Utilization of the Rapid RapidINTEL™ Plus Cartridge on the RapidHIT ID Instrument

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Introduction

The RapidHIT™ ID system generates a CODIS-eligible short tandem repeat (STR) profile in approximately 90 minutes. Originally built to be employed for reference samples by non-traditional users, advances in Rapid DNA technology have broadened its application to include low-quantity, aged, and degraded samples, as well as various biological materials beyond saliva. The Applied Biosystems Rapid INTEL™ Plus Sample Cartridge (Thermo Fisher Scientific) is the latest cartridge type for the RapidHIT™ ID system, designed for crime scene samples and developed to meet the requirements for future CODIS/national database access. It includes built-in internal quality control and quantification markers to flag inhibited, low-quantity, and degraded DNA samples among other improvements. The Rapid INTEL™ Plus cartridge also offers two protocols for the user: General ("G") for moderate DNA quantities and Specialized ("S") for low DNA quantities.

Materials and Methods

Figure 1. RapidHIT™ ID System.

Over two-hundred samples were run on the RapidHIT ID instrument to evaluate the suitability of the Rapid RapidINTEL™ Plus Sample Cartridge for crime scene samples. These samples were divided into three sub-studies to assess the following: sub-sampling thresholds for blood swabs ranging from 0.25 µL to 5 µL of blood, sensitivity on swabs with samples at 0.125 µL to 10 µL of blood, and sensitivity on DNA collection cards with saliva samples. Performance was assessed by various metrics, including evaluating profile completeness, type and number of flags, and predicted DNA.

Figure 3. A Puritan Cotton Swab (A) and a Puritan Micro Swab (B) used on the RapidHIT™ ID System

Figure 2. DNA Collection Cards used in the Sensitivity on DNA Collection Cards Study. (A) is a Bode Buccal DNA Collectors™ (Bode Technology, Lorton, VA), and (B) is a Nucleic-card™ (Copan).

Results and Discussion

Key metrics recorded by the RapidHIT™ ID System were used to evaluate performance within sub-studies. The first metric, shown in Figures 4, 6, and 8, was the first pass success rate status. A Quality Flag status is listed if any flags are called, while a Passing Status means no flags were recorded. The second metric, illustrated in Figures 5, 8, and 9, captured the number and type of flags or missing alleles. In addition, the utility of new features for the INTEL Plus cartridge such as predicted DNA amounts and the Stochastic (STO) quality flag are shown.

Sub-Sampling Study

Data from the Sub-Sampling Study were used to evaluate the success and threshold range of Sub-Sampled Swabs, and compare cotton and micro swabs. The swabs were prepared with varying blood volumes (0.25 – 5 µL) added to cotton and micro swabs. For sub-sampling, additional cotton swabs were treated and then swabbed with a micro swab to create the sub-sampled swab. Micro and sub-swabs were processed using the Specialized protocol, while cotton swabs were run under the Generalized protocol on the Rapid INTEL Plus cartridge.

First Pass Success Rate Based on Sub-Sampling Study

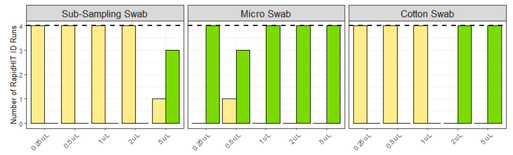

Figure 4. Bar graphs illustrating the first pass success rate for each of the swab types and their corresponding input amounts, with a dotted line to indicate the number of runs for each. All swab types increased the amount of passes as the volume of blood input increased. Sub-sampled swabs started to get passing status's at around 5 µL, micro swabs passed in all input values with an exception at 0.5 µL, and cotton swabs had all runs 2 µLs and greater pass.

Flagged & Missing Alleles Based on Sub-Sampling Study

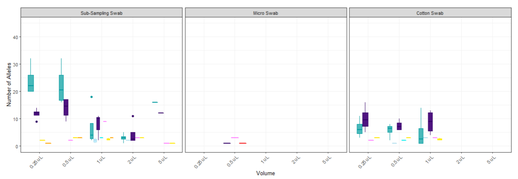

Figure 5. Box plot illustrating the number and types of flags, missing alleles, and drop in alleles, for each of the swab types and their corresponding input amounts. Sub-swabs had the most number of flags, followed by cotton swabs, and lastly micro swabs. Sub-swabs and cotton swabs both had a downward trend in the number of flags as the input increased, with cotton swabs having no flags for 2 µL and greater. Micro swabs only had one sample with quality flags at the 0.5 µL input, with the rest of the inputs having no flags.

Sensitivity on DNA Collection Cards

Data from this sensitivity study were used to assess the success and threshold range of DNA collection cards run on the RapidHIT™ ID System. The cards included Bode Buccal DNA Collectors™ (Bode Technology, Lorton, VA) and Nucleic-card™ (Copan), collected according to manufacturer protocols. Punches of various sizes (1.2 mm to 6 mm) were taken from DNA-containing sections using the Harris Uni-Core Punch (Ted Pella, Inc.). Bode cards were processed using the Specialized protocol for INTEL Plus cartridges, while Nucleic cards were run under both Generalized and Specialized protocols for INTEL Plus cartridges.

First Pass Success Rate Based on Sensitivity on DNA Collection Cards Study

Figure 6. Bar graphs illustrating the first pass success rate for Bode and Nucleic cards in the INTEL Plus cartridge at various saliva card punches, with a dotted line to indicate the number of runs for each. Bode 3 mm, Nucleic 3 mm, and Nucleic 1.2 mm punches were run using the specialized protocol. Nucleic 3 mm and Nucleic 6 mm punches were run using the general protocol. Bode 3 mm punch had 13/14 runs pass, Nucleic 1.2 mm punch had 5/6 runs pass, and Nucleic 3 mm punch (general) had 2/3 runs pass. Both the specialized 3 mm Nucleic punch and general 6 mm Nucleic punches passed for all their runs.

Flagged & Missing Alleles Based on Sensitivity on DNA Collection Cards Study

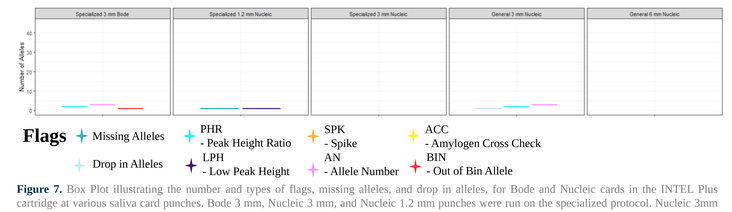

Figure 7. Box Plot illustrating the number and types of flags, missing alleles, and drop in alleles, for Bode and Nucleic cards in the INTEL Plus cartridge at various saliva card punches. Bode 3 mm, Nucleic 3 mm, and Nucleic 1.2 mm punches were run on the specialized protocol. Nucleic 3mm and Nucleic 6 mm punches were run on the general protocol. Few flags were called for all the DNA Collection cards regardless of factors.

Sensitivity Study

Data from the Sensitivity Study were used to evaluate the success and threshold range of cotton and micro swabs, as well as the effect of cartridge type (ACE or INTEL Plus), prepared with varying blood volumes (0.125 – 10 µL).

First Pass Success Rate Based on Sensitivity Study

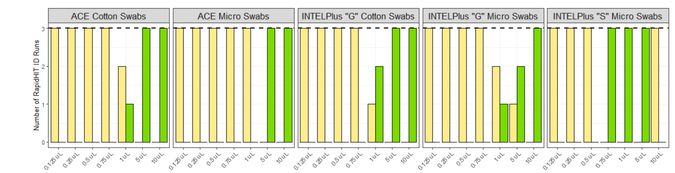

Figure 8. Bar graphs illustrating the first pass success rate for cotton and micro swabs in both the INTEL Plus and ACE cartridges at various blood inputs, with a dotted line to indicate the number of runs for each. Cotton swabs in the INTEL Plus cartridge were run using the general protocol, and micro swabs in the INTEL Plus cartridge were run using both specialized and general protocol. For all samples, excluding INTEL Plus Micro Swabs, as the volume of blood increase, the number of passes increased. INTEL Plus specialized micro swabs had a 100% pass rate at 0.75 µL, all samples excluding ACE micro swabs began passing at 1 µL, and by 5 µL 100% of the time swabs were passing (with the exception of Intel Plus general micro swabs, where one sample had a quality flag at 5 µL and for Intel Plus specialized micro swabs where at 10 µL of input all had quality flags due to pull-up).

Flagged & Missing Alleles Based on Sensitivity Study

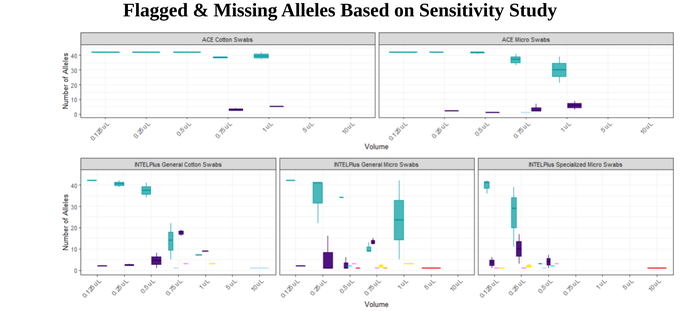

Figure 9. Box Plot illustrating the number and types of flags, missing alleles, and drop in alleles, for cotton and micro swabs in both the INTEL Plus and ACE cartridges at various blood inputs. Cotton swabs in the INTEL Plus cartridge were run using the general protocol, and micro swabs in the INTEL Plus cartridge were run using both specialized and general protocol. As the input amount increased, the number of flags decreased for all swab types and conditions. At 10 µL the INTEL Plus specialized micro swab samples each contained only the flag "BIN", likely due to pull-up.

Rapid INTEL™ Plus Features

INTEL Plus cartridges feature additional metrics of interest. The new STO quality flag predictor assesses the probability that a sample will have peak height imbalance's greater than 40% or allelic dropout. In addition, Quantification markers are used to predict the amount of DNA in a given run. The amount of DNA recovered from a set volume of blood can vary by donor, age of the sample, collection technique, etc. Thus, the quantification markers provide an opportunity to assess how much DNA was used in the reaction.

All Studies Run Status Assessment

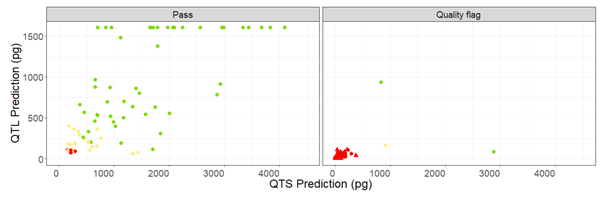

Figure 10. Graph illustrating the correlation between the Stochastic (STO) quality marker per sample, overall run status (Pass or Quality Flag), and allelic dropout. If a sample had drop out it was indicated by a triangle and if there was no dropout it indicated by a circle. The small qualification value (QTS) is on the x axis while the large quantification marker is on the y axis, both depict the predicted DNA in pg. No green or yellow STO flags contained dropout. Thus, all samples with dropout were labeled with the red STO flag. Samples with larger predicted DNA quantities were more likely to have an overall pass status and have a green STO flags. Samples that had the overall status of "quality flag", contained the majority of samples with red STO flags with a few exceptions for samples who had a pass status but extremely low DNA predicted amounts.

Expected Versus Predicted DNA Amounts

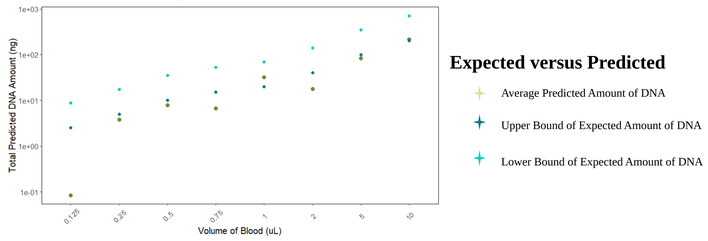

Figure 11. Graph depicting the accuracy of the quantification system included in the Rapid INTEL™ Plus cartridge. The y-axis has been log10 transformed. The range of expected DNA amounts was calculated from published numbers of white blood cells/uL of blood. The average predicted amount of DNA was based on the QTS estimates of DNA used in the reaction (values for sub-sampled swabs were removed from this calculation). The average predicted amount of DNA for this study was on the lower end of what was expected for each volume of DNA. As the RapidHIT™ ID system only consumes a fraction of the DNA extract, these calculations are hinging on the extracted DNA being uniformly present throughout the lysis buffer and complete elution of the DNA off of the swab.

Conclusions

This study demonstrated the RapidHIT™ ID System's capability to effectively process a diverse range of crime scene-grade samples, including both blood and saliva. Furthermore, the data generated provides valuable insights into best practices for using the RapidINTEL™ Plus cartridge with the RapidHIT™ ID System, helping to refine and establish user SOPs, highlighting additional features of the INTEL Plus cartridge. Future efforts with this data will include re-evaluating with modified rapid DNA analysis to assess the effect on the number of passing profiles for each sub-study.

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