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Validation of the Applied Biosystems™ RapidHIT™ ID System using ACE GlobalFiler™ Express sample cartridges for analysis of reference swabs

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INTRODUCTION

The Applied Biosystems™ RapidHIT™ ID System is fast, simple-to-use and produces trusted lab-quality forensic DNA profiles in as little as 90 minutes with one minute of hands-on time. The system integrates sample preparation, amplification and capillary electrophoresis to generate robust DNA profiles ideal for reference sample analysis outside of a central laboratory testing facility. The RapidHIT ID System (Figure 1) employs a single-use, disposable sample cartridge that is pre-loaded with all required chemistry. The system also utilizes a multi-use buffer and polymer design enabling sample capillary electrophoresis, completing the entire sample-to-profile workflow.

Figure 1. The Applied Biosystems RapidHIT ID System.

OBJECTIVE

The goal of this study was to assess the RapidHIT ID System for the analysis of single source, buccal, reference samples according to the Scientific Working Group for DNA Analysis Methods developmental validation guidelines (1). Performance of the RapidHIT ID using the RapidHIT ID ACE GlobalFiler Express sample cartridges was evaluated using to the following criteria: precision, accuracy, sensitivity, and the ability to detect mixed source DNA profiles.

MATERIALS AND METHODS

Sizing precision enables the determination of accurate and reliable genotypes. Precision was assessed by running five RapidHIT ID ACE GlobalFiler Express allelic ladder control cartridges across five different RapidHIT ID Systems. Precision was measured by calculating and plotting the standard deviation of the size values for each allele.

Accuracy was measured for repeatability and reproducibility to ensure conformity of the result to the expected DNA profile. Five replicate runs of RapidHIT ID ACE GlobalFiler Express positive control cartridges were performed across five different RapidHIT ID Systems and the results were compared. Additionally, accuracy was further evaluated by running pairs of buccal reference swabs on both the same and different systems. Finally, all DNA profiles were not only compared to replicates for accuracy, but also to the results of the standard GlobalFiler Express direct amplification workflow performed on Applied Biosystems™ 3500xL or 3130xL Genetic Analyzers.

Sensitivity testing, to assess upper and lower limits of DNA input, was performed using a practical approach. Sensitivity was measured by the ability of the RapidHIT ID System to generate DNA profiles from a series of varying amounts of deposited cells determined by swabbing method. This method tested four sets of swabs, representing 1, 5, 10, and 20 unidirectional passes or swipes, across four different systems. The quality of the generated profiles was assessed for the 16 samples.

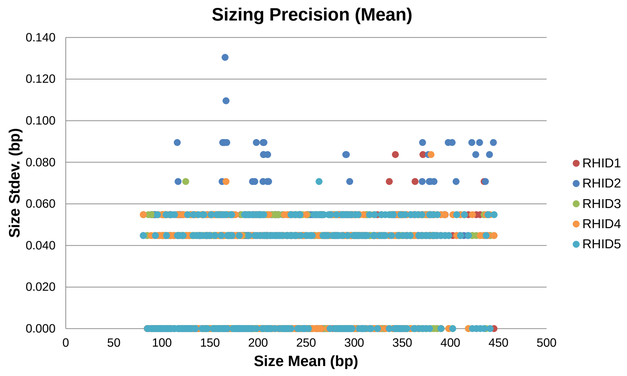
The ability to detect mixtures was assessed to confirm the system is capable of recognizing the presence of a mixture, or contamination, in the anticipated single source reference profile results. Four pairs of mock mixture swab samples were prepared by applying saliva in ratios of 1:1 and 1:10 from two known contributors. Each pair of swabs was analyzed using a different system. The ability of the RapidHIT ID System to detect the presence of a mixture for each of the eight mock samples was evaluated.

Peak height ratios, inter- and intracolor balance values, and signal variability measurements were calculated throughout the study. Intercolor balance was expressed as a ratio of the mean peak height of a dye over the mean sample peak height. Signal variability was determined by measuring the coefficient of variation for the mean size standard peak heights in positive control runs. Peak detection and stochastic thresholds were calculated for the concordance data set using previously published methods (2, 3).

RESULTS

Upon assessment of sizing precision (Figure 2), the standard deviation of the size of each allele in the allelic ladder did not exceed 0.14 base pairs when measured across the five tested systems. This observation confirms the ability of the system to accurately size allele differences of one base pair across the GlobalFiler Express sizing range.

Figure 2. Sizing precision data.



All replicate samples tested in the evaluation of accuracy yielded concordant profiles for called alleles with one exception. One sample, from the buccal reference swabs, generated a system-flagged profile indicating further review was required. An out-of-bin, off-ladder allele designation was assigned for a known allele. Reanalysis, with an alternate allelic ladder, resolved the discordant result. As a result, concordance was calculated to be 99.8% for the 564 tested buccal reference swab samples. All positive control replicates were concordant. Additionally, observations of stutter, pull-up, peak height ratio and color balance values were calculated for the 564 sample concordance data set.

Figure 3. Stutter example.

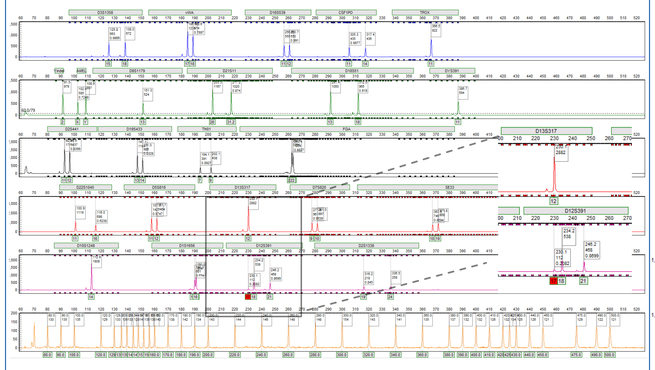


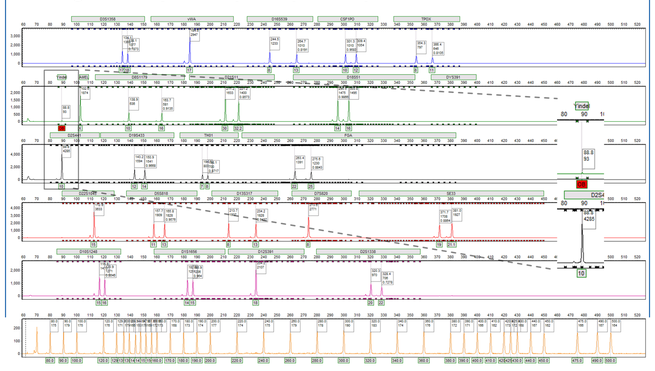
Figure 3, above, represents one of the 11 samples, of the 564 tested, that demonstrated stutter above the peak detection threshold. All 11 samples were accurately flagged for review by the RapidHIT ID System. Table 1 lists the mean, minimum, maximum, and mean plus 3 standard deviations calculated stutter values.

Table 1. Observed locus specific stutter values.

	Mean	Minimum	Maximum	Mean+3SD
D3S1358	10.5%	6.80%	17.3%	15.9%
vWA	9.58%	5.07%	14.0%	14.4%
D16S539	8.15%	4.78%	14.3%	13.8%
CSF1PO	8.12%	4.95%	14.8%	13.1%
TPOX	5.61%	4.69%	8.81%	7.83%
D8S1179	8.16%	4.79%	17.0%	14.0%
D21S11	10.9%	6.24%	23.0%	16.5%
D18S51	9.69%	5.05%	20.5%	17.2%
DYS391	8.21%	5.99%	12.1%	11.1%
D2S441	7.00%	4.72%	10.3%	10.9%
D19S433	8.62%	5.05%	23.1%	14.5%
TH01	6.85%	4.91%	12.0%	12.0%
FGA	9.78%	4.87%	17.6%	16.0%
D22S1045	10.7%	6.11%	19.8%	17.4%
D5S818	7.97%	4.90%	16.8%	12.7%
D13S317	7.39%	4.99%	13.4%	11.6%
D7S820	6.92%	4.94%	10.7%	10.5%
SE33	12.7%	5.18%	21.0%	20.6%
D10S1248	9.88%	5.99%	16.6%	15.3%
D1S1656	10.4%	5.06%	18.5%	18.1%
D12S391	11.9%	5.18%	26.3%	20.7%
D2S1338	11.2%	5.44%	21.8%	17.7%

Figure 4, below, represents one of the 10 samples, of the 564 tested, that demonstrated spectral pull-up above the peak detection threshold. All 10 samples were accurately flagged for review by the RapidHIT ID System.

Figure 4. Spectral pull-up example.



Evaluation of the sensitivity results demonstrated interpretable DNA profiles for each data point. One of the 1 pass, or swipe, replicates was system-flagged indicating the possibility of heterozygote allele drop-out. One of the alleles in the heterozygote pair was confirmed to be present below the defined detection threshold. The other 15 samples yielded the expected profiles. Figure 5 and Table 2 demonstrate an example of a 1 pass, or swipe, STR profile and summary sensitivity data results, respectively.

Figure 5. STR profile example for 1 swipe collection.

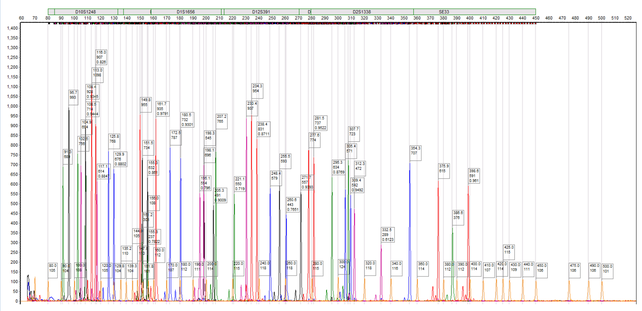


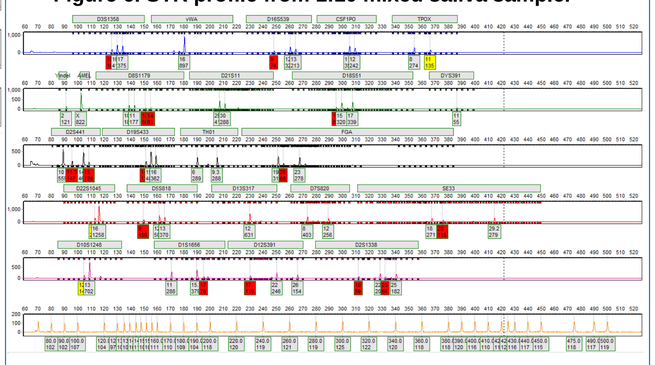
Table 2. Sensitivity summary data.

	Mean Peak Height (RFU)	Mean Peak Height Ratio*
1 swipe collection	**377**	**82%**
5 swipe collection	**646**	**84%**
10 swipe collection	**763**	**84%**
20 swipe collection	**860**	**87%**

*calculated for autosomal loci only

The ability to detect, and system-flag mixtures, was confirmed for all eight of the contrived mixed source samples across the four tested systems. Figure 6 demonstrates an example of an STR profile from a 1:10 saliva:saliva mixed source contrived sample. During the validation, contamination was assessed using negative controls following allelic ladder and high quantity sensitivity series runs. No data was detected above the peak detection threshold for the negative controls.

Figure 6. STR profile from 1:10 mixed saliva sample.



Peak height ratio (PHR) data, Table 3, was calculated and summarized on a locus specific basis throughout the validation study. The summary consists of minimum and maximum observed PHR values as well as the calculated mean for each locus.

Table 3. Observed locus specific PHR data summary.

	Minimum PHR	Maximum PHR	Mean PHR
D3S1358	0.51	0.99	0.86
vWA	0.51	0.99	0.83
D16S539	0.57	0.99	0.85
CSF1PO	0.43	0.99	0.84
TPOX	0.17	0.99	0.84
AMEL	0.25	0.99	0.87
D8S1179	0.56	0.99	0.85
D21S11	0.64	0.99	0.87
D18S51	0.51	0.99	0.87
D2S441	0.53	0.99	0.90
D19S433	0.6	0.99	0.89
TH01	0.34	0.99	0.86
FGA	0.47	0.99	0.89
D22S1045	0.46	0.99	0.83
D5S818	0.6	0.99	0.89
D13S317	0.66	0.99	0.86
D7S820	0.38	0.99	0.85
SE33	0.31	0.99	0.80
D10S1248	0.6	0.99	0.87
D1S1656	0.56	0.99	0.85
D12S391	0.4	0.99	0.85
D2S1338	0.33	0.99	0.82

Dye specific measurements were calculated and summarized in Table 4 for positive control data runs performed throughout the validation. Additionally, the table also includes run-to-run signal variability measurement for the positive control data.

Table 4. Dye specific summary data.

	Intercolor Balance	Signal Variability (%CV)	Intracolor Balance
6-FAM (Blue)	1.1		39%
VIC (Green)	0.8		42%
NED (Yellow)	0.8		39%
TAZ (Red)	1.3		38%
SID (Purple)	1.0		41%
LIZ (Orange)		5%	

Calculated locus specific detection and stochastic thresholds appear in Table 5, below. These thresholds were calculated using the 564 concordance sample set. A 50 RFU minimum peak detection threshold was applied where the calculated Receiver Operator Characteristic determined threshold was less than this value. The stochastic threshold for haploid loci was set to the locus specific peak detection threshold. Where the calculated stochastic threshold multiplicative factor for a given locus was determined to be below 2, a minimum value for the stochastic threshold was set at two times the peak detection threshold.

Table 5. Locus specific analytical and stochastic thresholds.

Dye	Marker	Analytical Threshold	Stochastic Threshold
6-FAM	D3S1358	50	100
6-FAM	vWA	50	100
6-FAM	D16S539	50	100
6-FAM	CSF1PO	50	100
6-FAM	TPOX	84	210
VIC	AMEL	50	100
VIC	Yindel	74	74
VIC	D8S1179	50	100
VIC	D21S11	50	100
VIC	D18S51	50	100
VIC	DYS391	50	50
NED	D2S441	68	136
NED	D19S433	50	100
NED	TH01	50	100
NED	FGA	50	100
TAZ	D22S1045	50	100
TAZ	D5S818	50	100
TAZ	D13S317	50	100
TAZ	D7S820	50	100
TAZ	SE33	50	150
SID	D10S1248	50	100
SID	D1S1656	50	100
SID	D12S391	50	100
SID	D2S1338	50	125

CONCLUSION

The results of this validation study conclude that the Applied Biosystems RapidHIT ID System is a robust solution for the generation of DNA profiles from buccal, reference samples. The design of the fast, easy-to-use system enables trusted, lab-quality results in non-traditional, decentralized settings.

REFERENCES

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