

Validation of the RapidINTEL™ Plus Cartridge for Investigative Lead Samples and to Meet the SWGDAM Rapid Guidelines

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Abstract

Although DNA profiles from *reference samples* run on rapid DNA instruments are allowed into CODIS, the FBI does not currently allow upload of DNA profiles from *crime scene samples* run on rapid DNA instruments. In 2020, SWGDAM, ENFSI, and the FBI Rapid DNA Crime Scene Technology Advancement Task Group outlined rapid DNA requirements that, when met, would allow DNA profiles from crime scene samples run on rapid DNA instruments to be uploaded into CODIS. In early 2024, Thermo Fisher released a new sample cartridge, RapidINTEL™ Plus, designed to meet these requirements while improving on the performance of crime scene samples run on the RapidHIT™ ID system.

The RapidINTEL™ Plus sample cartridge was developed in conjunction with the RapidHIT™ ID System Software v2.0 and RapidLINK™ Software v2.0. In addition to new features required for CODIS, improvements were made to the RapidINTEL™ Plus cartridge to produce high quality and reliable data from crime scene samples through the introduction of Internal Quality Control markers, Quantification markers, and PCR enhancements to increase sensitivity and improve peak height and inter-locus balance.

We performed a developmental validation to assess the newly optimized RapidHIT™ ID System v2.0 for the analysis of investigative lead samples with our RapidINTEL™ Plus cartridge. The system demonstrated substantial improvements in first pass success rate, sensitivity, and suitability for law enforcement personnel to generate investigative leads and identify suspects faster.

Introduction

The Applied Biosystems™ RapidINTEL™ Plus sample cartridge is an updated version of the RapidINTEL™ sample cartridge specifically developed for RapidHIT™ ID System v2.0 and RapidLINK™ Software v2.0 for use with single source crime scene samples. It was developed to increase first pass success and to meet the requirements outlined the position statement by ENFSI, SWGDAM and the Rapid DNA Crime Scene Technology Advancement Task Group.

The RapidINTEL™ Plus sample cartridge includes the following improvements:

- **Amplification chemistry** – Based on GlobalFiler™ Express chemistry, but with the following additions:
 - Internal Quality Control (IQC) markers to identify samples that contain PCR inhibitors
 - Quantification markers to estimate the DNA quantity in the PCR and to determine if the sample is degraded (QTS, QTL)
 - Master mix and primer mix optimizations for improved DNA profile balance
- **Dual instrument protocols** – Promotes flexibility by providing a dual protocol system which includes a Specialized protocol and General protocol. The Specialized protocol, in conjunction with the use of a micro/mini swab, enables higher sensitivity and is intended for samples that are expected to contain limited amounts of DNA.
- **System performance** – Increased DNA capture in PCR and reduced stochastic amplification by decreasing the number of PCR cycles.

Materials and Methods

The study included a total of 135 samples that were prepared or collected to simulate biological evidence found at crime scenes, including blood, saliva, touch, and other types of samples such as tooth, bone, nail, etc. A few samples contained measured DNA input amounts, and many samples were unmeasured input amounts from stains, cast-off blood, and used or worn items. Most of the samples were collected using a micro HydraFlock swab (Puritan, 25-3318) and were left to air-dry at ambient temperature overnight before running on the RapidHIT™ ID instrument using the Specialized protocol. Other samples such as the nail, tooth, gum, and fabric cuttings were run neat using either or both Specialized and General protocols. A total of 12 RapidHIT™ ID instruments were used for this study.

All results were taken from the RapidLINK™ v2.0 Software or GeneMapper™ ID-X v1.7.

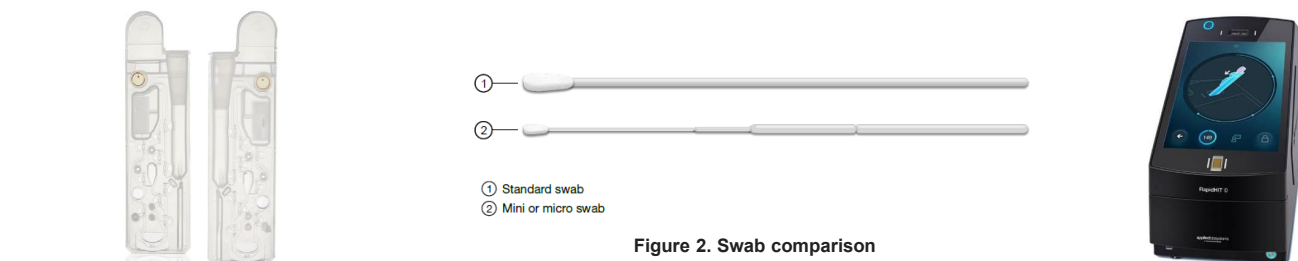


Figure 1. RapidINTEL™ Plus sample cartridge

Results

RapidINTEL™ Plus Chemistry

Figure 4 is an example of an electropherogram of the RapidINTEL™ Plus chemistry containing the Quantification/Quality Small (QQS) and Quantification/Quality Large (QQL) markers located at the front and back of the FAM (blue) dye channel.

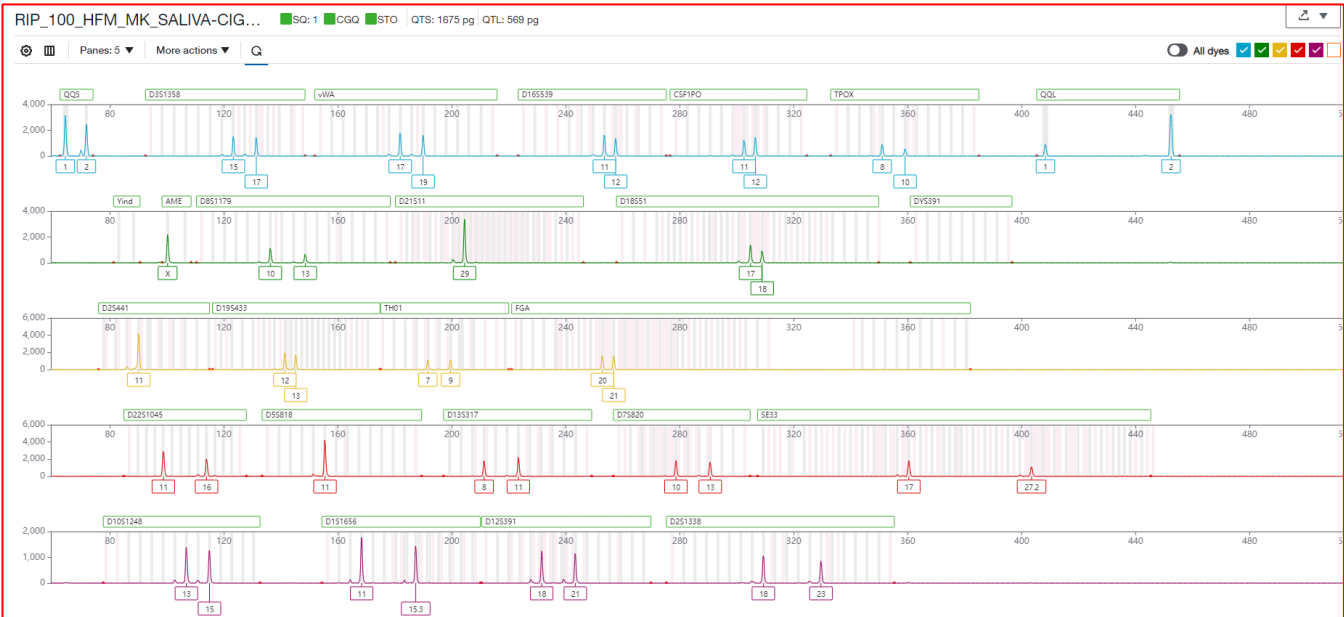


Figure 4. RapidINTEL™ Plus electropherogram of a cigarette butt sampled using HydraFlock micro swab and run under the Specialized protocol

Quantification System

Figure 4 displays the accuracy and precision of the quantification system incorporated into the RapidINTEL™ Plus chemistry. The blue dots are the estimated amount of DNA loaded on the swab in the blood sample after back calculating from the QTS value. The red boxes are the theoretical DNA quantities for the specified volumes of blood on the swab (x-axis). Theoretical values are based on a typical WBC of 4,500-11,000/μL (27-66 ng/μL). The dotted green boxes are the adjusted expected DNA quantities based on 50% recovery on cotton swab. The data indicates that a flocked swab is more efficient at releasing sample than the standard cotton swab.

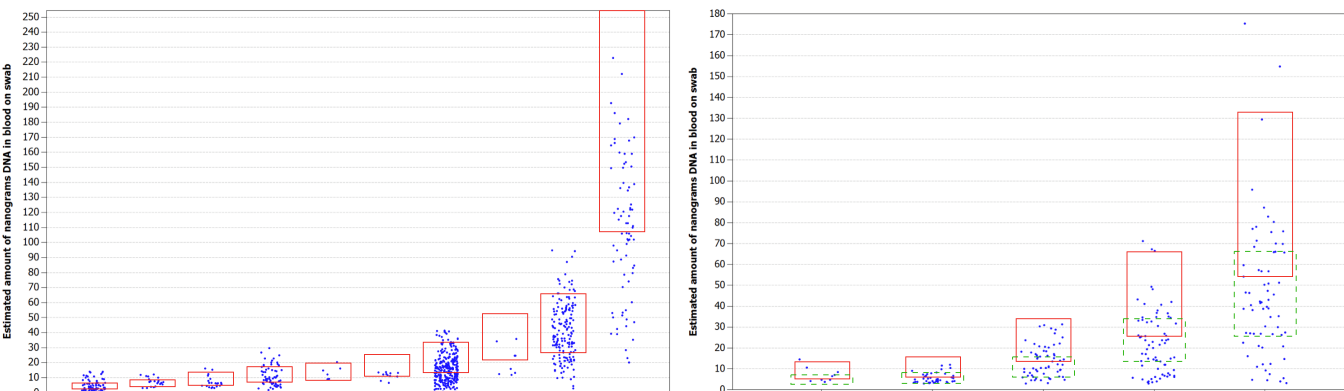


Figure 4. Performance of quantification system with blood on flocked and cotton swabs

Stochastic Flagging

Table 1 shows the percentage of samples that exhibited a red STO flag which contain allelic dropout (all samples run during development of RapidINTEL™ Plus).

Table 1. STO flag indicator correlation to allele dropout

STO indicator flag	Meaning	Number of samples	Percent of samples with dropout
	Extremely rare dropout	1,379	0.15
	Rare dropout	432	1.4
	Frequent dropout	531	75.3

Table 2. Percent alleles recovered (%) for each mock case sample

Sample Type	Substrate Category	Detailed Substrate/Sample Information	Protocol	Loading Method	Input Information	Percentage of Alleles Recovered	Unflagged Loci (Autosomal)	Allele Dropouts	QTS (ng)	QTL (ng)	Hit	Req	Inh	STO	RL Review Status
OTHER	Hair	Hair beard	Spec	DL	Plucked	100%	20	0	7,850	>1,600					Pass w. review
	Hair	Hair eyebrow	Spec	DL	Plucked	100%	20	0	8,442	>1,600					Pass w. review
	Hair	Hair head	Spec	DL	Plucked	100%	21	0	6,552	>1,600					Pass w. review
	Semen	Cosmoed tan	Spec	HFM	Scam	100%	19	0	4,954	>1,600					Pass w. review
	Hair	Hair head	Spec	DL	Plucked	100%	21	0	1,488	>1,600					Pass
	Semen	Denim	Spec	HFM	Scam	100%	21	0	2,809	862					Pass w. review
	Bone	Tooth powdered	Spec	DL	Clipped	100%	21	0	3,354	>1,600					Pass
	Tooth	Whole tooth ground	Spec	DL	Extracted	100%	21	0	10,054	789					Pass
	Hair	Hair head	Spec	DL	Plucked	100%	21	0	983	>1,600					Pass
	Fingernail	Fingernail, clipping	Spec	DL	Clipped	100%	21	0	2,462	1,089					Pass
	Semen	Denim	Spec	HFM	Scam	100%	21	0	1,643	802					Pass w. review
	Fingernail	Fingernail, clipping cleaned with EtOH	Spec	DL	Clipped	100%	21	0	1,097	422					Pass
	Tooth	Whole tooth ground	Spec	DL	Extracted	100%	21	0	772	193					Pass
	Fingernail	Fingernail, clipping cleaned with EtOH	Spec	DL	Clipped	100%	21	0	667	126					Pass
	Hair	Hair head	Spec	DL	Plucked	100%	21	0	292	644					Pass w. review
	Hair	Hair head	Spec	DL	Plucked	100%	20	0	120	209					Pass w. review
	Hard surface	Cement	Spec	HFM	Scam	100%	21	0	4,734	>1,600					Pass
	Fabric	White cotton, 4-mm cutting	Spec	DL	5 µL	100%	21	0	5,518	>1,600					Pass
	Hard surface	Glass slide	Spec	HFM	Scam	100%	21	0	2,684	>1,600					Pass
	Fabric	Carpet	Spec	HFM	Scam	100%	21	0	2,319	>1,600					Pass
BLOOD	Hard surface	Drywall	Spec	HFM	Scam	100%	21	0	3,873	>1,600					Pass
	Fabric	Fabric, 4-mm cutting	Spec	DL	7.5 µL	100%	21	0	1,969	>1,600					Pass
	Fabric	White cotton, 4-mm cutting	Spec	DL	1 µL	100%	21	0	3,698	>1,600					Pass
	Fabric	Colored cotton, 4-mm cutting	Spec	DL	2.5 µL	100%	21	0	3,660	>1,600					Pass
	Hard surface	Cement	Spec	HFM	Scam	100%	21	0	1,032	>1,600					Pass
	Hard surface	Cement	Spec	HFM	Scam	100%	21	0	2,047	>1,600					Pass
	Hard surface	Cement	Spec	HFM	Scam	100%	21	0	3,073	1,112					Pass
	Fabric	Fabric, 4-mm cutting	Spec	DL	7.5 µL	100%	21	0	2,045	>1,600					Pass
	Denim	4-mm cutting	Spec	DL	19	100%	21	0	1,471	80					Pass w. review
	Fabric	Colored cotton, 4-mm cutting	Spec	DL	2.5 µL	100%	21	0	2,056	>1,600					Pass
	Hard surface	Glass slide	Spec	HFM	Scam	100%	20	0	1,071	>1,600					Pass w. review
	Hard surface	Glass slide	Spec	HFM	Scam	100%	21	0	1,441	>1,600					Pass
	Fabric	Cotton swab, 4 mos. old	Spec	HFM	Cast-off	100%	21	0	1,601	>1,600					Pass
	Fabric	Cotton swab, 2 mos. old	Spec	HFM	Cast-off	100%	21	0	874	>1,600					Pass
	Fabric	Denim, 3.7 years old	Spec	HFM	Cast-off	100%	21	0	1,108	676					Pass
	Fabric	Carpet, low pile	Spec	HFM	Scam	100%	21	0	762	746					Pass
	Denim	Denim	Spec	HFM	Scam	100%	21	0	513	>1,600					Pass
	Fabric	Denim	Spec	HFM	Scam	100%	21	0	682	1,032					Pass
	Hard surface	Cement	Spec	HFM	Scam	100%	21	0	999	891					Pass
SALIVA	Fabric	White cotton	Spec	HFM	Scam	100%	21	0	510	887					Pass
	Hard surface	Glass slide	Spec	HFM	Scam	100%	21	0	1,232	1,176					Pass
	Fabric	Wood, 2 mos. old	Spec	HFM	Cast-off	100%	21	0	552	563					Pass w. review
	Hard surface	Glass slide	Spec	HFM	Scam	100%	21	0	652	430					Pass
	Hard surface	Tile	Spec	HFM	Scam	100%	21	0	556	486					Pass
	Fabric	Denim	Spec	HFM	Scam	100%	20	0	50	292					Pass w. review
	Fabric	Wood, 2 mos. old	Spec	HFM	Cast-off	100%	21	0	173	190					Pass
	Fabric	Denim	Spec	HFM	Scam	100%	21	0	576	141					Pass
	Fabric	White cotton	Spec	HFM	Scam	100%	21	0	384	203					Pass
	Fabric	Wood, 2 mos. old	Spec	HFM	Cast-off	100%	21	0	347	208					Pass
	Fabric	Cotton swab, 2 mos. old	Spec	HFM	Cast-off	100%	21	0	180	358					Pass
	Fabric	Electrical tape	Spec	HFM	Cast-off	100%	21	0	292	140					Pass
	Hard surface	Tile	Spec	HFM	Scam	100%	21	0	108	115					Pass
	Hard surface	Plywood, 2 mos. old	Spec	HFM	Cast-off	100%	21	0	213	66					Pass
	Fabric	Colored cotton	Spec	HFM	Scam	100%	18	0	162	38					Quality flag
	Fabric	Cotton swab, 4 mos. old	Spec	HFM	Cast-off	88%	19	0	318	56					Quality flag
	Fabric	Denim, 4-mm cutting	Spec	DL	10 µL	100%	20	2	367	ND					Quality flag
	Gum	Mint gum	Spec	HFM	Used	100%	21	0	4,495	>1,600					Pass w. review
	Hard surface	Soda can	Spec	HFM	Used	100%	21	0	1,692	>1,600					Pass
TOUCH	Fabric	White cotton	Spec	DL	Scam	100%	21	0	3,119	>1,600					Pass
	Hard surface	Plastic water bottle	Spec	HFM	Used	100%	21	0	2,583	>1,600					Pass w. review
	Gum	Fruit gum	Spec	HFM	Used	100%	21	0	1,144	691					Pass
	Hard surface	Glass slide	Spec	HFM	Scam	100%	21	0	2,541	>1,600					Pass
	Fabric	White cotton	Spec	DL	20 µL	100%	21	0	2,618	>1,600					Pass
	Hard surface	Toothbrush	Spec	HFM	Used	100%	21	0	1,556	>1,600					Pass
	Hard surface	Toothbrush	Spec	HFM	Used	100%	21	0	517	>1,600					Pass
	Cigarette	Cigarette paper	Spec	HFM	Smoked	100%	21	0	1,675	569					Pass
	Hard surface	Glass slide	Spec	HFM	Scam	100%	21	0	2,673	>1,600					Pass
	Gum	Fruit gum	Spec	DL	Used	100%	21	0	3,483	284					Pass
	Hard surface	Coffee cup, ceramic	Spec	HFM	Used	100%	21	0	1,071	>1,600					Pass
	Hard surface	Coffee cup, ceramic	Spec	HFM	Used	100%	21	0	1,788	927					Pass
	Hard surface	Soda can	Spec	HFM	Used	100%	21	0	888	366					Pass
	Cigarette	Cigarette paper	Spec	HFM	Smoked	100%	21	0	888	220					Pass
	Hard surface	Plastic water bottle	Spec	HFM	Used	100%	21	0	1,460	>1,600					Pass
	Hard surface	Toothbrush, electric	Spec	HFM	Used	100%	21	0	608	671					Pass
	Gum	Mint gum	Spec	DL	Used	100%	21	0	439	234					Pass
	Hard surface	Coffee cup, ceramic	Spec	HFM	Used	100%	21	0	507	234					Pass
	Cigarette	Cigarette paper half	Spec	DL	Smoked	100%	21	0	1,186	245					Pass
	Cigarette	Cigarette paper	Spec	HFM	Smoked	100%	21	0	399	141					Pass
	Cigarette	Cigarette paper	Spec	HFM	Smoked	100%	21	0	420	171					Pass
	Hard surface	Toothbrush, electric	Spec	HFM	Used	100%	21	0	1,709	88					Pass
TOUCH	Hard surface	Beer can	Spec	HFM	Used	100%	21	0	1,050	ND					Quality flag
	Gum	Mint gum	Spec	HFM	Used	100%	21	0	691	211					Pass
	Hard surface	Plastic straw	Spec	HFM	Used	100%	21	0	385	242					Pass
	Gum	Fruit gum	Spec	DL	Used	100%	21	0	473	77					Pass
	Cigarette	Cigarette paper whole	Spec	DL	Smoked	100%	21	0	203	77					Pass
	Cigarette	Cigarette paper half	Spec	DL	Smoked	100%	20	0	218	49					Quality flag
	Gum	Fruit gum	Spec	HFM	Used	100%	20	0	144	117					Pass w. review