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Evolution Of RAPIDHIT™ ID System for Investigative Leads

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Introduction

The RapidHIT™ ID System is a fully automated system for short tandem repeat (STR) based human identification in a sample-to-answer system. The system generates Combined DNA Index System (CODIS) compatible DNA profiles in less than 90 minutes. Sample processing requires less than one minute of hands on time and has been optimized for use in decentralized environments including police booking stations and border checkpoints. Our RapidHIT ID ACE GlobalFiler™ Express Sample Cartridge was designed for rapid human identification of single-source, buccal reference samples in a fully automated sample-to-answer workflow. To address the need for improved capabilities with investigative leads samples, we developed the RapidINTEL™ Sample Cartridge. The RapidINTEL Sample Cartridge uses the same trusted GlobalFiler Express STR PCR chemistry as the RapidHIT ID ACE system with two significant protocol changes; the number of PCR cycles is increased to 32 cycles and the lysis buffer volume is reduced to 300 µL. In conjunction with the improved sensitivity protocol the system thresholds were optimized for investigative lead sample types. The developmental validation studies for this system were performed with mock casework samples in accordance with the Scientific Working Group for DNA Analysis Methods (SWGDAM) guidelines. We describe the primary analysis of the resulting profiles, performed using the RapidINTEL Sample Cartridge by GeneMarker® HID STR Human Identity Software on the instrument. The software uses optimized system threshold settings to perform the analysis and displays the result. When the primary analysis indicates that secondary review is required, the DNA profile can be reviewed and edited in the GeneMarker HID software using the guidance described here. Finally, if the sample is determined to be acceptable after review, the file can be manually exported for database searching. The results show that the RapidHIT ID System with RapidINTEL Sample Cartridge provides a useful solution, allowing law enforcement personnel to generate investigative leads and identify suspects faster.

Objective of the Validation

The objective of the validation was to assess the performance of the RapidINTEL™ Sample Cartridges on the RapidHIT ID System v1.1.3 when analyzing high quality, single-source blood and saliva samples. The validation was performed according to guidelines from the Scientific Working Group for DNA Analysis (SWGDAM, December, 2016). The results for the verification sample set, mixture studies and casework studies are presented here. The complete set of results is available in the User Bulletin, “RapidINTEL™ Sample Cartridge for blood and saliva samples” available on the Thermo Fisher Scientific website.

Instrument and System Background Information

In a traditional Applied Biosystems™ forensic workflow, Thermo Fisher Scientific validates an STR kit on a variety of samples. The validation data is provided to customers for reference when they optimize system thresholds for peak detection, genotyping, and quality value flagging.

In the RapidHIT™ ID workflow, Thermo Fisher Scientific optimizes and validates system thresholds on a specific verification sample set, then performs complete validation studies on the verification sample set and a variety of other samples. The customer does not have access to system threshold settings.

Table 1 RapidINTEL Sample Cartridge on RapidHIT ID System v1.1.3 system thresholds

Thresholds and Filters	Setting
Analytical threshold	50 RFU
Stochastic threshold	1,600 RFU*
Minimum peak height ratio threshold	40%, 99% for Y indel and DYS391
Minimum heterozygous peak intensity threshold	640 RFU
Stutter filters	Locus-specific
Locus-specific filter	21% for samples, 30% for positive control
Maximum number of expected peaks in a marker	2
Global filter (between loci)	21% for samples, 30% for positive control
Minimum off-ladder (OL) intensity	30 RFU

*Stochastic threshold = 50 RFU for Y indel and DYS391 because they are hemizygous.

Primary analysis with RapidHIT ID system

The primary analysis is automatically performed on the RapidHIT ID System by an internal version of the GeneMarker HID STR Human Identity Software on the instrument. The software uses the system threshold settings in Table 1 to perform the analysis. When the primary analysis is complete, the system generates one of three status results; green checkmark, yellow checkmark or red "X”.

Table 2 Primary analysis results on the RapidHIT ID System

Status	DNA profile is generated	Action
Green	Yes	Green: All peaks and markers in the DNA profile met system threshold criteria.
Yellow	Yes	Samples only, not positive or negative controls. Yellow: One or more system threshold criteria was not met. An internal quality score flag is triggered. Sample requires manual secondary analysis in the RapidLINK™ Software/GeneMarker™ HID software
Red	No	Red: The DNA profile was not generated.

Secondary analysis with RapidHIT ID system

When primary analysis on the instrument yields a yellow check mark, it indicates that quality score flags are present and the sample requires manual secondary analysis in the RapidLINK™ Software. A primary analysis result from the instrument is marked requires review in the RapidLINK Software. When a sample is selected for review in the RapidLINK Software, a version of the GeneMarker™ HID STR Human Identity Software opens on the RapidLINK Software computer. The DNA profile can be reviewed and edited in the GeneMarker HID software. If a sample is determined to be acceptable after review, a CMF file can be manually exported for database searching.

The increased sensitivity of the RapidINTEL Sample Cartridge may exacerbate stochastic effects in low-level samples, primarily attributable to the increased PCR cycle number. Due to these increased stochastic effects in the system, the thresholds used are more conservative than those applied in the ACE GFE cartridge system and therefore an increase in samples requiring secondary analysis will be seen. A single marker can prevent a sample from passing primary analysis (for example due to an elevated stutter peak). For this reason, all markers in the samples must be evaluated, as opposed to analyzing each marker in isolation. In addition, a locus specific filter is not applied to the Y indel and DYS391 markers in female samples and pull-up artifact peaks may be called. These alleles can be deleted during secondary analysis.

Two former forensic analysts performed blind analysis on each profile. Both analysts reviewed the profile before final secondary analysis profiles were generated. Guidelines were developed (as described in the User Bulletin) and used in the analysis of data generated during validation. According to these guidelines:
-The stochastic threshold, analytical threshold and local filter will not be overridden.
-Stutter greater than the marker filter can be overridden at analyst discretion.
-The analyst can confirm inconclusive heterozygote allele(s) provided there is no indication of a mixture, no marker drop-out in the profile, the average peak height across the profile is approximately 250 RFU or greater and for low-level samples, peak height ratio imbalance is >30%.

Methods

Six RapidHIT™ ID System v1.1.3 instruments were used for validation studies. All instruments were not used in every study; however, each instrument was used in at least one study.

The verification sample set included blood samples at 5 different volumes from 46 unique donors and saliva samples from 51 unique donors at 6 different volumes, giving a thorough data set for setting RapidINTEL Sample Cartridge thresholds. For each sample, genotype concordance between the RapidHIT ID cartridge and our standard forensics workflow was verified by also processing with a standard forensic DNA workflow including DNA extraction/purification with PrepFiler *Express*™ Forensic DNA Extraction Kit, quantification of DNA with Quantifiler™ Trio DNA Quantification Kit, PCR amplification of 1 ng DNA input with 29 PCR cycles using the GlobalFiler™ PCR Amplification Kit and CE analysis with 3500xL Genetic Analyzer.This verification sample set was also useful as a Sensitivity/Population study because of the range of sample donors and input volumes.

Methods Continued

To study the performance of the RapidINTEL Sample Cartridges with samples containing more than one contributor, three mixture studies were performed with low input saliva or blood mixtures to approximate investigative leads samples. For Mixture Study 1, a total of 4 µL of saliva from two donors was run on 6 instruments at mixture ratios of Donor A: Donor B of 1:0, 1:1, 1:3, 1:8, 8:1, 3:1, 0:1. For Mixture Studies 2 and 3, varying inputs of blood from 2 donors were run on 6 instruments at mixture ratios of Donor A: Donor B of 1:1, 1:2, 1:4, 1:8, 1:16, 16:1, 8:1, 4:1.

To mimic the types of samples commonly encountered in forensics laboratories, blood and saliva samples were prepared at multiple sample volumes on various substrates. In addition, a limited number of touch and hair samples were tested. For a subset of the mock casework samples, a dual wet-swab technique was used for the purpose of testing on both the RapidINTEL Sample Cartridge and ACE GFE cartridge. The samples were prepared as follows:
-Blood and saliva samples were deposited on various substrates, allowed to dry, and then swabbed. Punches were obtained from denim and some cotton samples.
-Cigarette butt and gum samples were swabbed directly, or were cleaned, handled by a single individual, then swabbed.
-Hair sample was pulled from the head, inspected for the presence of root, the placed in a sample cartridge.
-Touch samples were cleaned, handled by a single individual, then swabbed.

Results

The complete set of results is available in the User Bulletin, “RapidINTEL™ Sample Cartridge for blood and saliva samples” available on the Thermo Fisher Scientific website.

-Verification Sample Set

The verification study used blood samples (at 5 different volumes) from 46 unique donors and saliva samples from 51 unique donors (at 6 different volumes) (Figure 8 and Figure 9 and Table 10).

Table 3 Percent of correct, unflagged markers after primary analysis and markers recovered/ confirmed after secondary analysis for the 398 blood and buccal samples.

Sample Type	Volume	N	Percent of correct, unflagged markers after primary analysis	Percent of correct, unflagged markers plus confirmed markers during secondary analysis
Blood	0.25 µL	11	16%	29%
	0.5 µL	45	34%	48%
	1 µL	51	49%	72%
	2 µL	50	58%	81%
	4 µL	44	77%	89%
Saliva	1 µL	49	19%	34%
	2 µL	50	35%	53%
	4 µL	50	44%	61%
	6 µL	46	55%	75%

Overall, the data presented in Table 3 demonstrates marker recovery increased 18% for blood samples and 17% for saliva samples after secondary analysis. The Y indel and DYS391 markers were not included in the calculations.

-Mixture Sample Set

Three studies evaluated the performance of RapidINTEL™ Sample Cartridges with samples containing more than one contributor. Mixtures should trigger the ploidy flag (PL) in primary analysis if the peak height of the minor contributor peak in a mixture exceeds the analytical threshold and the locus-specific filter.

Mixture study 1

Mixture study 1 used a total of 4 µL of saliva from two donors run on 6 instruments at mixture ratios of donor A: donor B of 1:0, 1:1, 1:3, 1:8, 8:1, 3:1, 0:1. Primary analysis generated results (PL flag) for mixtures down to a 1:8 and 8:1 ratio. One of the eight 1:8 samples generated a result, which indicates that the mixture was not detected (Table 4). All of the alleles the 1:8 sample were from the major contributor. This data was analyzed with a 21% locus-specific filter, which can mask minor contributor alleles in low mixed-source ratio samples.

Table 4 Mixture study 1: Saliva mixture samples showing that 1 of the 1:8 mixture samples did not trigger the PL flag. As expected, the 0:1 and 1:0 single-source samples did not trigger the PL flag.

Mixture ratio	Donor A (µL)	Donor B (µL)	Yellow	Green	Replicates
0:1	0	1	1	1	2
1:0	1	0	1	1	2
1:1	2	2	8	—	8
1:3	1	3	8	—	8
1:8	0.45	3.55	7	1	8
3:1	3	1	9	—	8
8:1	3.55	0.45	8	—	8

Donor B.

Mixture studies 2 and 3

Mixture studies 2 and 3 used varying inputs of blood from 2 donors on 6 instruments. Primary analysis generated results (PL flag) for mixtures down to 1:16 and 16:1 ratios. Two of the 5 (16:1) samples generated a result, which indicates that the mixture was not detected (Table 5). All of the alleles in the 16:1 samples were from the major contributor. This data was analyzed with a 21% locus- specific filter which can mask minor contributor alleles in low mixed-source ratio samples.

Table 5 Mixture studies 2 and 3: Blood mixture samples showing that only two of the 16:1 mixture samples did not trigger the PL flag

Mixture ratio	Donor A (µL)	Donor B (µL)	Yellow	Green	Replicates
Test case 2: Higher input blood samples					
1:1	0.5	0.5	5	0	5
1:2	0.5	1	5	0	5
1:4	0.25	1	5	0	5
1:8	0.125	1	5	0	5
1:16	0.062	1	5	0	5
16:1	1	0.062	3	2	5
8:1	1	0.125	5	0	5
4:1	1	0.25	5	0	5
Test case 3: Lower input blood samples					
1:1	0.33	0.33	5	0	5
1:2	0.33	0.67	5	0	5
1:4	0.165	0.67	5	0	5
1:8	0.083	0.67	5	0	5
1:16	0.042	0.67	5	0	5
16:1	0.67	0.042	5	0	5
8:1	0.67	0.083	5	0	5
4:1	0.67	0.165	5	0	5

-Mock Casework Sample Set

Blood, saliva, hair, and touch samples were prepared at multiple sample volumes on different substrates to mimic casework-type samples. Two mock-casework studies were performed to evaluate primary analysis pass rate, the average number of correct, unflagged markers after primary analysis, and secondary analysis marker recovery by sample and substrate type.

Table 6 Samples for mock-casework study 1

Sample type	Substrate	Volume or sample size[1]	Replicates
Blood	Cotton	2 µL drop, swab	n=4
	Denim: Blood was spotted and dried in 2013, then stored at room temperature.	1 µL drop, swab	n=5
Saliva	Coffee cup: Entire contents consumed, then top was swabbed	Swab	n=4
	Soda can: Entire contents consumed, then mouth area was swabbed	Swab	n=4
	Cigarette butts	Entire wrapper paper on the filter portion of the cigarette[2]	n=4
	Gum: Chewed for 30 minutes, rolled into slender shape, stored in refrigerator until processing.	Entire piece of gum[2]	n=4
Hair	Pulled hair	Hair root [2]	n=1
Touch	Firearm handled by a single donor	Swab	n=5
	Face mask worn by a single donor	Swab	n=4
	Hat worn by single donor	Swab	n=2
	Pen handled by a single donor	Swab	n=4

[1] Dual wet-swab technique was used for swab collections, one swab was processed. Therefore, the swab that was processed contained half the total amount of DNA collected.
[2] The item was placed directly into a sample cartridge.

Mock-casework study 1 processed a limited number of blood, saliva, epithelial/touch samples, and a hair sample. Results are shown in Table 7. Results show that secondary analysis generally improved marker recovery. Yindel and DYS391 are not included in the markers recovered (therefore, the total possible number of markers is 22).

Table 7 Mock-casework study 1: Percent of correct, unflagged markers after primary analysis and markers recovered/ confirmed after secondary analysis

Sample type	Substrate type	Percent of correct, unflagged markers after primary analysis	Percent of correct, unflagged markers plus confirmed markers during secondary analysis
Blood	Cotton (n=4)	0%	0%
	Denim (n=5)	23%	33%
Epithelial	Cap (n=2)	2%	2%
	Firearm (n=5)	0%	0%
	Mask (n=4)	34%	47%
	Pen (n=4)	0%	0%
Saliva	Cigarette butt (n=4)	2%	2%
	Coffee cup (n=4)	20%	20%
	Gum (n=4)	91%	100%
	Soda can (n=4)	17%	44%
Hair Root	Hair root (n=1)	100%	100%

Mock-casework study 2 included differing inputs of blood and saliva on various substrates and hair roots. The sampling information and data are shown in Tables 8-9.

Table 8 Samples for mock-casework study 2

Sample type	Substrate	Volume or sample size[1]	Replicates
Blood	Glass	7.5 µL, 15 µL, 30 µL drop	3 swabs from each volume
	Tile (ceramic)	25 µL, 50 µL, 100 µL smear	3 or 6 swabs from each volume
	Tarred surface	15 µL, 30 µL drop	4 or 5 swabs from each volume
	Drywall	4 µL, 7.5 µL, 15 µL drop	3 swabs from each volume
	Black underwear	3.75 µL, 7.5 µL, 15 µL, 30 µL, 60 µL drop	1 to 5 swabs from each
	White cotton: Blood was spotted and dried in 2010, then stored at room temperature.	2 µL drop, 5 mm punch[2]	n=3
	Denim: Blood was spotted and dried in 2013, then stored at room temperature.	1 µL drop, 5 mm punch[2]	n=1
Saliva	Water bottle: Entire contents was consumed, then the top was swabbed.	Swab	n=3
	Coffee cup: Entire contents was consumed, then the top was swabbed.	Swab	n=4

[1] Dual wet-swab technique was used for swab collections, one swab was processed (unless otherwise noted). Therefore, the swab that was processed contained half the total amount of DNA collected.
[2] The item was placed directly into a sample cartridge.

Secondary analysis for casework test case 2 improved marker recovery for all samples where DNA was recovered with the exception of the blood on white cotton samples which remained the same, as seen in Table 9. Multiple samples did not meet system thresholds during primary analysis. However, secondary analysis showed a full profile with a single marker flagged. For example, the blood on glass, tarred surface, and tile recovered over 90% of markers after secondary analysis.

Table 9 Mock-casework study 2: Percent of correct, unflagged markers after primary analysis and markers recovered/ confirmed after secondary analysis

Sample type	Substrate type	Percent of correct, unflagged markers after primary analysis	Percent of correct, unflagged markers plus confirmed markers during secondary analysis
Blood	Denim (n=1)	0%	0%
	Drywall (n=9)	33%	57%
	Glass (n=9)	88 %	91%
	Tarred surface (n=9)	86 %	93%
	Tile (n=15)	97%	99%
	Underwear (n=14)	55 %	69%
	White cotton (n=3)	6%	6%
Saliva	Cigarette butt (n=9)	8%	11%
	Coffee cup (n=4)	49 %	72%
	Gum (n=3)	0%	44%
	Water bottle (n=3)	0%	0%
	Hair root (n=2)	50%	50%

Overall, blood samples show higher primary analysis pass rates and higher numbers of correct, non-flagged markers than saliva and touch samples. Variation in results between the sensitivity study and the mock casework study may be due to sampling technique or interferences from inhibitors present on the substrate.

Conclusions

RapidINTEL Sample Cartridges that are run on RapidHIT ID System v1.1.3 can accurately analyze moderate- to high-DNA content samples. This workflow expands the capability of the system to allow analysis of blood and saliva samples. Samples that do not meet the validated system thresholds are appropriately identified during primary analysis. Any samples that do not pass primary analysis require manual secondary analysis before results can be reported. This workflow supports the collaboration of decentralized locations for automatic primary analysis and forensic laboratories for required secondary analysis. The results of this study conclude that the Applied Biosystems RapidHIT ID System provides a useful solution, allowing law enforcement personnel to generate investigative leads and identify suspects faster.

TRADEMARKS/LICENSING

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