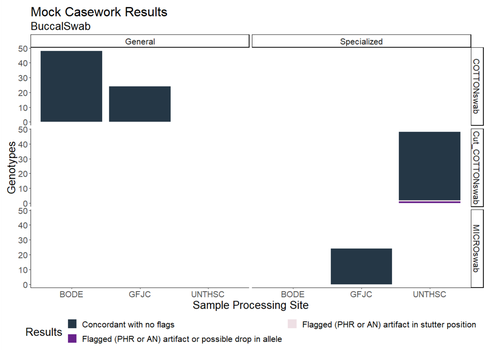

Figure 6. Bar graphs illustrating the automated genotyping results for the six buccal swabs processed in this study. Buccal swabs were collected with 10 swipes on Puritan cotton swabs. Data are grouped by protocol selected and any processing completed prior to running on the RapidHIT ID instrument in order to illustrate the workflow choices that each site made and the potential effect on the genotyping results. UNTHSC's decision to cut the cotton swab prior to running with the Specialized protocol resulted in the most quality flags.

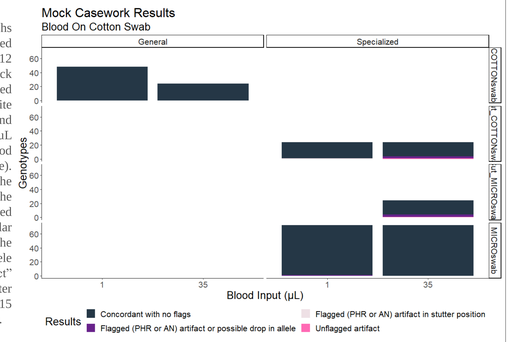

Figure 7. Bar graphs illustrating the automated genotyping results for the 12 "blood on cotton swab" mock casework samples processed in this study. Each site processed a cotton swab and Micro Flock swab with 35 µL of blood and 1 µL of blood (four swabs in total per site). Running swabs with the higher volume of blood in the Specialized protocol resulted in more quality flags (similar to results seen in the Sensitivity study). Each allele in the "Unflagged artifact" category was a high stutter peak of a homozygous 15 allele in the D19S433 locus.

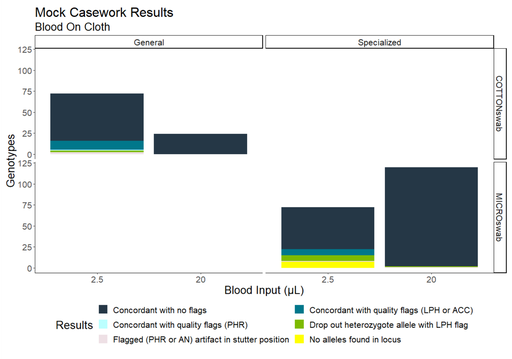

Figure 8. Bar graphs illustrating the automated genotyping results for the 12 "blood on cloth" mock casework samples processed in this study. Each site processed two samples with 20 µL of blood and two samples with 2.5 µL of blood. More quality flags were seen when processing the smaller blood sample, regardless of the swab used to sub-sample or the protocol chosen.

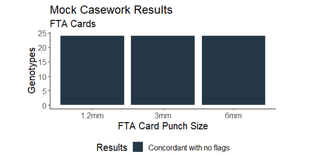

Figure 9. Bar graphs illustrating the automated genotyping results for FTA card punches at three different sizes. Profiles for each FTA card punch size were concordant with no quality flags.

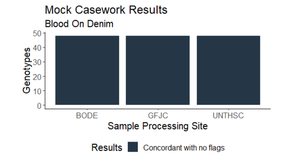

Figure 10. Bar graphs illustrating the automated genotyping results for six "blood on denim" mock casework samples. Profiles for each sample were concordant with no quality flags despite differences in how the samples were sub-sampled and run on the instrument.

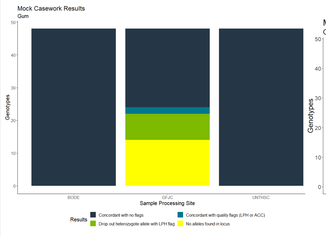

Figure 11. Bar graphs illustrating the automated genotyping results for six gum samples chewed for 30 minutes. Profiles for each sample were concordant with no quality flags with the exception of one sample run at GFJC, where two methods of processing were tested. When sub-sampling the cotton swab used to swab the chewed gum, GFJC produced a low level partial profile.

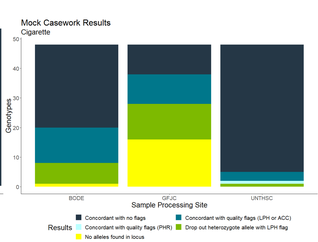

Figure 12. Bar graphs illustrating the automated genotyping results for six cigarette samples. Each site chose to swab the used cigarette before running on the RapidHIT ID instrument. The drop out and/or quality flags seen in each profile suggest future work should evaluate alternative methods of processing.

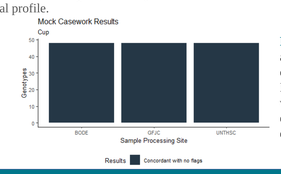

Figure 13. Bar graphs illustrating the automated genotyping results for six used cup samples. Each site sub-sampled with the Micro Flock swab and ran on the instrument with the Specialized protocol. Profiles for each sample were concordant with no quality flags.

Conclusions

Data from this study illustrate the sensitivity and robustness of the RapidINTEL Plus Sample cartridge on a variety of sample inputs and sample types. Additionally, data generated in this study provide necessary information for laboratories considering processing crime scene samples on RapidHIT ID instruments and development of standard operating procedures.

Acknowledgements

Reagents for this study were provided by Thermo Fisher Scientific. This study was funded in part by Thermo Fisher Scientific (Sponsored Research Agreement RP0223).

Questions?

THE UNIVERSITY *of* NORTH TEXAS HEALTH SCIENCE CENTER *at* FORT WORTH

Due to space limitations, only a portion of the generated data could be illustrated on this poster.