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Validation Planning for the Applied Biosystems RapidHIT ID System

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ABSTRACT

Rapid DNA systems are a useful addition to the otherwise complex forensic DNA workflow. These “sample in – answer out” systems develop DNA data in under two hours, offering expedited sample processing for both laboratory and law enforcement needs. Whether data is used for booking stations, database hit confirmations, human remains identification and comparison, investigative leads, or any other rapid DNA need, it is recommended that the user understand the system’s analytical capability to enhance their evidence triage policies and identify the most appropriate samples for rapid DNA processing.

Here HID Professional Services (HPS) describes a range of options for internal validation and/or performance check of the Applied Biosystems™ RapidHIT™ ID System and sample cartridges. This allows laboratories to customize the validation study design based on user needs and the anticipated use of the instrument and chemistry.

INTRODUCTION

The efficacy and reliability of procedures used for forensic sample analysis are demonstrated through the validation process, which includes both developmental and internal validations. Developmental validation of the Applied Biosystems RapidHIT ID System with the ACE GlobalFiler™ Express and RapidINTEL™ sample cartridges was completed by Thermo Fisher Scientific in 2019.[1,2]

Internal validations and performance checks demonstrate that established methods and procedures perform as expected.[3] Accumulated validation data is used to establish requirements for training users, interpreting data, and reporting results. Validation is the gold standard for ensuring compliance with accreditation requirements.

In the United States, the FBI Quality Assurance Standards recommend that users design an internal validation or performance check of a rapid DNA system based on how the system will be used.[3] A fully automated workflow developing a high-quality DNA profile from a casework reference sample requires a performance check at a minimum. Users that will interpret DNA electropherograms developed using a rapid DNA instrument require a validation to accumulate data for establishing analysis and interpretation guidelines. Planning for internal validations can be difficult due to vague language within the quality assurance standards and confusion over whether a Rapid DNA System validation needs to be as complex as a conventional forensic DNA laboratory workflow validation. HPS has developed validation and performance check plans to meet the needs a variety of different of Rapid DNA use cases.

THE RAPIDHIT ID SYSTEM

The Applied Biosystems RapidHIT ID System is fast and simple to use. It produces trusted lab-quality forensic DNA profiles in approximately 90 minutes with one minute of hands-on time. The system integrates sample preparation, amplification, and capillary electrophoresis to generate robust DNA profiles outside of a central laboratory testing facility. The RapidHIT ID System employs a single-use, disposable sample cartridge that is pre-loaded with all the required chemistry. The system also utilizes a multi-use buffer and polymer cartridge enabling sample capillary electrophoresis of multiple samples, completing the entire sample-to-profile workflow.

Thermo Fisher Scientific extensively validated the RapidHIT ID System thresholds for the expert system-capable software to determine whether a DNA profile is of high quality.

The developmental validation of the RapidHIT System set the parameters for:

1) The Rapid DNA instrument
2) The chemistry and/or concentration of the PCR STR DNA typing kit (Rapid DNA cartridge)
3) The settings of the expert system-capable software
4) Other system software parameters

Figure 1. System Sample Run Quality Flags

Green check mark: DNA profile is flag-free*
Yellow check mark: At least one flag at a locus*
Red “X”: no analyzable profile, not considered for this analysis

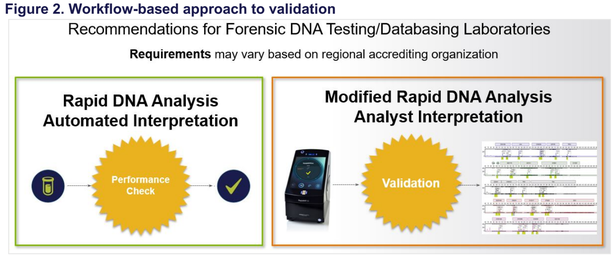
*The Y indel and DYS391 are excluded from the quality assessment

PLANNING A VALIDATION

Validation plans should meet the needs of the laboratory. Samples should be chosen to ensure all validation goals are met; both samples and controls should be representative of the sample types that the RapidHIT ID System is intended to process.

The intended workflow determines whether the user requires a performance check or a validation (Figure 2), and the sample number and type required (Figure 3). If a particular set of samples will meet the needs of more than one validation study, there is no need to duplicate them. Each sample type (blood, saliva, bone, etc.) and collection method (swab, cutting, scraping, etc.) should be included in the validation to fully understand the capabilities of the rapid DNA system and develop data that supports analysis of each sample type with the rapid DNA workflow.

Figure 2. Workflow-based approach to validation



In the US, FBI approval of the RapidHIT ID expert system is currently pending. There are no FBI-approved Rapid DNA systems for forensic DNA casework samples at this time.

Designing a validation project:

Determine the scope of the validation based on the intended workflow. The extent of the interpretation guidelines desired by the user determines how many validation samples are required. Samples should be carefully selected to provide relevant data for setting interpretation guidelines.

Optimize the number and selection of samples used to meet the validation requirements. Using samples across multiple studies, as outlined in Figure 3, will maximize efficiency. Create a plan for data analysis prior to beginning the validation to ensure that the study design captures the information required by the scope.

VALIDATION STUDIES

HPS RapidHIT ID System validation and performance check service offerings are similar to HPS direct amplification validation projects. Profiles from control, known, and non-probative samples are analyzed to meet the required studies as detailed in Figure 3.

Figure 3. Sample selection considerations

Sample Set	Study	Sample selection and analysis notes	Validation	Perf. Check
Total samples	**All**	**Chosen and provided by the laboratory/agency**	**Up to 50**	**Up to 18**
Controls (RHID cartridges or lab designated samples)	Contamination		~3 negatives	~3 negatives
	Accuracy	Alternate positives with negatives Option: add a substrate control	~3 positives	~2 positives
Test Samples (combination of single source, mixture source, high and low-level samples – all chosen carefully to meet goals)	Sensitivity and Stochastic	Options: include swipe study to optimize collection protocol, dilution series to demonstrate peak height correlates to input	18 - 44	Up to 13
	Precision	Use test samples and control samples		
	Mixture	Options: include one or more mixed samples to confirm PL flag will fire		
	Known and Non-Probative	Ensure samples represent the range of typical laboratory rapid DNA samples – this may include fresh, aged, and/or properly and improperly collected and stored samples		
	Artifacts	Use test samples and control samples		

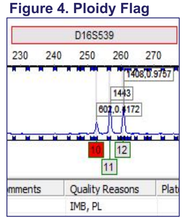
Contamination Study

Contamination is the unintentional introduction of exogenous DNA, through various means, into a DNA sample or test reaction. The contamination study gives a snapshot of lab contamination at the time of the validation and provides a baseline of what to expect under ideal conditions.

This study also assesses if the ploidy flag (PL) and/or imbalance flag (IMB) are triggered when contamination is detected in single-source samples. PL is triggered when more than two alleles are detected at a single locus and IMB is triggered when sister alleles do not meet system thresholds (Figure 4). Positive control and negative control cartridges provide confidence the system is not contaminated and is running as expected. Control samples also provide data for understanding baseline noise.

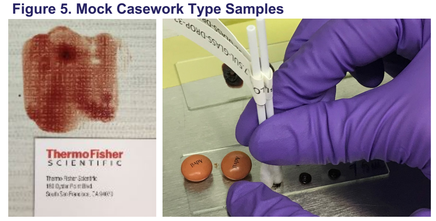
Substrate controls provide confidence that the substrate does not introduce contamination. These controls result in a yellow checkmark, indicating that manual analysis is required and confirming that the size standard ran correctly.

Figure 4. Ploidy Flag



A PL and/or IMB flag in a sample that is expected to be single source may be an indication of contamination in the sample.

Figure 5. Mock Casework Type Samples



Develop practical validation sample sets (e.g. 50 µL stain = ½ business card) that would meet laboratory collection guidelines for collecting duplicate swabs for analysis.

Sensitivity & Stochastic Study

This study confirms whether user collection methods produce reliable results with the Rapid System. Remember to expect variability in results due to variability in different individuals and between different collection events from the same individual. Rapid DNA samples do not require quantification; therefore, sensitivity studies may identify a range of sample inputs that yield interpretable profiles but that would not be extrapolated to estimate DNA input.

For a performance check or simple validation, it is sufficient to run a variety of swabs collected using normal protocols. The results are used to understand what constitutes passing and failing data for the system.

A more intensive validation may include swabs to confirm that the current collection method is optimized for the RapidHIT ID System. An example sample set could include swabs collected with “a few” cheek swipes compared to duplicate swabs collected with “many” check swipes. Comparing passing and failing results for the “low” and “high” swipe samples can be used to optimize buccal sample collection protocols for a high success rate.

If approximate sample amounts are documented, a dosing study-type assessment may be performed to understand the limits of the system and chemistry. Peak height ratios (Figure 6) can be used as a metric for assessing imbalance. These ratios may be affected by factors such as substrate inhibitors, low input amounts, and/or saturation.

Figure 6. Peak height ratio assessment

Approx. Saliva Input (µL)	D3S1358	vWA	D16S539	CSF1PO	D8S1179	TH01	FGA	D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338
12.5	57%	79%	70%	97%	83%	75%	67%	94%	99%	76%	47%	79%	74%	78%	95%
	97%	83%	58%	94%	93%	87%	79%	90%	85%	100%	70%	76%	73%	62%	85%
	94%	64%	67%	64%	78%	94%	98%	67%	80%	99%	85%	96%	74%	86%	84%
6.25	70%	56%	89%		67%	75%	68%	56%	91%	88%	80%	53%	97%	74%	76%
	100%	58%				86%		87%	92%	70%		79%	45%	82%	
	78%	60%	98%		81%	88%	58%	74%	90%	70%	58%	66%			

Precision & Accuracy Studies

Known and control samples are used to measure the repeatability of allele sizing and reproducibility of sample genotyping. This provides confidence in the system, improving efficiency of both data analysis and review.

Each sample is analyzed with a ladder selected from a ladder pool by the RapidHIT ID System software as the best match to the specific sample run. In a precision study, the selected ladder can be examined when off-ladder alleles are observed to evaluate the efficacy of ladder selection.

Known & Non-Probative Study

It is critical to test typical, routine sample types to demonstrate that the RapidHIT ID System will perform as expected (Figure 5). This is because some substrates may clog the sample cartridge or inhibit PCR. Additionally, this study may be used to develop protocols for Rapid DNA data interpretation.

A sample set designed to assess sample handling and storage protocols may include the following: aged samples, fresh samples, samples stored under varying environmental conditions such as humidity and temperature, and other samples as appropriate.

Profiles from this study may be compared between rapid DNA replicates or with data from a traditional DNA workflow to assess concordance. This study demonstrates that the system successfully generates correct genotype results from typical evidence and reference samples, as well as any variability and limitations of the system that may be observed.

Mixture Study

Samples run as part of the mixture study provide confidence that the system will flag contamination in single source samples by demonstrating the capability of the system to flag results with the PL flag when a mixture is processed.

A performance check or basic validation may evaluate all single-source samples for PL and IMB flags. Instances of elevated stutter, noisy baseline, pull-up and/or drop-in events will trigger PL flag functions, demonstrating successful flags when more than two alleles are present at a locus.

More intensive validations may include one or more known, mixed samples to demonstrate that a sample with more than one contributor will trigger the PL flag. Some laboratories may require more information to understand the rapid DNA system capabilities as it relates to mixture resolution. For these users, sample sets may include volumetric mixtures of saliva or blood; however, sample variation is expected with these sample types.

Artifact Study

Use all samples to assess and describe the frequency and severity of artifacts which may differ from those observed in the traditional forensic DNA workflow due to sample input amount, differences in thermal cycling and injection parameters, and/or hardware.

This study assesses the suitability of chemistry-specific global filters for reproducible artifacts such as minus stutter, plus stutter, minus A, and pull-up while confirming that artifacts are properly flagged.

CONCLUSIONS

Internal validations test procedures to demonstrate that the protocols work as expected. Validations provide the foundation for every standard operating procedure. Validation data serves as a benchmark of system performance that can be referenced as needed.

Carefully chosen samples and a well-designed RapidHIT ID System validation project will provide data to support standard operating procedures from sample collection through interpretation and reporting guidelines. With thoughtful preparation, a RapidHIT ID System validation can assess sample data for all the studies that would be expected for a conventional forensic DNA validation: contamination, sensitivity and stochastic, precision and accuracy, known and non-probative samples, mixtures, and artifacts.

REFERENCES

1. Hennessy LK et al. (2014). Developmental validation of the GlobalFiler™ Express kit, a 24-marker STR assay, on the RapidHIT™ System. Forensic Science International: Genetics (13): 247-258.
2. User Bulletin: RapidINTEL Sample Cartridge for blood and saliva samples.
3. Federal Bureau of Investigation, “Quality Assurance Standards or Forensic DNA Testing Laboratories” Effective July 1, 2020

TRADEMARKS/LICENSING

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