

RapidINTEL™ Plus Sample Cartridge

USER BULLETIN

For use with RapidHIT™ ID System v2.0 or later

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
C	2 April 2025	The following section was updated: "RapidINTEL™ Plus allelic ladder cartridges" on page 11. The following sections were added: "General run recommendations for various sample types" on page 15 and Chapter 3, "Equivalency and validation studies (one-punch cartridge)". Minor text updates were made to align with current documentation style (throughout the document).
B00	18 March 2024	The following sections were added: Chapter 2, "Validation and verification studies" and Appendix A, "Thresholds, flags, and artifacts". Minor text updates were made for clarification (throughout the document).
A.0	15 November 2023	New document for the RapidINTEL™ Plus Sample Cartridge.

The information in this guide is subject to change without notice.

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IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The RapidINTEL™ Plus Sample Cartridge is a new version of the RapidINTEL™ Sample Cartridge specifically developed for RapidHIT™ ID System v2.0 and RapidLINK™ Software v2.0.

Like the RapidINTEL™ Sample Cartridge, the RapidINTEL™ Plus Sample Cartridge is for use with single-source crime scene samples. However, 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
 - Master mix and primer mix optimizations for improved DNA profile balance
- **Dual instrument protocols**—Protocol selection is based on the sample substrate or the expected amount of DNA in the sample
- **PCR optimizations**—PCR chamber and thermal cycling enhancements

Table 1 Cartridge type abbreviations used in this document

Type	Abbreviation
RapidHIT™ ID ACE GlobalFiler™ Express	ACE GFE sample, allelic ladder, or control cartridge
RapidINTEL™ Plus	RapidINTEL™ Plus sample, allelic ladder, or control cartridge

System compatibility

The RapidINTEL™ Plus Sample Cartridge runs only on RapidHIT™ ID System v2.0 or later. The cartridge is not compatible with RapidHIT™ ID System v1.x.

RapidHIT™ ID System v2.0 supports the following cartridges:

- RapidINTEL™ Plus cartridges
- ACE GFE cartridges
- RapidHIT™ ID Primary Cartridge GlobalFiler™ Express 150 Kit cartridges

Protocols

When used with a RapidINTEL™ Plus cartridge, RapidHIT™ ID System v2.0 allows users to select from two different protocols: **General** or **Specialized**:

Table 2 RapidHIT™ ID System v2.0 protocols for use with the RapidINTEL™ Plus cartridge

Protocol	Application	Swab type
General	Crime scene samples that have a moderate quantity of DNA	• Standard swabs, similar to the RapidINTEL™ cartridge • Mini or micro swabs
Specialized	• Crime scene samples that have a low quantity of DNA • Samples that require a high degree of sensitivity	Mini or micro swabs only Note: If needed, you can transfer a portion of the sample from a standard swab to a mini or micro swab. (The transfer process is referred to as "subsampling".)

Note: RapidLINK™ Software administrators can configure the instrument to automatically select only the **General** protocol or only the **Specialized** protocol when running a RapidINTEL™ Plus cartridge. If the instrument is configured for only one protocol, the **Select sample protocol** screen is not displayed during run setup.

For more information on the protocols, see "Instrument protocols" on page 12.

Amplification chemistry

Additional markers

The RapidINTEL™ Plus Sample Cartridge uses the same core locus set as the RapidHIT™ ID ACE GlobalFiler™ Express Sample Cartridge and the RapidINTEL™ Sample Cartridge, but includes the additional markers described in Table 3.

Table 3 Additional markers in the RapidINTEL™ Plus Sample Cartridge

Marker	Purpose
Internal Quality Control (IQC) markers	- Confirm successful PCR amplification - Identify samples that contain PCR inhibitors
Quantification markers	Identify samples that contain low quantities of DNA and/or degraded DNA

In combination, the IQC markers and quantification markers are able to determine when a sample is degraded in the absence of PCR inhibition. The addition of these new markers resulted in two new loci: Quant/Quality Small (QQS) locus and Quant/Quality Large (QQL) locus.

Note: For the complete list of loci, see “RapidINTEL™ Plus assay markers and dye labels” on page 104.

IQC markers

The RapidINTEL™ Plus cartridge contains two IQC markers:

- One marker at ~72 bp in the QQS locus (referred to as the "small IQC marker" in this document)
- One marker at ~452 bp in the QQL locus (referred to as the "large IQC marker" in this document)

These markers are the same IQC markers that are found in the GlobalFiler™ IQC PCR Amplification Kit. Similar to the GlobalFiler™ IQC kit, the template DNA for the IQC markers resides in the PCR primer mix and amplifies in the presence or absence of sample DNA.

- The two IQC markers are used to confirm that thermal cycling was successful.
- The peak height ratio of the two IQC markers is used to indicate the presence of PCR inhibitors in the sample. In the presence of a PCR inhibitor, the peak height of the large IQC marker is substantially less than the peak height of the small IQC marker.

The following is a list of some common PCR inhibitors (not a comprehensive list):

- An abundance of sample DNA
- Clothing dyes found in denim (for example, indigo dye)
- Heme found in blood
- Humic acid found in soils

Note: For information about the IQC marker flags in RapidLINK™ Software v2.0, see “Process Quality Value (PQV) flags” on page 17.

Quantification markers

The RapidINTEL™ Plus cartridge contains two quantification markers:

- One marker at ~64 bp in the QQS locus (referred to as the "small quant marker" in this document)
- One marker at ~408 bp in the QQL locus (referred to as the "large quant marker" in this document)

The quantification markers are not STR loci—they are unique, stable regions of the human genome with minimal polymorphisms. The template DNA for these markers is contained in the sample DNA itself, and therefore amplifies only in the presence of human genomic DNA. The peak heights of these markers increase or decrease with the amount of DNA present in the PCR. RapidHIT™ ID System v2.0 translates the peak heights into a quantity of DNA expressed in picograms (pg), then separately displays the estimated DNA quantity from each of the markers.

- The peak heights of the two quantification markers represent the estimated DNA quantity.
- For samples that contain degraded DNA or PCR inhibitors, the peak height of the large quant marker is less than the peak height of the small quant marker.
- For samples that do *not* contain degraded DNA or PCR inhibitors, the peak heights of the two quantification markers are similar.

Note: For information about the quantification marker flags in RapidLINK™ Software v2.0, see "Process Quality Value (PQV) flags" on page 17.

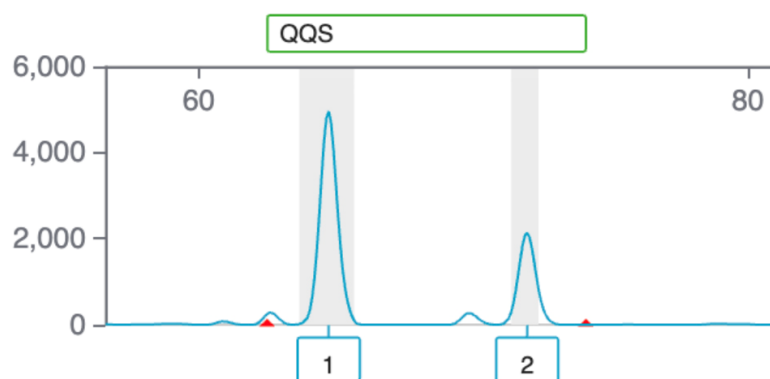


Figure 1 QQS locus—RapidINTEL™ Plus Positive Control Cartridge The QQS locus contains the small quant marker and the small IQC marker. The small quant marker has an allele label of "1". The small IQC marker has an allele label of "2".

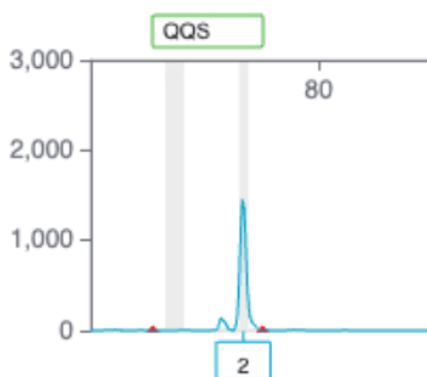


Figure 2 QQS locus—RapidINTEL™ Plus Negative Control Cartridge In the absence of sample DNA, only the small IQC marker amplifies.

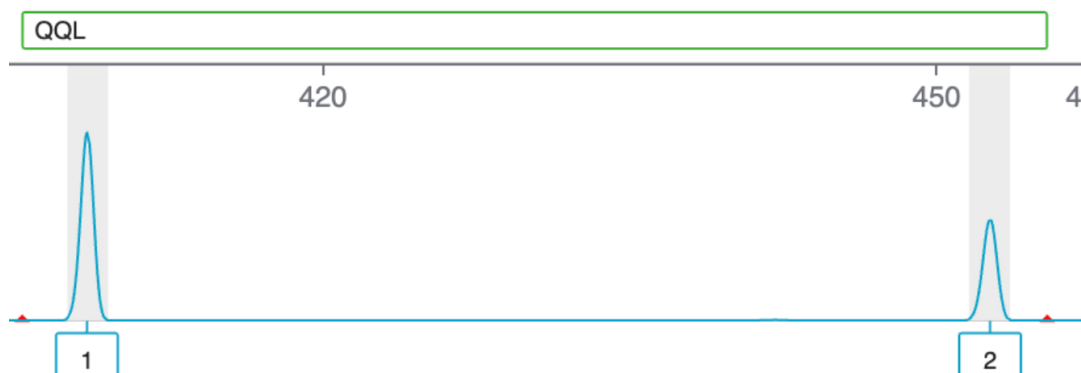


Figure 3 QQL locus—RapidINTEL™ Plus Positive Control Cartridge The QQL locus contains the large quant marker and the large IQC marker. The large quant marker has an allele label of “1”. The large IQC marker has an allele label of “2”.

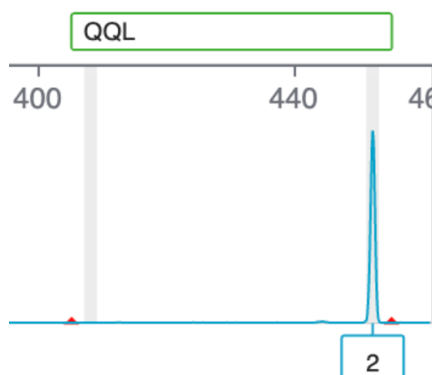
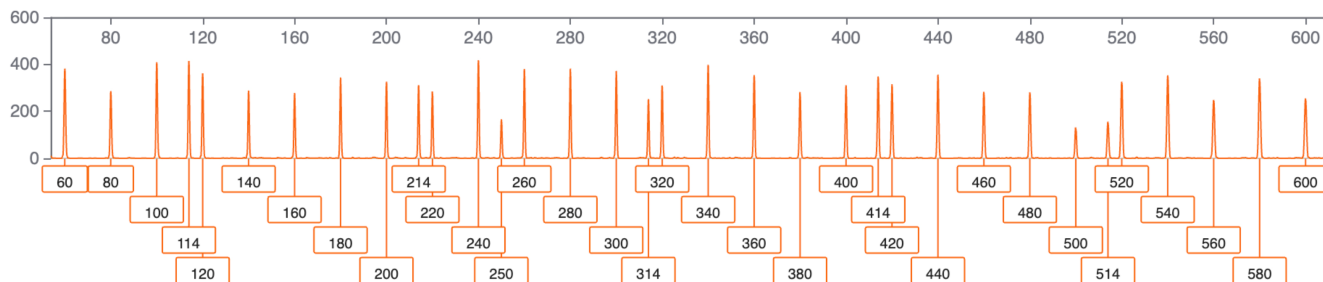


Figure 4 QQL locus—RapidINTEL™ Plus Negative Control Cartridge In the absence of sample DNA, only the large IQC marker amplifies.

Size standard

The RapidINTEL™ Plus cartridge uses GeneScan™ 600 LIZ™ Size Standard v2.0 (also referred to as “GS600”), which is new to the RapidHIT™ ID System. The GS600 size standard functions the same way as the size standard in the ACE GFE cartridge.



RapidINTEL™ Plus positive control cartridges

RapidHIT™ ID System v2.0 requires a positive control to set the spectral matrix that is used to separate the fluorescence emitted by the dyes used in the amplification chemistry. Because of this, a positive control must be the first run when a new primary cartridge is inserted into the RapidHIT™ ID instrument.

The spectral matrix is chemistry-specific; therefore, you must run the following cartridges after every primary cartridge installation:

- Run an ACE GFE positive control cartridge before running ACE GFE allelic ladder, negative control, or sample cartridges.
- Run a RapidINTEL™ Plus positive control cartridge before running RapidINTEL™ Plus allelic ladder, negative control, or sample cartridges.

RapidINTEL™ Plus allelic ladder cartridges

Because the RapidINTEL™ Plus cartridges include two added loci and a size standard that is different from the ACE GFE and RapidINTEL™ cartridges, there is a separate RapidINTEL™ Plus allelic ladder cartridge.

When a RapidINTEL™ Plus allelic ladder cartridge is run, the ladder file is added to the allelic ladder library and becomes available for use with all samples that are subsequently run on the current primary cartridge. When a RapidINTEL™ Plus allelic ladder cartridge is not run, sample runs rely on the allelic ladder library for allele calling. After a new primary cartridge is inserted into the RapidHIT™ ID instrument, running the RapidINTEL™ Plus allelic ladder cartridge is optional.

Note: For more information about running allelic ladder cartridges, see the *Technical Note: Impact on Genotyping Results when a Native Allelic Ladder is not Available in the Applied Biosystems™ RapidHIT™ ID Allelic Ladder Library* (Pub. No. [TCF1000105](#)).

PCR optimizations

The following PCR optimizations produce well-balanced DNA profiles for the recommended sample amounts and generate a low peak height ratio threshold for challenging single-source samples:

- One large, oblong collection punch in the PCR chamber, which captures more DNA than the RapidINTEL™ cartridges
- Decreased thermal cycling time (30 cycles for the RapidINTEL™ Plus cartridges compared to 32 cycles for the RapidINTEL™ cartridges)

Table 4 RapidINTEL™ Plus Sample Cartridge thermal cycler conditions

The thermal cycler conditions are controlled internally by RapidHIT™ ID System v2.0.

Step	Temperature	Time
Activation hold	95°C	1 minute
Cycle (30 cycles)	94°C	3 seconds
	61.5°C	30 seconds
Final hold	60°C	8 minutes

Instrument protocols

During run setup, the instrument software may show the **Select sample protocol** screen. You can select the **General** protocol or the **Specialized** protocol. The only difference between the protocols is the amount of lysis solution that is used during DNA extraction. Select a protocol based on the substrate (the material that is being inserted into the instrument) and the expected amount of DNA in the sample.

Note: RapidLINK™ Software administrators can configure the instrument to automatically select only the **General** protocol or only the **Specialized** protocol when running a RapidINTEL™ Plus cartridge. If the instrument is configured for only one protocol, the **Select sample protocol** screen is not displayed during run setup.

General protocol

When the **General** protocol is selected, 500 µL of lysis solution is delivered during the DNA extraction step. The **General** protocol is intended for use with samples that contain moderate amounts of DNA (for example, 2–35 µL of blood). Blood volumes >35 µL may produce PCR saturation artifacts. Blood volumes well in excess of 35 µL may cause contamination in subsequent runs (see “Contamination study” on page 85).

Specialized protocol

When the **Specialized** protocol is selected, 100 µL of lysis solution is delivered during the DNA extraction step. Because the sample is concentrated into a smaller volume of lysis solution, the **Specialized** protocol provides greater sensitivity than the **General** protocol. The **Specialized** protocol is intended for use with samples that are expected to contain limited amounts of DNA.

IMPORTANT! The **Specialized** protocol *must* be used with a mini swab, micro swab, or substrate that absorbs and retains <40 µL of liquid (or no liquid). Using standard swabs will cause most or all of the 100 µL of lysis solution to be absorbed, and none of it transferred to the PCR chamber. For more information, see “Swabs and subsampling” on page 14.

Selecting the appropriate protocol for the RapidINTEL™ Plus cartridge

The RapidINTEL™ Plus cartridge is designed to use either protocol on the RapidHIT™ ID instrument, depending on the swab type or substrate:

- **Specialized protocol (100-µL lysis volume)**—The sample inserted into the cartridge should absorb ≤40 µL of lysis solution. Recommended for the Puritan™ HydraFlock™ 6" Sterile Micro Ultrafine Flock Swab with Polystyrene Handle.
- **General protocol (500-µL lysis volume)**—The sample inserted into the cartridge should absorb ≤150 µL of lysis solution. Recommended for the Puritan™ 3" Sterile Standard Cotton Swab and other substrates.

Exceeding these volumes may decrease the lysate volume to a level that is insufficient for transfer to the PCR chamber.

To help you select the correct protocol for non-swab samples, see Figure 5.

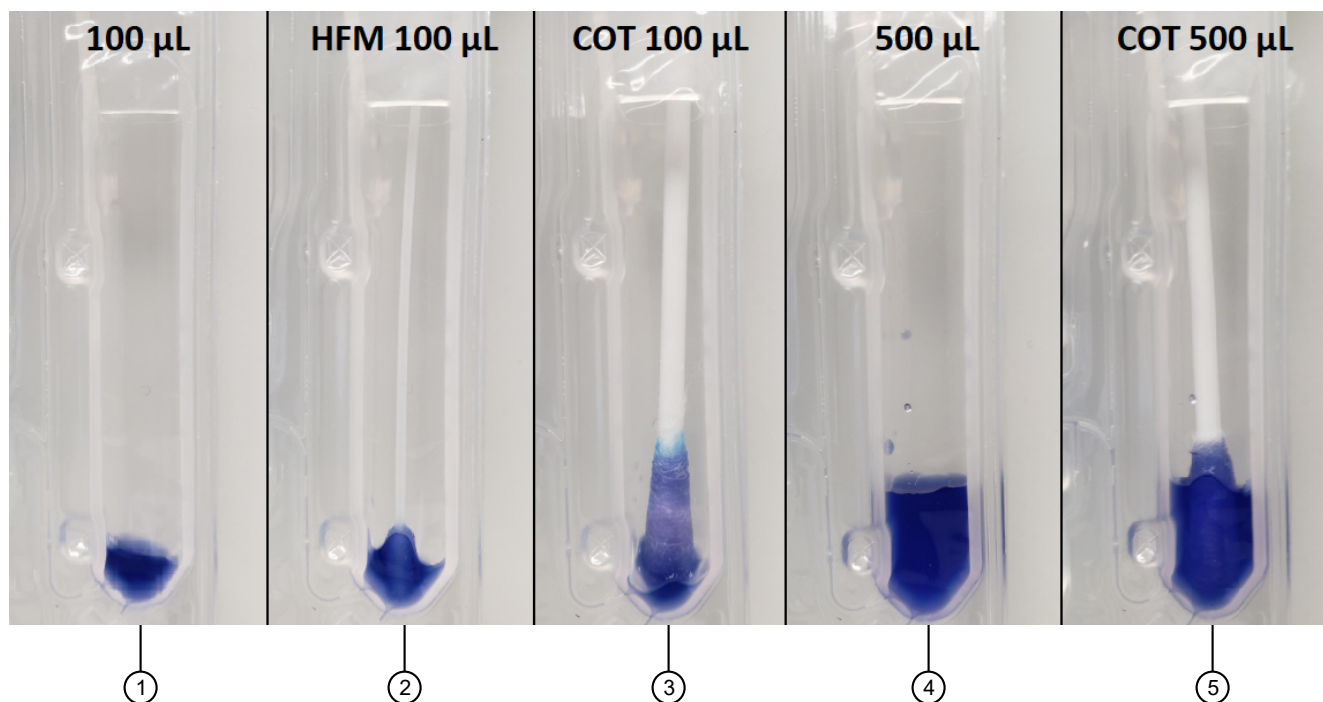


Figure 5 Swab and sample compatibility with the Specialized and General protocols Blue dye was added to help visualize the lysis buffer volumes.

- ① Specialized protocol, 100 µL of lysis solution level in the cartridge.
- ② Specialized protocol, with insertion of a Puritan™ HydraFlock™ 6" Sterile Micro Ultrafine Flock Swab with Polystyrene Handle ("HFM").
- ③ Specialized protocol, with insertion of a standard cotton swab ("COT"). A COT swab will absorb *all* 100 µL of lysis solution, which results in no sample in the PCR chamber.
- ④ General protocol, 500 µL of lysis solution level in cartridge.
- ⑤ General protocol, with insertion of a COT swab.

Swabs and subsampling

Mini and micro swabs

Note: In this document, "mini swab" and "micro swab" refer to swabs that absorb and retain <40 µL of liquid (or no liquid).

Mini and micro swabs have a much smaller swab tip than the standard swabs typically used for rapid DNA collection. Mini and micro swabs can fit into smaller grooves and absorb much less liquid.

Mini and micro swabs provide the following benefits compared to standard swabs:

- **Less sample required**—Mini and micro swabs use a smaller portion of the sample. This is especially true when using a mini or micro swab to subsample from a standard swab; see "Subsampling" on page 15.
- **Increased sensitivity for the Specialized protocol**—When using the **Specialized** protocol with a RapidINTEL™ Plus cartridge, a mini or micro swab must be used. For more information, see "Specialized protocol" on page 13.



Figure 6 Swab comparison

- ① Standard swab
- ② Mini or micro swab

Subsampling

Subsampling is a technique that involves transferring a portion of sample that is on a standard swab to a mini or micro swab. Subsampling allows you to:

- Save the original swab for further testing
- Achieve maximum sensitivity when using the **Specialized** protocol
- Triage samples for subsequent laboratory processing

General run recommendations for various sample types

Blood

Recommended input volumes of blood for the **General** protocol (with standard, mini, or micro swabs) and the **Specialized** protocol (with mini or micro swabs) are provided in Table 5. Targeting an input volume of 2–5 μL of blood allows you to use either protocol.

Note: Swabs that contain more than the recommended volume of blood can be subsampled onto another standard swab or mini or micro swab. See “Swabs and subsampling” on page 14.

Table 5 Recommended input volumes of blood

- ■—Indicate volumes that are likely to produce a complete and balanced profile.
- ▲—Indicate volumes that are likely to produce a usable partial profile.
- ▲—Indicate volumes that are unlikely to produce a usable profile.
- ●—Indicate volumes that are likely to produce profiles with input-overload artifacts and an increased potential for DNA contamination in subsequent runs.

Blood input volume	General protocol	Specialized protocol
>35 μL	●	●
35 μL	▲	●
20 μL	▲	●
15 μL	■	●
10 μL	■	▲
5 μL	■	■
2 μL	■	■

Table 5 Recommended input volumes of blood *(continued)*

Blood input volume	General protocol	Specialized protocol
1 µL		
0.5 µL		
0.2 µL		
0.1 µL		
<0.1 µL		

Saliva

Recommended input volumes of saliva for the **General** protocol (with standard, mini, or micro swabs) and the **Specialized** protocol (with mini or micro swabs) are provided in Table 6.

Table 6 Recommended input volumes of saliva

- —Indicate volumes that are likely to produce a complete and balanced profile.
- —Indicate volumes that are likely to produce a usable partial profile.
- —Indicate volumes that are unlikely to produce a usable profile.
- —Indicate volumes that are likely to produce profiles with input-overload artifacts and an increased potential for DNA contamination in subsequent runs.

Saliva input volume	General protocol	Specialized protocol
>35 µL		
35 µL		
15 µL		
8 µL		
4 µL		
1 µL		
<1 µL		

Buccal swabs

If you are collecting buccal swabs specifically for the RapidINTEL™ Plus cartridges, we recommend the following steps:

1. Swipe the inside of the person's inner cheek once, moving in one direction using moderate pressure.
2. Run the swab with the **General** protocol.

We recommend the following steps for processing buccal swabs collected with multiple swipes of the inside of the person's inner cheek:

1. Subsample onto another swab (standard, mini, or micro swab). See "Swabs and subsampling" on page 14.
2. Run the swab with the **General** protocol.

Table 7 Buccal swab processing (RapidINTEL™ Plus cartridges)

Buccal swab collection method	Subsampling	Protocol
Single swipe, one direction	Not required	General
Multiple swipes	Recommended	General

Process Quality Value (PQV) flags

Inhibition (INH) flag

In RapidLINK™ Software v2.0, the INH flag identifies samples that contain a PCR inhibitor.

INH flag indicator	Description
	The ratio of the QQS 2 peak height (small IQC marker) to the QQL 2 peak height (large IQC marker) is >5: QQS 2:QQL 2 is >5 
N/A (no flag is shown)	The ratio of the QQS 2 peak height (small IQC marker) to the QQL 2 peak height (large IQC marker) is ≤5: QQS 2:QQL 2 is ≤5

Note: The INH flag indicates that the sample displays the typical symptom of inhibition and the effect may be severe enough to cause allelic dropout.

Degradation (DEG) flag

In RapidLINK™ Software v2.0, the DEG flag identifies samples that contain degraded DNA.

DEG flag indicator	Description
	The ratio of the QQS 1 peak height (small quant marker) to the QQL 1 peak height (large quant marker) is >5: QQS 1:QQL 1 is >5 
N/A (no flag is shown)	The ratio of the QQS 1 peak height (small quant marker) to the QQL 1 peak height (large quant marker) is ≤ 5: QQS 1:QQL 1 is ≤ 5

Note: The DEG flag indicates that the sample displays the typical symptom of degradation and the effect *may* be severe enough to cause allelic dropout.

Stochastic (STO) flag

In RapidLINK™ Software v2.0, the STO flag assesses the likelihood of a sample having peak height imbalances $<40\%$ or allelic dropout. This assessment is based on the combined average peak height of a subset of larger STR loci (CSF1PO, D18S51, FGA, SE33, and D2S1338).

In RapidLINK™ Software v2.0, the STO flag is displayed as follows:

STO flag indicator	Confidence level	Average peak height of indicator loci
	Very low quantity	≤ 225 RFU
	Low quantity	>225 RFU and ≤ 575 RFU
	Optimal quantity	>575 RFU

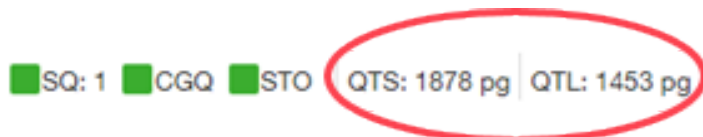
Note: For more information, see “Stochastic (STO) quality flag” on page 97 in Appendix A, “Thresholds, flags, and artifacts”.

Quantification (QTS and QTL) values

RapidLINK™ Software v2.0 displays two quantification values for each sample that is run with the RapidINTEL™ Plus cartridge:

- **QTS (small quantification value)**—Estimates the PCR DNA quantity in the size range of the small quant marker (~64 bp) and is based on the peak height of the QQS 1 peak.
- **QTL (large quantification value)**—Estimates the PCR DNA quantity in the size range of the large quant marker (~408 bp) and is based on the peak height of the QQL 1 peak.

In samples that do *not* contain degraded DNA or PCR inhibitors, both quantification values will be similar to each other (see below). Notice that this sample is above the stochastic range (the STO flag is ) and the presence of DNA degradation or PCR inhibitors was *not* detected (neither the DEG or INH flag is displayed).



In samples that *do* contain degraded DNA or PCR inhibitors, the QTL value will be substantially less than the QTS value (see below). Notice that this sample is likely to exhibit allelic dropout (the STO flag is ) and the presence of DNA degradation or PCR inhibitors was detected (both the DEG and INH flags are displayed).



Note: Because the large quant marker (QQL 1 peak) cannot determine DNA quantities >1,600 pg, the QTL value displays **>1,600 pg** for samples that contain DNA quantities above this value. Both the QTS and QTL values display **ND** (not determined) for any sample that results in no peak height values for the QQS 1 or QQL 1 peaks.

Note: For more information, see “Quantification markers” on page 98 in Appendix A, “Thresholds, flags, and artifacts”.

Summary of the RapidINTEL™ Plus cartridge features

With RapidHIT™ ID System v2.0 and RapidLINK™ Software v2.0, the RapidINTEL™ Plus cartridge allows better evaluation of DNA profiles and greater confidence in the results. Features include:

- The first-pass success rate of RapidHIT™ ID System v2.0 was increased by improving the intra locus balance and reducing stochastic PCR events as follows:
 - Optimizing the primer and master mixes for the RapidHIT™ ID instrument.
 - Increasing the punch size in the RapidINTEL™ Plus cartridge PCR chamber, doubling the amount of DNA captured in the PCR. The increased DNA capture improves the limit of detection and allele balance, allowing the PCR cycle number to be decreased from 32 cycles to 30 cycles without sacrificing sensitivity.

Note: The two circular punches in the RapidINTEL™ Plus cartridge have been replaced with one oblong punch. The oblong punch is approximately the same size as the two circular punches combined. For more information, see Chapter 3, “Equivalency and validation studies (one-punch cartridge)”.

- Setting system thresholds for a wide range of blood (0.1–35 µL) and saliva (4–35 µL) inputs. The system works best with samples that contain ~1 µL of blood and ~4 µL of saliva, and is more finely tuned for low input samples.
- Targets for quantification estimates and PCR amplification success were added. The quantification targets allow you to determine if the sample input is excessive for the system (QQS 1 peak height >3,800 RFU), which increases the potential for amplification artifacts, or if the input is stable across the STR range (the QTS and QTL values similar).



Validation and verification studies

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Objective of the validation and verification studies

The objective of the validation and verification studies was to assess the performance of the RapidINTEL™ Plus Sample Cartridge on RapidHIT™ ID System v2.0.

The studies were performed according to the Scientific Working Group on DNA Analysis Methods (SWGDM) *Validation Guidelines for DNA Analysis Methods* (December 2016) and the FBI Quality Assurance Standards (July 2020).

The following studies were performed:

- Positive control
- Negative control (carryover)
- Population and concordance
- Sensitivity
- Precision and accuracy
- PCR inhibition
- DNA degradation
- Mixture
- Species specificity

- Subsampling
- Case-type samples

The validation and verification studies assessed:

- Overall performance using the system thresholds developed for the RapidINTEL™ Plus cartridges
- Accurate detection and flagging of peaks that do not comply with the system thresholds
- Ability to detect PCR inhibition
- Ability to detect DNA degradation
- Ability to detect DNA profiles at risk of PCR stochastic effects
- Ability to estimate DNA load at the time of the PCR
- Ability to detect mixtures
- Primary analysis pass rate (the percentage of samples that meet all system thresholds and generate full, single-source profiles without manual review)
- Secondary analysis pass with review recovery rate (the percentage of samples that meet all system thresholds and generate full, single-source profiles after manual review)
- Sample input amounts to determine the optimal sample type and amounts to provide accurate results

Materials and methods

Instruments, cartridges, and software

12 RapidHIT™ ID System v2.0 instruments were used for the validation and verification studies. Each instrument was used in at least one study, however, not all instruments were used in every study.

Before the studies were run, a RapidINTEL™ Plus positive control cartridge and a RapidINTEL™ Plus allelic ladder cartridge were run on all instruments.

Note: Data presented in this chapter was obtained using RapidINTEL™ Plus cartridges run on RapidHIT™ ID System v2.0 and analyzed on RapidLINK™ Software v2.0 or GeneMapper™ ID-X Software v1.7. Capillary Electrophoresis sizing analysis was done using GeneScan™ 600 LIZ™ Size Standard v2.0.

Instruments, cartridges, and software

Item	Catalog number
RapidHIT™ ID System v2.0	A41810
RapidHIT™ ID Primary Cartridge GlobalFiler™ Express 150 Kit	A53084
RapidINTEL™ Plus 50 Sample Cartridge Kit	A54338
RapidINTEL™ Plus Positive Control Cartridge	A54342
RapidINTEL™ Plus Negative Control Cartridge	A54343
RapidINTEL™ Plus Ladder Cartridge Kit (allelic ladder)	A54344

Thermal cycler conditions

Optimal assay performance was determined by evaluating assay sensitivity and peak height balance.

Table 8 RapidINTEL™ Plus Sample Cartridge thermal cycler conditions

The thermal cycler conditions are controlled internally by RapidHIT™ ID System v2.0.

Step	Temperature	Time
Activation hold	95°C	1 minute
Cycle (30 cycles)	94°C	3 seconds
	61.5°C	30 seconds
Final hold	60°C	8 minutes

Sample collection, preparation, and loading

Sample collection

Swabbed samples were collected with standard swabs or with micro or mini swabs, as described in Table 9. Not all swab types were used in every study.

Some samples were collected without swabs (for example, gum).

Table 9 Swab types used

"MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Swab type	Tip size	Item	Cat. no.	Swab type abbreviations used in this document	Use cases
Spun cotton tip	Standard	Puritan™ 3" Sterile Standard Cotton Swab with Semi-Flexible Polystyrene Handle	25-803 2PC (MLS)	COT	Routine sampling; used with the General (500-µL) protocol
Nylon or polyester tip (flocked tip)	Micro or mini	Puritan™ HydraFlock™ 6" Sterile Micro Ultrafine Flock Swab with Polystyrene Handle, with 100-mm Breakpoint	25-3318-H (MLS)	HFM	Specialized sampling of smaller sample amounts that require maximum detection sensitivity; used with the Specialized (100-µL) protocol or the General (500-µL) protocol
		FLOQSwabs™ 516CS01 Ultra Minitip Flocked Swab with 100-mm Breakpoint	516CS01 (Copan)	CPM	

Sample preparation

Samples were prepared as described in each study section, under "Cartridge and sample summary".

Whether obtained from commercial suppliers or from known individuals, all human biological samples (blood, saliva, semen, hair, bone, fingernails, and tooth) were obtained with informed consent.

Sample loading

Samples were loaded into the RapidINTEL™ Plus cartridges in one of the following ways:

- Inserting a swab with sample into a cartridge. Swab types used were COT, CPM, or HFM.
- Directly loading a sample into a cartridge (no swab was used). In this document, this method may be referred to as "direct load" or "DL".

Calibration data set

Before performing the validation and verification studies, a calibration data set was used to establish various analysis thresholds for the RapidINTEL™ Plus assay. The calibration data set includes 1,513 samples that were processed with RapidINTEL™ Plus cartridges on RapidHIT™ ID System v2.0.

The calibration data set is listed in Table 10. For each sample type, the table provides the input amount, collection method, run protocol, and number of samples processed.

The resulting analysis thresholds are provided in Appendix A, "Thresholds, flags, and artifacts".

Table 10 Calibration data set: Sample types and input amounts

Sample type	Input amount	Collection method	Run protocol	Number of samples
Blood—936 blood samples 0.1–35 µL on CPM, COT, or HFM (from ~350 unique donors)				
Blood	0.1 µL	HFM	Specialized	81
Blood	0.1 µL	COT	General	14
Blood	0.2 µL	HFM	Specialized	15
Blood	0.2 µL	COT	General	10
Blood	0.25 µL	HFM	Specialized	62
Blood	0.25 µL	COT	General	57
Blood	0.4 µL	HFM	Specialized	5
Blood	0.5 µL	HFM	Specialized	262
Blood	0.5 µL	CPM	Specialized	4
Blood	0.5 µL	COT	General	69
Blood	0.8 µL	HFM	Specialized	8
Blood	1 µL	HFM	Specialized	91
Blood	1 µL	CPM	Specialized	4
Blood	1 µL	COT	General	70
Blood	2 µL	COT	General	63
Blood	4 µL	HFM	General	3
Blood	5 µL	HFM	Specialized	8

Table 10 Calibration data set: Sample types and input amounts (*continued*)

Sample type	Input amount	Collection method	Run protocol	Number of samples
Blood	5 µL	COT	General	4
Blood	10 µL	HFM	Specialized	14
Blood	10 µL	CPM	Specialized	4
Blood	15 µL	HFM	Specialized	10
Blood	15 µL	COT	General	10
Blood	20 µL	HFM	Specialized	10
Blood	25 µL	HFM	Specialized	10
Blood	25 µL	COT	General	10
Blood	35 µL	HFM	Specialized	18
Blood	35 µL	CPM	Specialized	8
Blood	35 µL	COT	General	12
Saliva—175 saliva samples, 1–35 µL on HFM and 1–8 µL on COT (from ~25 unique donors)				
Saliva	1 µL	HFM	Specialized	26
Saliva	1 µL	COT	General	24
Saliva	2 µL	COT	General	24
Saliva	4 µL	HFM	Specialized	29
Saliva	4 µL	COT	General	26
Saliva	8 µL	HFM	Specialized	6
Saliva	8 µL	COT	General	26
Saliva	12 µL	HFM	Specialized	4
Saliva	35 µL	HFM	Specialized	6
Saliva	Buccal	COT	General	4
gDNA—228 DNA Control 007 samples				
gDNA	1.32 ng	Direct load	Specialized	33
gDNA	2.64 ng	Direct load	Specialized	32
gDNA	3.96 ng	Direct load	Specialized	31
gDNA	5.28 ng	Direct load	Specialized	36
gDNA	6.6 ng	Direct load	Specialized	34

Table 10 Calibration data set: Sample types and input amounts *(continued)*

Sample type	Input amount	Collection method	Run protocol	Number of samples
gDNA	11 ng	Direct load	Specialized	31
gDNA	22 ng	Direct load	Specialized	31
gDNA—76 RapidINTEL™ Plus Positive Control Cartridge swabs				
gDNA	330 ng	COT	General	76
gDNA—31 DNA Control 007 and Control DNA 9947A samples				
gDNA, inhibited	30 ng	Direct load (inhibition) ^[1]	Specialized	31
gDNA—23 DNA Control 007 and Control DNA 9947A samples				
gDNA, degraded	30 ng	Direct load (degradation) ^[2]	Specialized	23
44 case-type samples				
Blood on denim	20 µL	HFM	Specialized	4
Blood on cloth	20 µL	HFM	Specialized	4
Blood on cloth	2.5 µL	HFM	Specialized	4
Blood on tile	20 µL	HFM	Specialized	4
Blood on tile	2.5 µL	HFM	Specialized	4
Coffee cup	Used	HFM	Specialized	5
Cigarette	Smoked	HFM	Specialized	4
Straw	Used	HFM	Specialized	4
Face mask	Worn	HFM	Specialized	3
Gum	Chewed	HFM	Specialized	2
Touch	Used	HFM	Specialized	2
Water bottle	Used	HFM	Specialized	2
Mobile phone	Used	HFM	Specialized	1
Sunglasses	Worn	HFM	Specialized	1

^[1] The sample was directly loaded into a sample cartridge and run under inhibition conditions with humic acid or hematin.

^[2] The sample was directly loaded into a sample cartridge and run after being subjected to degradation by heating at 95°C.

Validation and verification data set

Study	No. of runs	No. of instruments	Protocol	Sample	Additional information
Positive control	25	5	General ^[1]	Positive control cartridges	COT swabs are pre-loaded in the positive control cartridges.
Negative control	20	10	Specialized ^[1]	Negative control cartridges	Each run was performed after a 35-µL blood sample run that used the General protocol.
Population	216	10	General or Specialized	75 unique blood samples, male and female 25 unique saliva samples, male and female	Blood: 1 µL and 4 µL of on HFM swabs; 35 µL on COT swabs Saliva: 4 µL on HFM swabs; 35 µL on COT swabs Blood or saliva was loaded onto the swab, then dried before the swab was loaded into the cartridge lysis chamber.
Sensitivity	114	6	Specialized	Blood and saliva samples (male and female)	Blood: 0.1 µL, 0.2 µL, 1 µL, 15 µL, 25 µL, and 35 µL on HFM and COT swabs Saliva: 1 µL, 4 µL, and 35 µL on HFM swabs; 1 µL, 2 µL, 4 µL, and 8 µL on COT swabs Blood or saliva was loaded onto the swab, then dried before the swab was loaded into the cartridge lysis chamber.
Precision	30	6	General ^[1]	Allelic ladder cartridges	—
PCR inhibition with single-source DNA	15	3	Specialized	gDNA: Male (DNA Control 007), 30 ng	The gDNA was mixed with humic acid and directly loaded into the cartridge lysis chamber. Humic acid concentrations: 0, 50, 100, 200, and 300 ng/µL
PCR inhibition with DNA mixtures	9	3	Specialized	gDNA: Mixture of female (Control DNA 9947A) and male (DNA Control 007), 60 ng total	The gDNA was mixed with humic acid and directly loaded into the cartridge lysis chamber. DNA ratios 1:1, 1:2, 1:3 (female:male) Humic acid concentration: 295 ng/µL

(continued)

Study	No. of runs	No. of instruments	Protocol	Sample	Additional information
DNA degradation with single-source DNA	12	3	Specialized	gDNA: Male (DNA Control 007), 30 ng	The gDNA was heat-degraded at 95°C, then directly loaded into the cartridge lysis chamber. Time points: 0, 4, 8, 12, and 15 minutes at 95°C
DNA degradation with DNA mixtures	18	3	Specialized	gDNA: Mixture of female (Control DNA 9947A) and male (DNA Control 007), 30 ng total	The gDNA was heat-degraded at 95°C, then directly loaded into the cartridge lysis chamber. DNA ratios: 2:1 and 5:1 (female:male) Time points: 0, 12, and 15 minutes at 95°C
Mixture	104	2	General or Specialized	Mixtures of male and female blood samples	Female:male ratios: 1:1, 1:2, 1:3, 1:4, 1:9, 1:19, and 1:40 Male:female ratios: 1:1, 1:2, 1:3, 1:4, 1:9, 1:19, and 1:40
Species specificity	8	1	General or Specialized	Blood or buccal samples from different species	Blood: 35 µL on HFM swabs Buccal: COT swab The HFM and COT swabs were dried before being loaded into the cartridge lysis chamber.
Subsampling	39	5	Specialized	Blood and buccal samples (male and female)	Blood: 35 µL on COT swabs Buccal: COT swabs After the samples were collected with COT swabs, they were subsampled onto HFM swabs. The HFM swabs were dried before being loaded into the cartridge lysis chamber.
Case-type samples	193	10	General or Specialized	Blood, saliva, touch, semen, bone, fingernail, hair, tooth	Mock casework samples, various preparations See “Case-type samples study” on page 59 for all case-type sample preparations.

^[1] The protocol type was read from the cartridge RFID tag.

Positive control study

Cartridge and sample summary

RapidINTEL™ Plus positive control cartridges were used.

- The cartridges are preloaded with COT swabs.
- Each swab contains 330 ng total human male gDNA (DNA Control 007).

Run and analysis summary

There were 25 RapidINTEL™ Plus positive control cartridge runs performed on 5 RapidHIT™ ID instruments.

- The **General** (500-μL) protocol was used.
- All 25 runs passed with no quality flags (✓).
- The run results were assessed for intra color balance and intra locus balance. See Figure 7 and Figure 8.

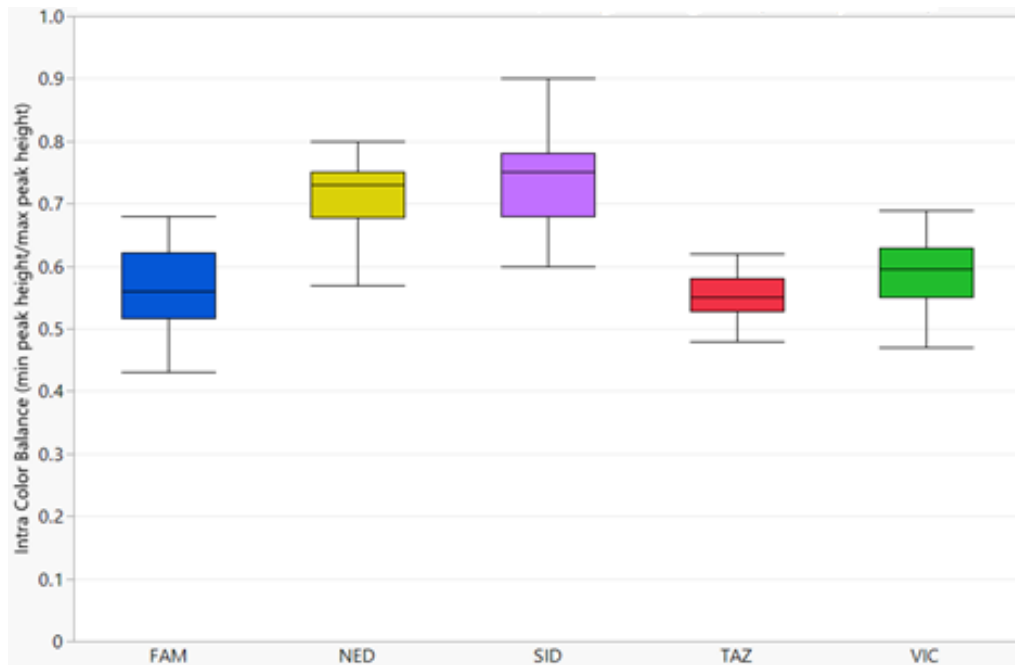


Figure 7 Results: Positive control study—intra color balance The intra color balance (ICB) is shown for each dye channel across 25 positive control runs. The ICB is calculated as the lowest average allele peak height divided by the highest average allele peak height in each dye channel, expressed as a percentage.

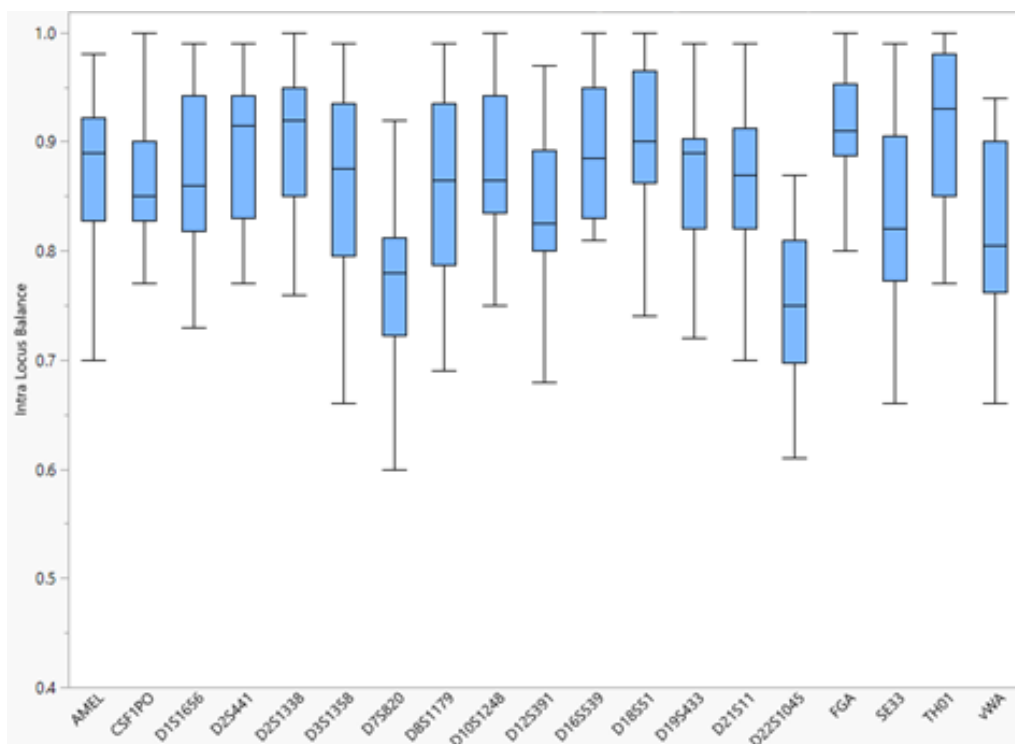


Figure 8 Results: Positive control study—intra locus balance The intra locus balance (ILB) is shown for all heterozygous markers across 25 positive control runs. The ILB is calculated as the lower allele peak height divided by the greater allele peak height in each heterozygote locus, expressed as a percentage.

Negative control study

Cartridge and sample summary

RapidINTEL™ Plus negative control cartridges were used.

Run and analysis summary

There were 20 RapidINTEL™ Plus negative control cartridge runs performed on 10 RapidHIT™ ID instruments.

- The **Specialized** (100-μL) protocol was used.
- Each negative control run was performed after a 35-μL blood sample run that used the **General** protocol.
- The run results were assessed for any reproducible peaks in the assay read region. None of the runs displayed reproducible peaks above the 35 RFU peak detection threshold within the assay read region. See Figure 9.

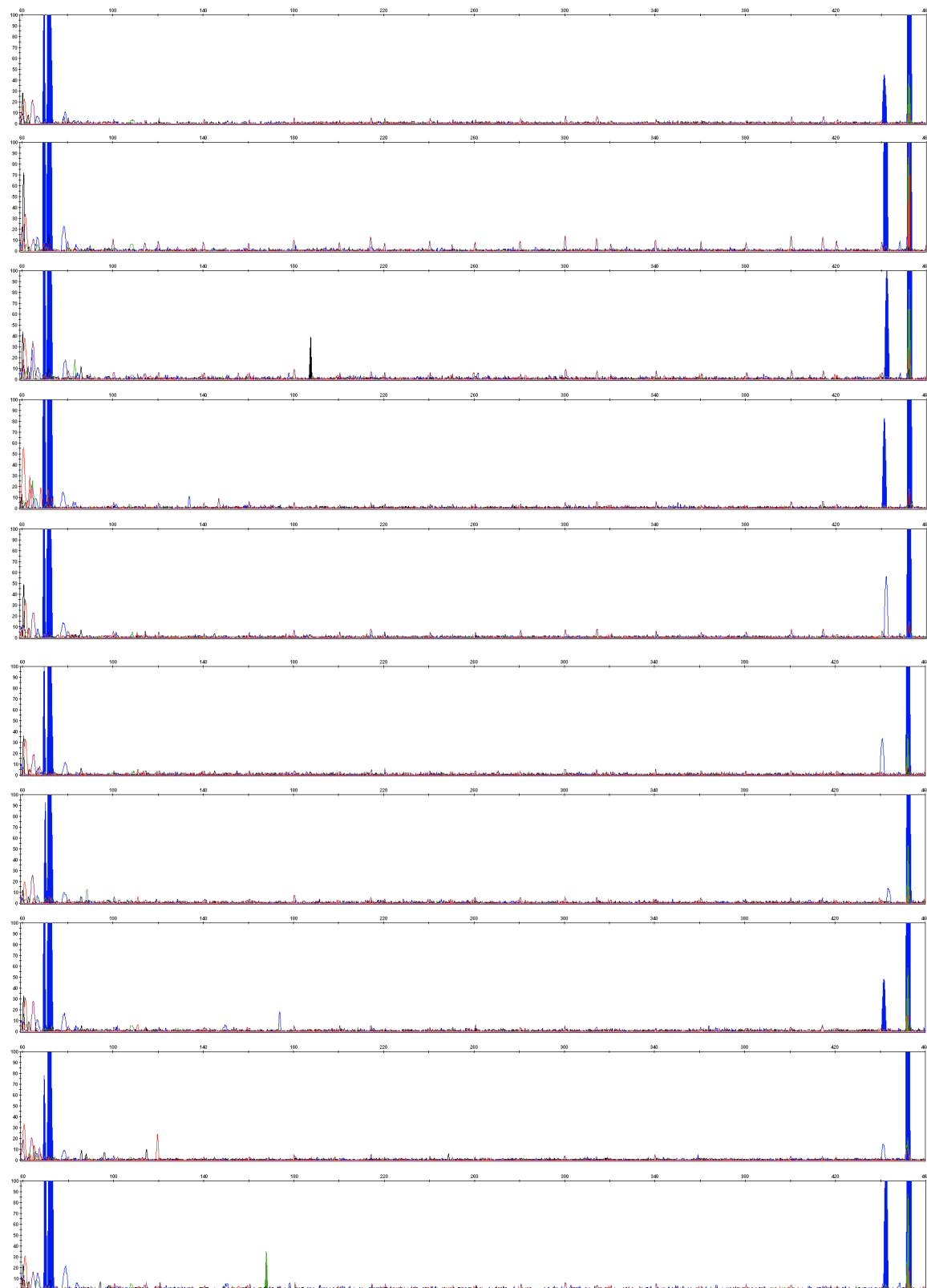


Figure 9 Results: Negative control study Electropherograms are shown for 10 of the negative control runs from 10 different instruments. The STR dyes are overlaid. The observed peaks in the FAM™ dye channel (blue) are expected products produced by the internal quality controls (IQCs).

Population study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- A total of 100 single-source blood and saliva samples from individuals of various ethnicities was used.
- Blood samples were deposited on HFM swabs at 1 µL and 4 µL or on COT swabs at 35 µL.
- Saliva samples were deposited on HFM swabs at 4 µL or on COT swabs at 35 µL.
- All swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.

Table 11 Population study—sample details

Sample type	No. of runs	Input amount	No. of females	No. of males	Ethnic breakdown
Whole blood	166	• 1 µL and 4 µL on HFM swabs • 35 µL on COT swabs	38	43	• 21 African American • 20 Asian • 20 Caucasian • 20 Hispanic
Saliva	50	• 4 µL on HFM swabs • 35 µL on COT swabs	12	24	Various, not tallied

Run and analysis summary

There were 216 RapidINTEL™ Plus sample cartridge runs performed on 10 RapidHIT™ ID instruments.

- For blood and saliva samples on HFM swabs, the **Specialized** (100-µL) protocol was used.
- For blood and saliva samples on COT swabs, the **General** (500-µL) protocol was used.
- The run results were assessed for first pass success rate, allele detection sensitivity, intra locus balance (ILB), genotype concordance, and allele sizing accuracy. See Figure 11 through Figure 14.

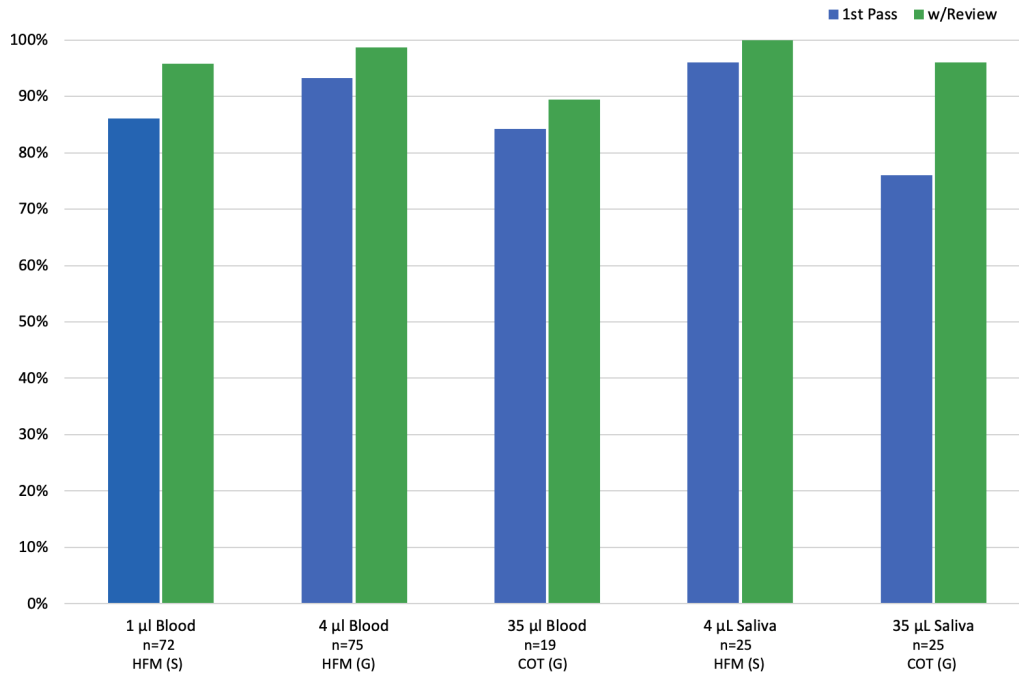


Figure 10 Results: Population study—first pass success rate The first pass success rate is shown for 216 population sample runs. The first pass success rate is broken down by sample type, input level, and swab type. The blue bars show the first pass success rate; the green bars show the pass rate with review. S=**Specialized** (100-µL) protocol. G=**General** (500-µL) protocol.

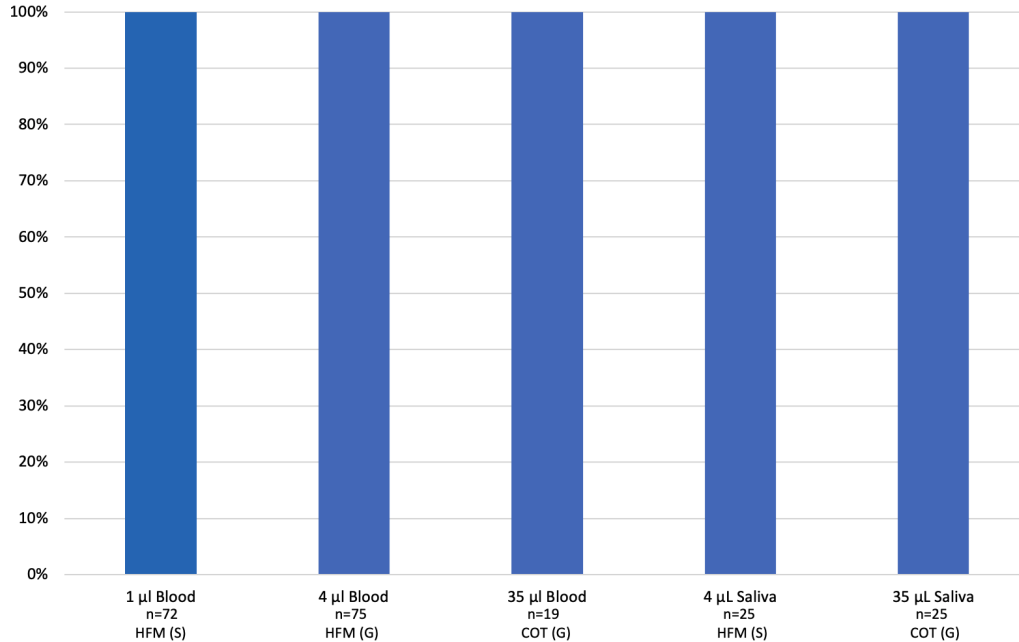


Figure 11 Results: Population study—allele detection sensitivity The allele detection sensitivity (% of a full profile) is shown for 216 population sample runs. The allele detection sensitivity is broken down by sample type, input level, and swab type. All samples gave full profiles for all input levels, swab types, and lysis protocols. S= **Specialized** (100-µL) protocol. G=**General** (500-µL) protocol.

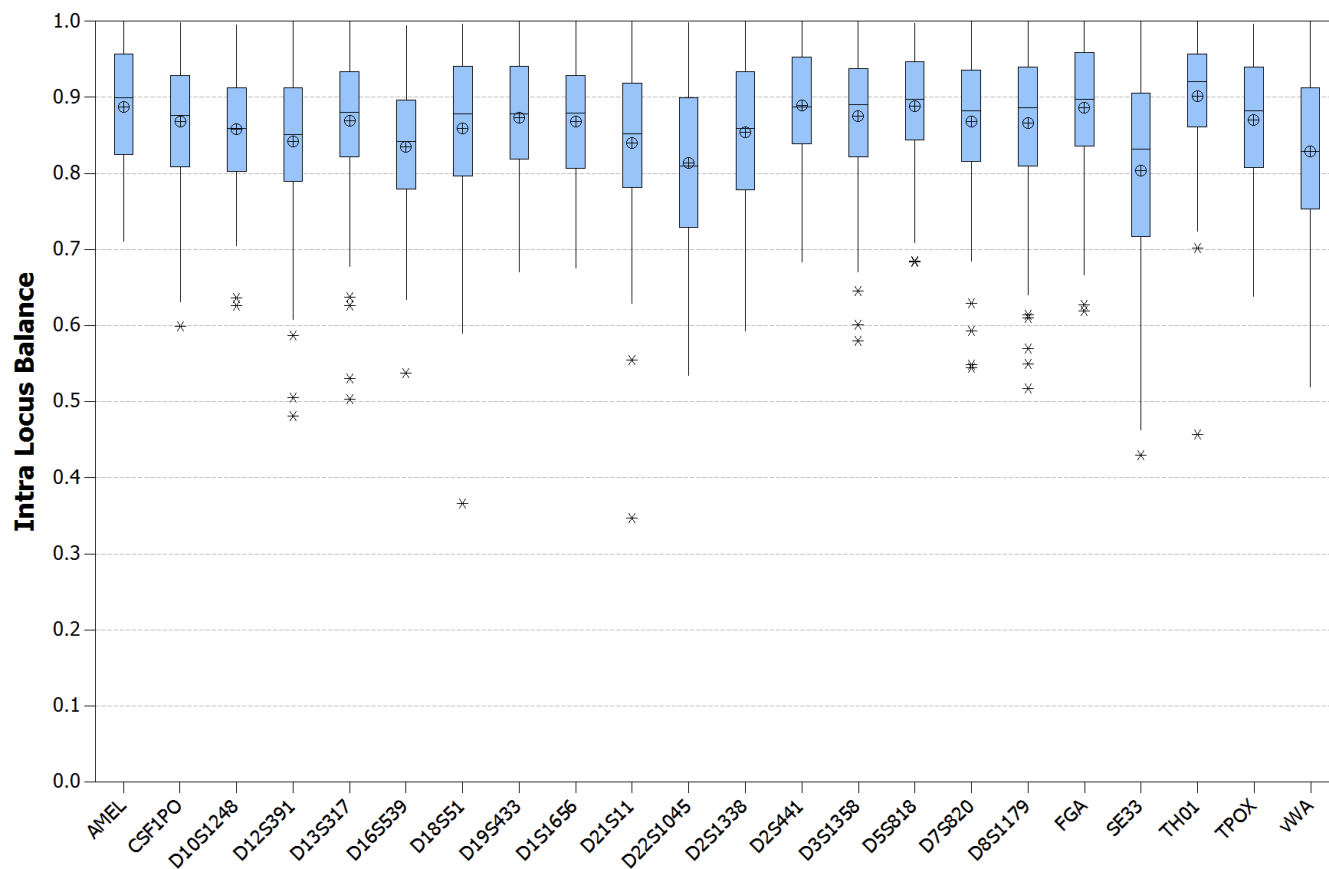


Figure 12 Results: Population study—intra locus balance The intra locus balance (ILB) is shown for 216 population sample runs.

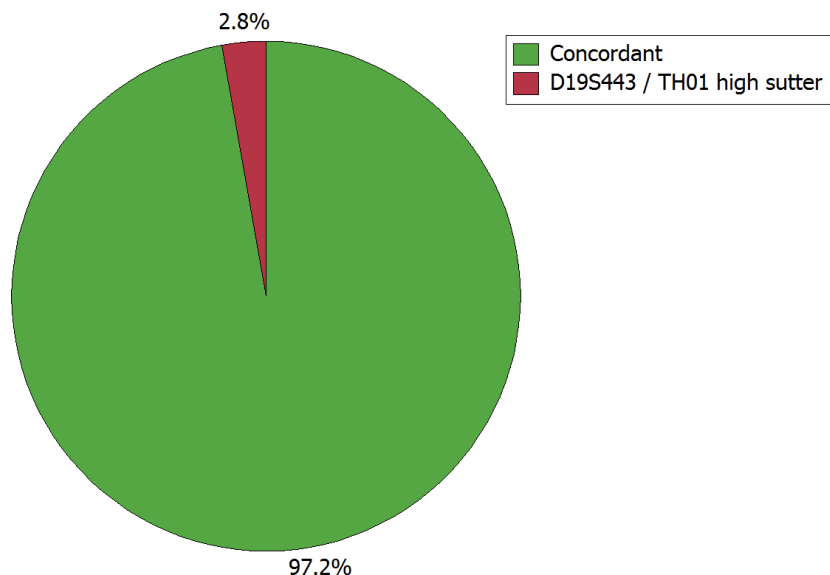


Figure 13 Results: Population study—genotype concordance Concordance is shown for 216 population sample runs. 210 out of 216 runs (97.2%) gave completely concordant genotypes. For individual allele calls, 4,972 out of 4,978 alleles (99.87%) were concordant. Known artifacts, flagged stutter, low-level single spurious peaks, and flagged OL (off-ladder) were removed during analysis of the data set. Non-concordant allele calls were the result of 6 high-stutter alleles in the D19S443 and TH01 marker regions that were called in runs that had >2 ng of sample input.

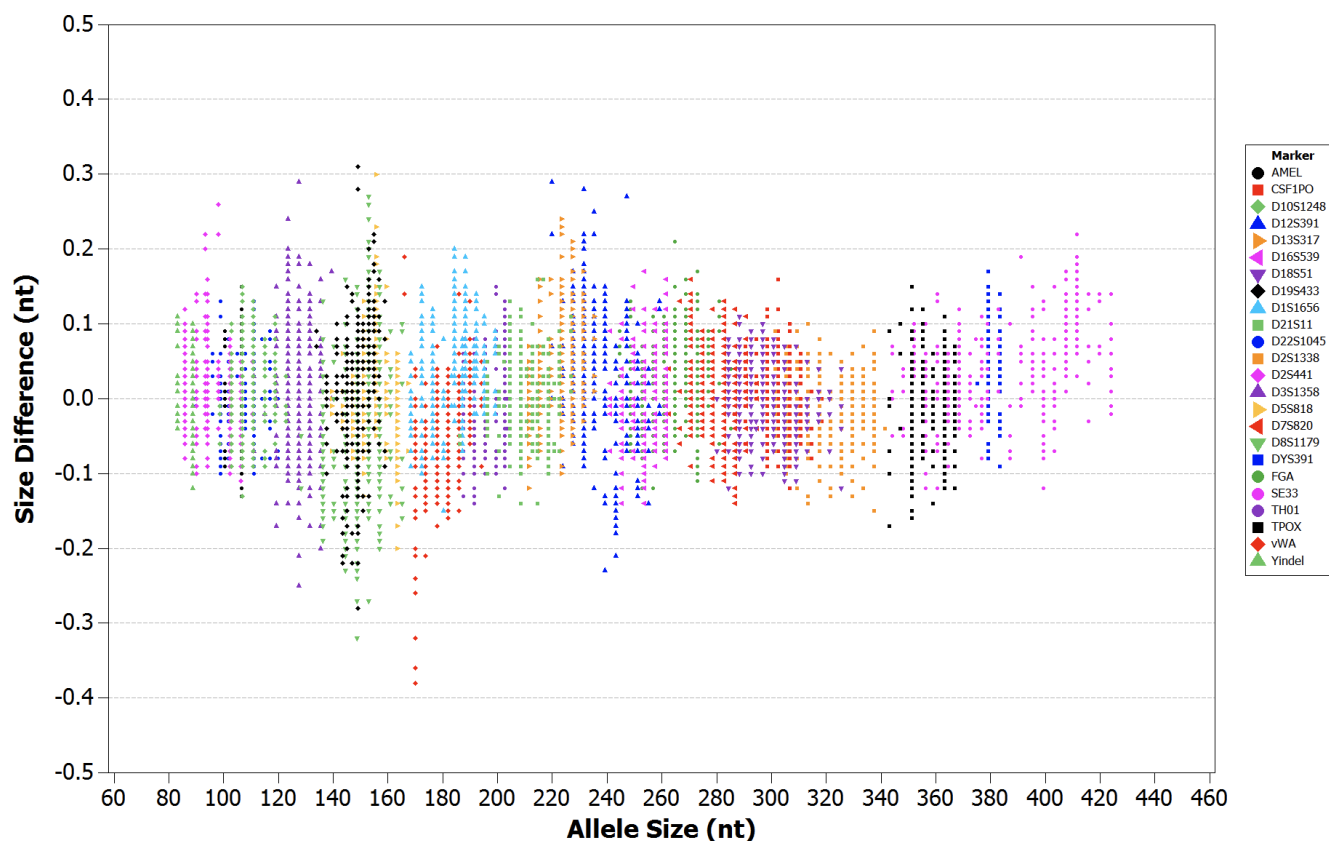


Figure 14 Results: Population study—allele sizing accuracy The allele sizing accuracy is shown for 216 population sample runs. Sizing differences between population sample alleles and corresponding allelic ladder alleles are plotted against allele size. The vast majority of the data (4,973 out of 4,978 allele calls) fell within ± 0.3 bp difference between the sample allele and allelic ladder allele, demonstrating that sample alleles can be accurately sized and genotyped.

Sensitivity study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Blood samples were provided by 6 unique human donors (male and female).
- Saliva samples were provided by 6 unique human donors (male and female).
- Blood samples were deposited on HFM swabs and COT swabs at varying input amounts.
- Saliva samples were deposited on HFM swabs and COT swabs at varying input amounts.
- All swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.

Table 12 Sensitivity study—sample details

Swab type	No. of runs	Input amount	Protocol
Blood (6 unique donors)			
HFM swabs	36	0.1 µL, 0.2 µL, 1 µL, 15 µL, 25 µL, and 35 µL	Specialized (100-µL)
COT swabs	36	0.1 µL, 0.2 µL, 1 µL, 15 µL, 25 µL, and 35 µL	General (500-µL)
Saliva (6 unique donors)			
HFM swabs	18	1 µL, 4 µL, and 35 µL	Specialized (100-µL)
COT swabs	24	1 µL, 2 µL, 4 µL, and 8 µL	General (500-µL)

Run and analysis summary

There were 114 RapidINTEL™ Plus sample cartridge runs performed on 6 RapidHIT™ ID instruments.

- For the 36 blood samples on HFM swabs, the **Specialized** (100-µL) protocol was used.
- For the 36 blood samples on COT swabs, the **General** (500-µL) protocol was used.
- For the 18 saliva samples on HFM swabs, the **Specialized** (100-µL) protocol was used.
- For the 24 saliva samples on COT swabs, the **General** (500-µL) protocol was used.
- The run results were assessed for the percent of alleles recovered. See Figure 15 through Figure 17.

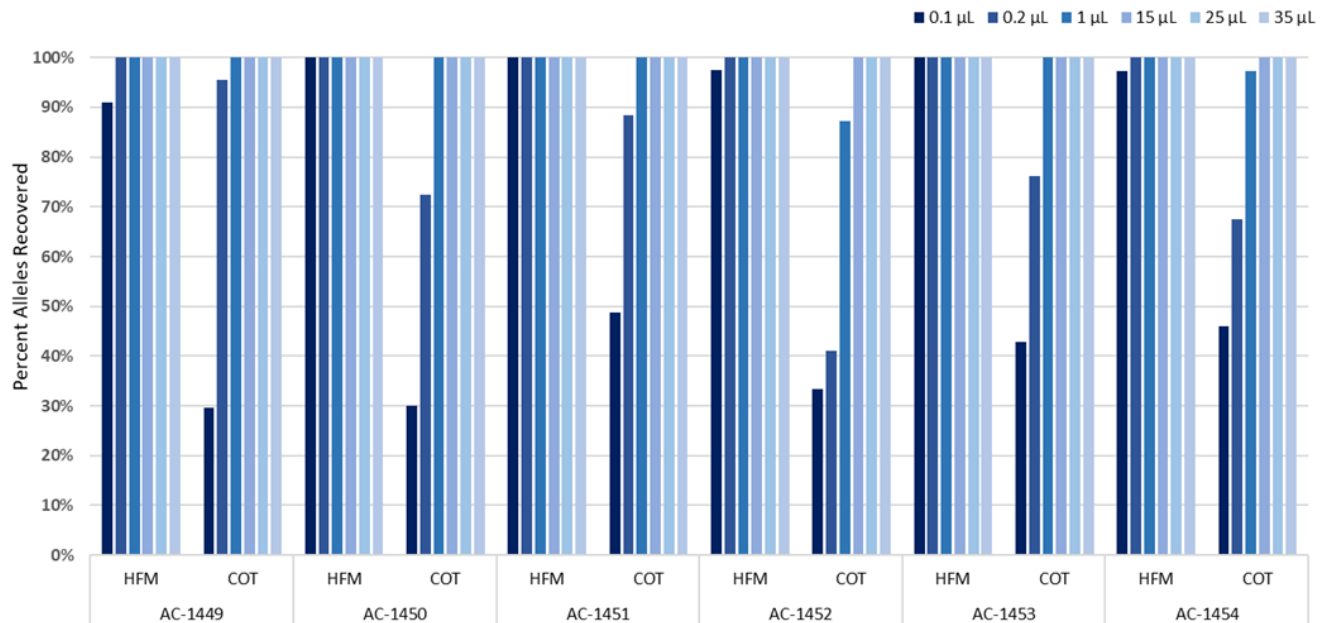


Figure 15 Results: Sensitivity study—blood samples, HFM vs. COT swabs The percent of alleles recovered on HFM vs. COT swabs is shown for 6 blood donors and 6 input amounts.

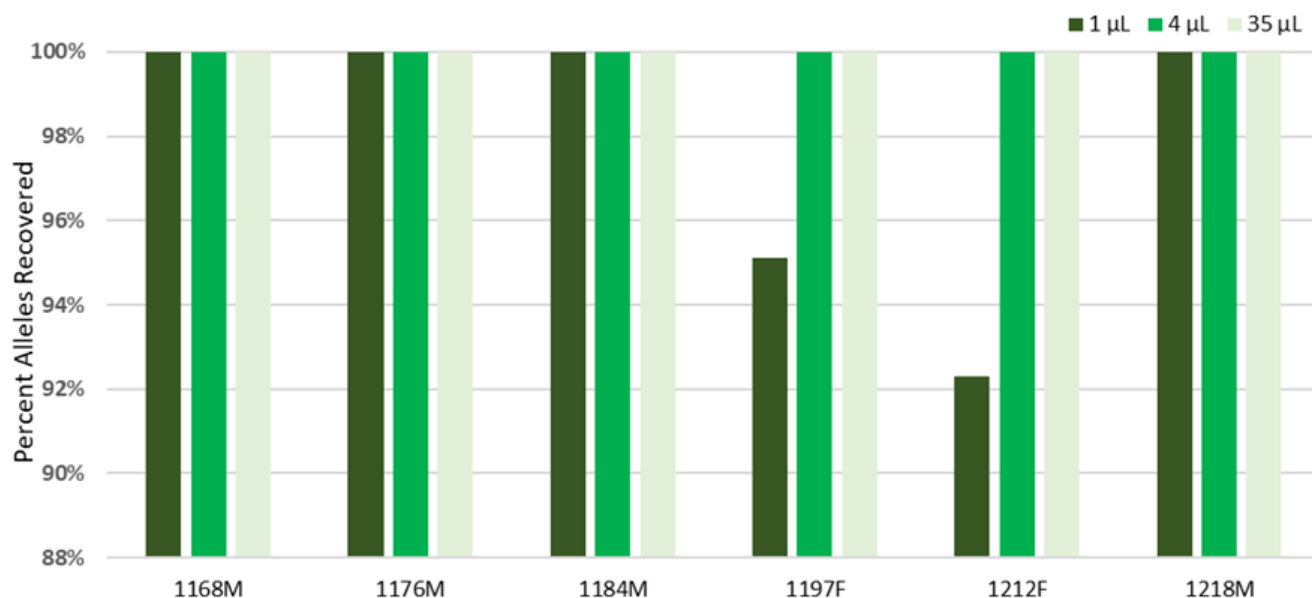


Figure 16 Results: Sensitivity study—saliva samples, HFM swabs The percent of alleles recovered on HFM swabs is shown for 6 saliva donors and 3 input amounts.

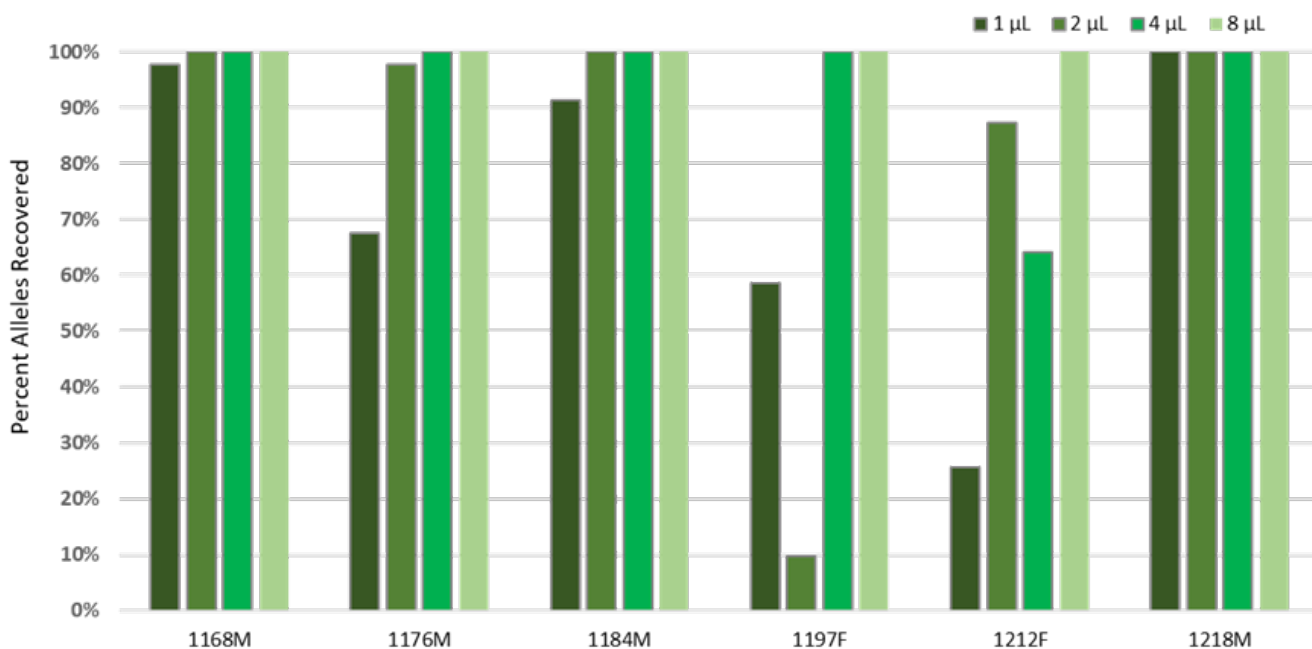


Figure 17 Results: Sensitivity study—saliva samples, COT swabs The percent of alleles recovered on COT swabs is shown for 6 saliva donors and 4 input amounts.

Precision study

Cartridge and sample summary

RapidINTEL™ Plus allelic ladder cartridges were used. Reliable genotyping of DNA evidence samples requires allele peaks to be sized with both accuracy and precision.

Run and analysis summary

There were 30 RapidINTEL™ Plus allelic ladder cartridge runs performed on 6 RapidHIT™ ID instruments.

- All 30 runs passed with no quality flags (✅), which indicates that all allele peaks were correctly assigned to the appropriate bins.
- The run results were assessed for sizing precision. All standard deviation (SD) results were <0.08 bp, which confirms the ability of the assay to achieve 1 bp resolution across the assay read range and demonstrates that STR alleles can be accurately genotyped. See Figure 18.

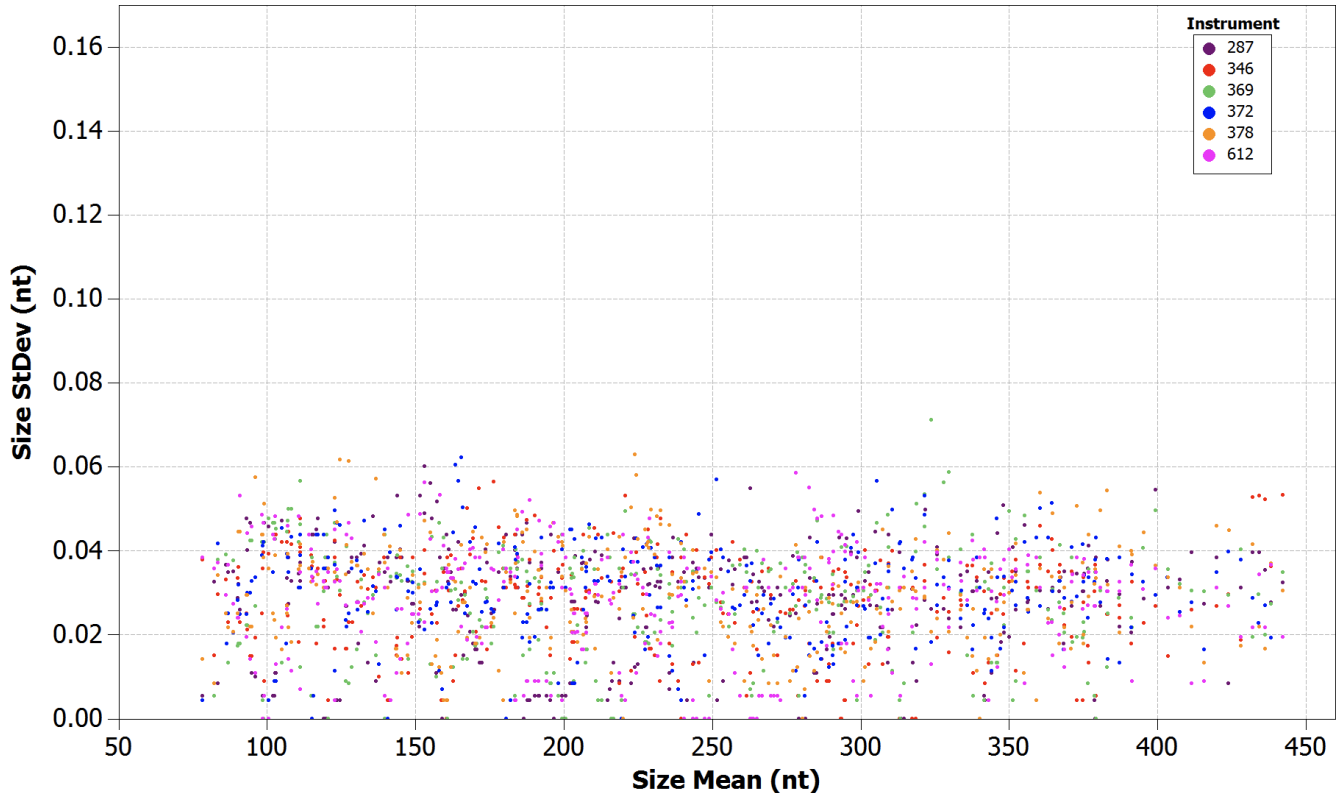


Figure 18 Results: Precision study—sizing precision The sizing precision (standard deviation) is shown for alleles across 30 repeated RapidINTEL™ Plus allelic ladder cartridge runs on 6 RapidHIT™ ID instruments.

PCR inhibition and DNA degradation studies

A series of experiments was performed to simulate the kind of challenging sample types that might be encountered in forensic casework DNA analysis:

- **PCR inhibition**—Humic acid, a chemical compound often found in soil-containing samples, served as a model system to simulate PCR inhibition.
- **DNA degradation**—DNA degradation was modeled using purified human gDNA that was exposed to high temperatures (95°C) for varying times in vitro.

PCR inhibition and DNA degradation treatments were used with single-source DNA samples and 2-individual DNA mixtures. These experiments allowed the robustness of the PCR chemistry to be tested, as well as the ability of RapidHIT™ ID System v2.0 to generate INH, DEG, and MIX quality flags (▲) in response to a range of challenging sample inputs.

PCR inhibition study with single-source DNA

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Samples were prepared with human male gDNA (DNA Control 007) and a series of increasing concentrations of humic acid PCR inhibitor:
 - Total DNA amount per run: 30 ng of DNA Control 007
 - Humic acid series: 0, 50, 100, 200, and 300 ng/μL final concentration in the PCR reaction
- Each sample was directly loaded into the RapidINTEL™ Plus sample cartridge lysis chamber (no swabs were used).

Run and analysis summary

There were 15 RapidINTEL™ Plus sample cartridge runs performed on 3 RapidHIT™ ID instruments.

- For each inhibited sample, 3 replicate runs were performed.
- The **Specialized** (100-μL) protocol was used.
- The run results were assessed for QQS 2:QQL 2 peak height ratio, number of alleles detected, and whether the INH quality flag (▲) was triggered. The trigger threshold was a QQS 2:QQL 2 peak height ratio >5. See Table 13 and Figure 19.

Table 13 Results: PCR inhibition study—humic acid series

Sample no.	Humic acid concentration	QQS 2/QQL 2	INH flag (>5)	No. of alleles detected
1	0 ng/μL	1.1	No	43 ^[1]
2	0 ng/μL	1.1	No	43 ^[1]
3	0 ng/μL	1.1	No	43 ^[1]
4	50 ng/μL	3.0	No	43 ^[1]
5	50 ng/μL	1.6	No	43 ^[1]
6	50 ng/μL	1.4	No	43 ^[1]

Table 13 Results: PCR inhibition study—humic acid series *(continued)*

Sample no.	Humic acid concentration	QQS 2/QLL 2	INH flag (>5)	No. of alleles detected
7	100 ng/μL	2.0	No	43 ^[1]
8	100 ng/μL	4.9	No	43 ^[1]
9	100 ng/μL	5.4	Yes	43 ^[1]
10	200 ng/μL	3.2	No	43 ^[1]
11	200 ng/μL	7.5	Yes	39
12	200 ng/μL	Undefined	Yes	14
13	300 ng/μL	7.5	Yes	2
14	300 ng/μL	Undefined	Yes	9
15	300 ng/μL	Undefined	Yes	3

^[1] 43 alleles is a full profile.

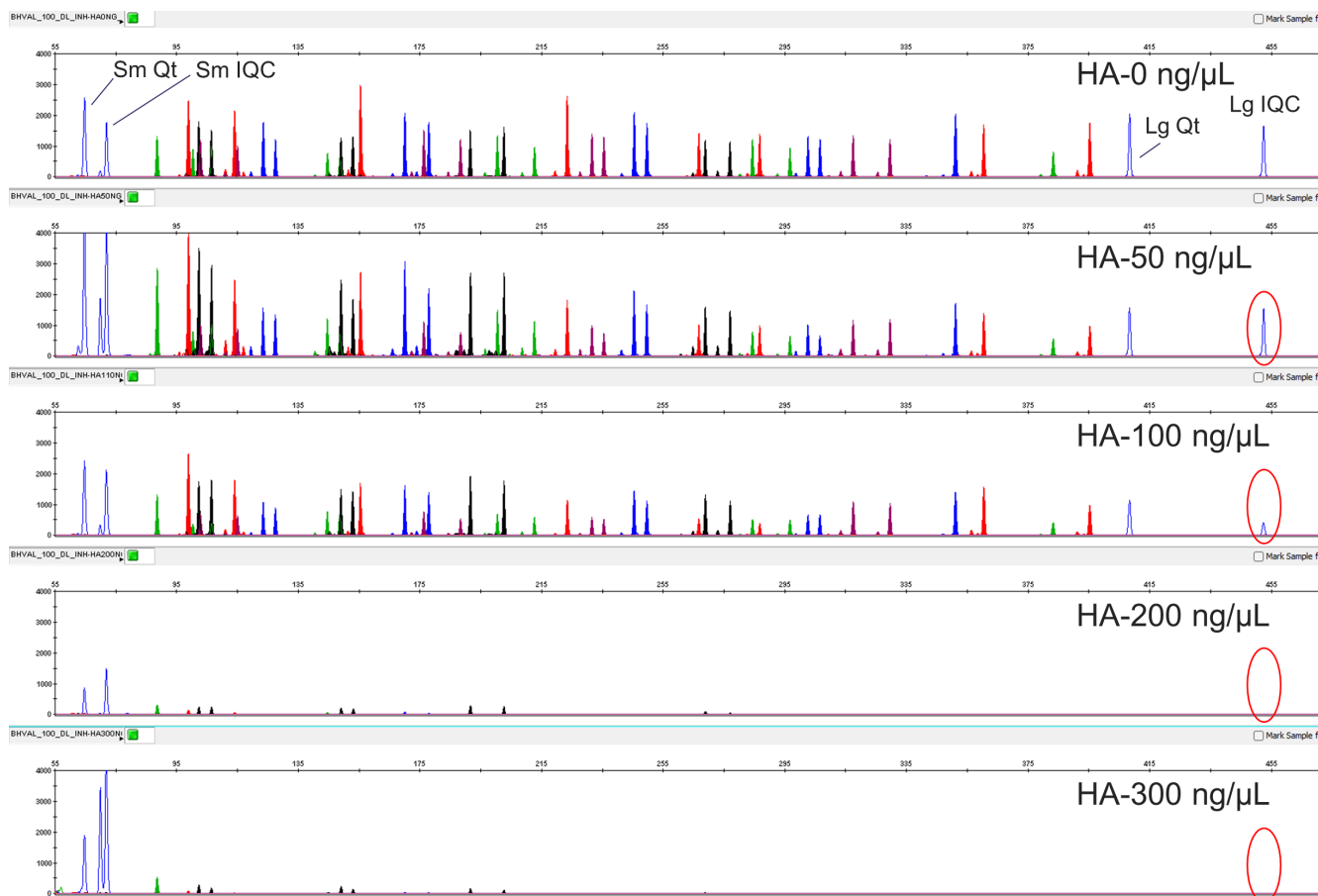


Figure 19 Results: PCR inhibition study—humic acid series Capillary electrophoresis data are shown for a series of samples that contained a fixed input (30 ng) of DNA Control 007 with increasing concentrations of humic acid (HA) PCR inhibitor (0, 50, 100, 200, and 300 ng/μL). The Large IQC peak (circled in red) gradually disappeared over the course of the series, until the Large IQC:Small IQC peak height ratio was >5.0 and triggered the INH quality flag for fractions with ≥100 ng/μL humic acid concentrations.

PCR inhibition study with DNA mixtures

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Mixture samples of purified human female gDNA (Control DNA 9947A) and human male gDNA (DNA Control 007) were prepared at 3 different mixture ratios:
 - Total DNA amount per run: 60 ng total of each DNA mixture (that is, female + male = 60 ng total)
 - Major contributor: female
 - Mixture ratios: 1:1, 1:2, and 1:3 (female:male)
- A constant level of humic acid at 295 ng/μL was added to the cartridge PCR chamber.
- Each mixture sample was directly loaded into the RapidINTEL™ Plus sample cartridge lysis chamber (no swabs were used).

Run and analysis summary

There were 9 RapidINTEL™ Plus sample cartridge runs performed on 3 RapidHIT™ ID instruments.

- For each inhibited sample mixture, 3 replicate runs were performed.
- The **Specialized** (100-µL) protocol was used.
- The run results were assessed for major and minor contributor STR alleles, and whether MIX or INH quality flags (▲) were triggered. See Table 14.

Table 14 Results: PCR inhibition study—humic acid series with 2-person mixture samples

Sample number	Humic acid concentration	Mixture ratio (female:male)	MIX flag	INH flag	No. of drop-ins (minor contributor)	No. of alleles (major and minor contributors)
1	295 ng/µL	1:1	Yes	Yes	24	61
2	295 ng/µL	1:1	Yes	Yes	19	55
3	295 ng/µL	1:1	Yes	Yes	12	47
4	295 ng/µL	1:2	Yes	Yes	9	37
5	295 ng/µL	1:2	No ^[1]	Yes	1	17
6	295 ng/µL	1:2	No ^[1]	Yes	3	11
7	295 ng/µL	1:3	Yes	Yes	9	44
8	295 ng/µL	1:3	Yes	Yes	7	42
9	295 ng/µL	1:3	Yes	Yes	3	13

^[1] The minor alleles detected were male markers.

DNA degradation study with single-source DNA

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Samples were prepared with human male gDNA (DNA Control 007) that was heat-treated in a thermal block for various times to create a series of samples with differing degrees of DNA degradation:
 - Total DNA amount per run: 30 ng of DNA Control 007, relative to the undegraded control sample (that is, the same amount of DNA was loaded for all degraded fractions, which was equivalent to 30 ng of the undegraded control sample)
 - Heat-treated series: 0, 4, 8, 12, and 15 minutes at 95°C, with 0 minutes being the undegraded control and 15 minutes being a severely degraded fraction
- Each sample was directly loaded into the RapidINTEL™ Plus sample cartridge lysis chamber (no swabs were used).

Run and analysis summary

There were 12 RapidINTEL™ Plus sample cartridge runs performed on 3 RapidHIT™ ID instruments.

- For each degraded DNA level, 3 replicate runs were performed.
- The **Specialized** (100-µL) protocol was used.
- The run results were assessed for QQS 1:QQL 1 peak height ratio, number of alleles detected, and whether the DEG quality flag (▲) was triggered. The trigger threshold is a QQS 1:QQL 1 peak height ratio >5. See Table 15 and Figure 20.

Table 15 Results: DNA degradation study—heat-treated series

Sample no.	Degradation severity (time at 95°C)	QQS 1/QQL 1	DEG flag (>5)	No. of alleles detected
1	0 minutes	0.9	No	43 ^[1]
2	0 minutes	1.2	No	43 ^[1]
3	0 minutes	2.0	No	43 ^[1]
4	4 minutes	2.1	No	43 ^[1]
5	4 minutes	3.0	No	43 ^[1]
6	4 minutes	4.3	No	43 ^[1]
7	8 minutes	5.2	Yes	43 ^[1]
8	8 minutes	8.4	Yes	43 ^[1]
9	8 minutes	8.5	Yes	43 ^[1]
10	12 minutes	26.3	Yes	43 ^[1]
11	12 minutes	46.2	Yes	42
12	12 minutes	Undefined	Yes	39
13	15 minutes	Undefined	Yes	35
14	15 minutes	Undefined	Yes	34
15	15 minutes	Undefined	Yes	31

^[1] 43 alleles is a full profile.

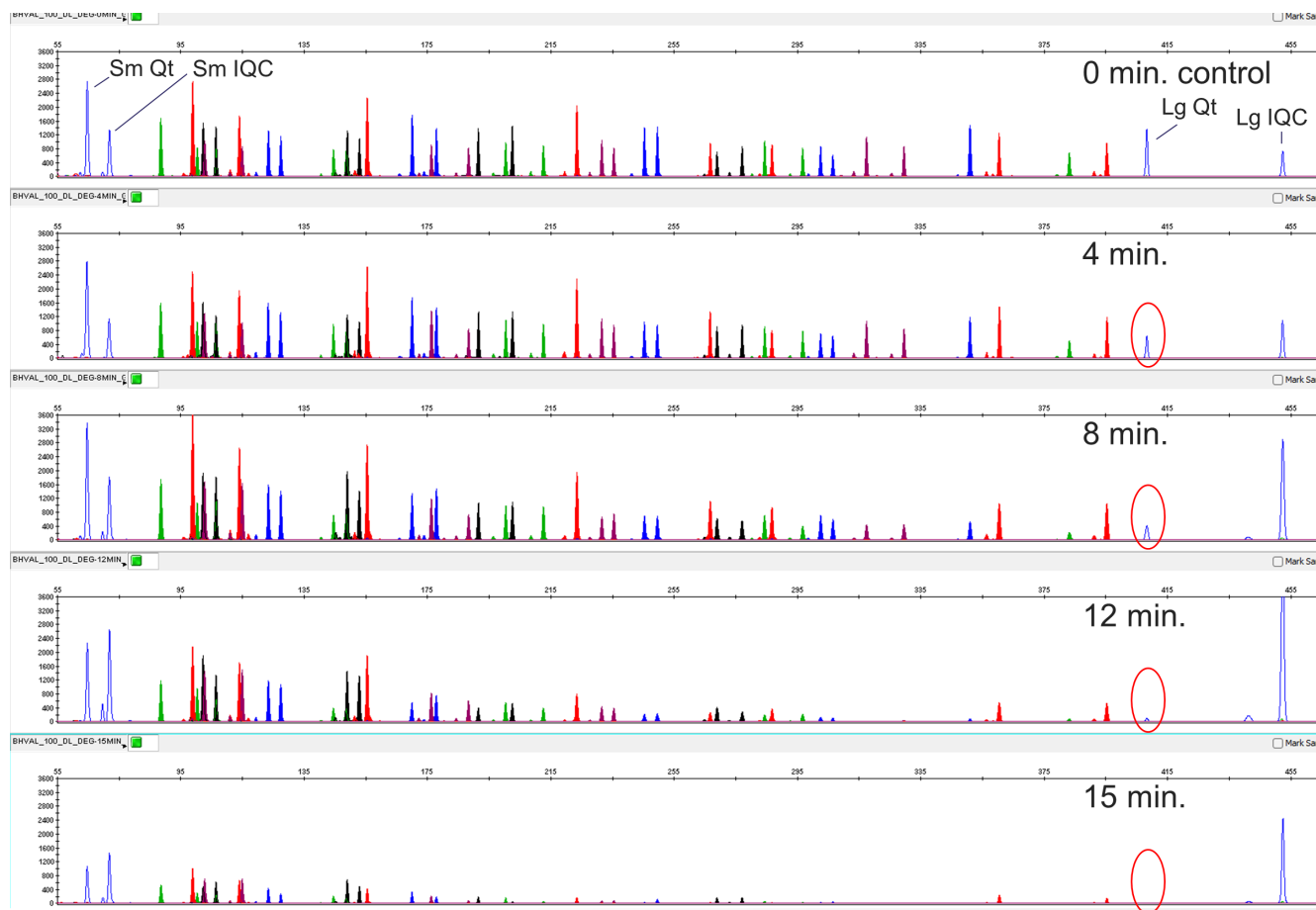


Figure 20 Results: DNA degradation study—heat-treated series Capillary electrophoresis data are shown for DNA Control 007 fractions from the degraded DNA series. Heat-treated DNA fractions (0, 4, 8, 12, and 15 minutes at 95°C) showed a range of degradation from the undegraded control (0 minutes) to the severely degraded 15-minute fraction. The Large Quant peak (circled in red) gradually disappears over the course of the series, until the Large Quant:Small Quant peak height ratio was > 5.0 and triggered the DEG flag for fractions heated ≥ 8 minutes.

DNA degradation study with DNA mixtures

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Mixture samples of purified human female gDNA (Control DNA 9947A) and human male gDNA (DNA Control 007) were prepared at 2 different mixture ratios, then heat-treated in a thermal block for various times to create a series of mixture samples with differing degrees of DNA degradation:
 - Total DNA amount per run: 30 ng total of each DNA mixture (that is, female + male = 30 ng total)
 - Major contributor: female
 - Mixture ratios: 2:1 and 5:1 (female:male)
 - Heat-treated series: 0, 12, and 15 minutes at 95°C, with 0 minutes being the undegraded control and 15 minutes being a severely degraded fraction
- Each sample was directly loaded into the RapidINTEL™ Plus sample cartridge lysis chamber (no swabs were used).

Run and analysis summary

There were 18 RapidINTEL™ Plus sample cartridge runs performed on 3 RapidHIT™ ID instruments.

- For each degraded DNA level, 3 replicate runs were performed.
- The **Specialized** (100-µL) protocol was used.
- The run results were assessed for major and minor contributor STR alleles, and whether the MIX or DEG quality flags (▲) were triggered. See Table 16.

Table 16 Results: DNA degradation study—heat-treated series with 2-person mixture samples

Sample number	Mixture ratio (female:male)	Degradation severity (time at 95°C)	MIX flag	DEG flag	No. of drop-ins (minor contributor)	No. of alleles (major and minor contributors)
1	2:1	0 minutes	Yes	No	26	63
2	2:1	0 minutes	Yes	No	26	63
3	2:1	0 minutes	Yes	No	26	63
4	2:1	12 minutes	Yes	Yes	17	43
5	2:1	12 minutes	Yes	Yes	13	34
6	2:1	12 minutes	Yes	Yes	9	32
7	2:1	15 minutes	Yes	Yes	9	26
8	2:1	15 minutes	Yes	Yes	5	23
9	2:1	15 minutes	Yes	Yes	5	22
10	5:1	0 minutes	Yes	No	24	61
11	5:1	0 minutes	Yes	No	23	60

Table 16 Results: DNA degradation study—heat-treated series with 2-person mixture samples (*continued*)

Sample number	Mixture ratio (female:male)	Degradation severity (time at 95°C)	MIX flag	DEG flag	No. of drop-ins (minor contributor)	No. of alleles (major and minor contributors)
12	5:1	0 minutes	Yes	No	22	59
13	5:1	12 minutes	Yes	Yes	11	36
14	5:1	12 minutes	Yes	Yes	6	30
15	5:1	12 minutes	Yes	Yes	5	29
16	5:1	15 minutes	Yes	Yes	4	21
17	5:1	15 minutes	No ^[1]	Yes	1	19
18	5:1	15 minutes	No ^[1]	Yes	1	13

^[1] The limit of detection for highly degraded samples is at 5:1.

Mixture study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Mixture samples of human male blood and human female blood were prepared twice—once with the female donor as the major contributor (female:male), and once with the male donor as the major contributor (male:female). Ratios for each mixture were as follows:
 - Female:male ratios**—1:1, 1:2, 1:3, 1:4, 1:9, 1:19, and 1:40
 - Male:female ratios**—1:1, 1:2, 1:3, 1:4, 1:9, 1:19, and 1:40
- Samples were deposited on HFM swabs or COT swabs at 5 µL per swab.

Run and analysis summary

There were 104 RapidINTEL™ Plus sample cartridge runs performed on 2 RapidHIT™ ID instruments.

- For the HFM swabs, the **Specialized** (100-µL) protocol was used.
- For the COT swabs, the **General** (500-µL) protocol was used.
- The run results were assessed for major and minor contributor STR alleles, and whether the MIX or ACC quality flags (▲) were triggered. See Table 17.

Table 17 Results: Mixture study—blood mixture analysis

Ratio	Swab type	MIX flag	ACC flag	Major contributor	No. of unique minor contributor alleles detected	Percent of minor contributor alleles detected	No. of alleles (major and minor contributors)
1:1	HFM	Yes	No	Female	29	100.0	65
1:1	HFM	Yes	No	Female	29	100.0	65
1:1	HFM	Yes	No	Female	31	100.0	71
1:1	HFM	Yes	No	Female	31	100.0	71
1:1	COT	Yes	No	Female	29	100.0	65
1:1	COT	Yes	No	Female	29	100.0	65
1:1	COT	Yes	No	Female	29	100.0	65
1:1	COT	Yes	No	Female	31	100.0	71
1:2	HFM	Yes	No	Female	29	100.0	65
1:2	HFM	Yes	No	Female	29	100.0	65
1:2	HFM	Yes	No	Male	23	100.0	65
1:2	HFM	Yes	No	Male	23	100.0	65
1:2	HFM	Yes	No	Female	31	100.0	71
1:2	HFM	Yes	No	Female	31	100.0	71
1:2	HFM	Yes	No	Male	27	100.0	71
1:2	HFM	Yes	No	Male	27	100.0	71
1:2	COT	Yes	No	Female	29	100.0	65
1:2	COT	Yes	No	Female	29	100.0	65
1:2	COT	Yes	No	Female	29	100.0	65
1:2	COT	Yes	No	Male	23	100.0	65
1:2	COT	Yes	No	Male	23	100.0	65
1:2	COT	Yes	No	Male	23	100.0	65
1:2	COT	Yes	No	Female	31	100.0	71
1:2	COT	Yes	No	Male	27	100.0	71
1:3	HFM	Yes	No	Male	18	78.3	60
1:3	HFM	Yes	No	Male	21	91.3	63

Table 17 Results: Mixture study—blood mixture analysis (continued)

Ratio	Swab type	MIX flag	ACC flag	Major contributor	No. of unique minor contributor alleles detected	Percent of minor contributor alleles detected	No. of alleles (major and minor contributors)
1:3	HFM	Yes	No	Female	29	100.0	65
1:3	HFM	Yes	No	Female	29	100.0	65
1:3	HFM	Yes	No	Female	29	93.5	69
1:3	HFM	Yes	No	Female	30	96.8	70
1:3	HFM	Yes	No	Male	27	100.0	71
1:3	HFM	Yes	No	Male	27	100.0	71
1:3	COT	Yes	No	Male	21	91.3	63
1:3	COT	Yes	No	Male	21	91.3	63
1:3	COT	Yes	No	Male	21	91.3	63
1:3	COT	Yes	No	Female	28	96.6	64
1:3	COT	Yes	Yes	Female	28	96.6	64
1:3	COT	Yes	No	Female	29	100.0	65
1:3	COT	Yes	No	Male	24	88.9	68
1:3	COT	Yes	No	Female	29	93.5	69
1:4	HFM	Yes	No	Female	25	86.2	61
1:4	HFM	Yes	No	Male	20	87.0	62
1:4	HFM	Yes	No	Male	21	91.3	63
1:4	HFM	Yes	No	Female	28	96.6	64
1:4	HFM	Yes	No	Female	27	87.1	67
1:4	HFM	Yes	No	Female	28	90.3	68
1:4	HFM	Yes	No	Male	27	100.0	71
1:4	HFM	Yes	No	Male	27	100.0	71
1:4	COT	Yes	Yes	Female	24	82.8	60
1:4	COT	Yes	No	Female	24	82.8	60
1:4	COT	Yes	No	Male	19	82.6	61
1:4	COT	Yes	No	Female	25	86.2	62

Table 17 Results: Mixture study—blood mixture analysis (continued)

Ratio	Swab type	MIX flag	ACC flag	Major contributor	No. of unique minor contributor alleles detected	Percent of minor contributor alleles detected	No. of alleles (major and minor contributors)
1:4	COT	Yes	No	Male	20	87.0	62
1:4	COT	Yes	No	Male	20	87.0	62
1:4	COT	Yes	Yes	Female	26	83.9	66
1:4	COT	Yes	No	Male	25	92.6	69
1:9	HFM	No	Yes	Female	3	10.3	39
1:9	HFM	Yes	Yes	Female	4	13.8	40
1:9	HFM	Yes	Yes	Female	9	29.0	49
1:9	HFM	Yes	Yes	Female	10	32.3	50
1:9	HFM	Yes	No	Male	12	44.4	56
1:9	HFM	Yes	No	Male	12	44.4	56
1:9	HFM	Yes	No	Male	14	60.9	56
1:9	HFM	Yes	No	Male	14	60.9	56
1:9	COT	Yes	Yes	Female	5	17.2	41
1:9	COT	Yes	Yes	Female	5	17.2	41
1:9	COT	Yes	Yes	Female	7	24.1	43
1:9	COT	Yes	Yes	Female	8	25.8	48
1:9	COT	Yes	No	Male	11	47.8	53
1:9	COT	Yes	No	Male	12	52.2	54
1:9	COT	Yes	No	Male	13	56.5	55
1:9	COT	Yes	No	Male	16	59.3	60
1:19	HFM	No	Yes	Female	2 ^[1]	6.9	38
1:19	HFM	No	Yes	Female	2 ^[1]	6.9	38
1:19	HFM	No	Yes	Female	2 ^[1]	6.5	42
1:19	HFM	No	Yes	Female	3	9.7	43
1:19	HFM	Yes	No	Male	2	8.7	44
1:19	HFM	Yes	No	Male	3	13.0	45

Table 17 Results: Mixture study—blood mixture analysis (continued)

Ratio	Swab type	MIX flag	ACC flag	Major contributor	No. of unique minor contributor alleles detected	Percent of minor contributor alleles detected	No. of alleles (major and minor contributors)
1:19	HFM	Yes	No	Male	3	11.1	47
1:19	HFM	Yes	No	Male	3	11.1	49
1:19	COT	No	No	Female	0	0.0	36
1:19	COT	No	Yes	Female	1 ^[1]	3.4	37
1:19	COT	No	Yes	Female	1	3.4	37
1:19	COT	Yes	Yes	Female	3	9.7	43
1:19	COT	Yes	No	Male	3	13.0	45
1:19	COT	Yes	No	Male	3	13.0	45
1:19	COT	Yes	No	Male	4	17.4	46
1:19	COT	Yes	No	Male	4	14.8	48
1:40	HFM	No	No	Female	0	0.0	36
1:40	HFM	No	Yes	Female	2 ^[1]	6.9	38
1:40	HFM	No	No	Female	0	0.0	40
1:40	HFM	No	No	Female	0	0.0	40
1:40	HFM	No	No	Male	0	0.0	42
1:40	HFM	No	No	Male	0	0.0	42
1:40	HFM	No	No	Male	0	0.0	44
1:40	HFM	No	No	Male	0	0.0	44
1:40	COT	No	No	Female	0	0.0	36
1:40	COT	No	No	Female	0	0.0	36
1:40	COT	No	Yes	Female	1 ^[1]	3.4	37
1:40	COT	No	No	Female	0	0.0	40
1:40	COT	No	No	Male	0	0.0	42
1:40	COT	No	No	Male	0	0.0	42
1:40	COT	No	No	Male	0	0.0	42
1:40	COT	Yes	No	Male	2	7.4	46

^[1] The minor alleles detected were male markers.

Species specificity study

Testing was performed to confirm that common, domestic, non-human animal species would have a minimal likelihood of interfering or cross-reacting with the RapidINTEL™ Plus assay, and to document the extent of any cross-species detection.

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Seven (7) whole blood samples from the following non-human species were purchased from a commercial supplier: dog, sheep, cow, pig, horse, rabbit, and rhesus monkey. The blood samples were deposited on HFM swabs at 35 µL.
- One (1) fresh buccal swab was collected from a dog using a COT swab.
- One (1) blood sample was collected from a human male donor. The blood sample was deposited on a HFM swab at 5 µL.
- All swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.

Run and analysis summary

There were 8 RapidINTEL™ Plus sample cartridge runs performed on 1 RapidHIT™ ID instrument.

- For all blood samples (HFM swabs), the **Specialized** (100-µL) protocol was used.
- For the dog buccal sample (COT swab), the **General** (500-µL) protocol was used.
- The run results were assessed for amplified peaks over the 35 RFU peak amplitude threshold (PAT). See Table 18 and Figure 21.

Table 18 Results: Species specificity study—peak tally

Peaks above the 35 RFU PAT were tallied for runs with non-human species whole blood or buccal cells on swabs. Out of the animal species tested, only the rhesus monkey produced cross-reactive peaks above the PAT due to low-level DNA homology with human-specific primers. For most species, the DNA did not amplify the QQS 1 or QQL 1 peaks; however, the rhesus monkey DNA exhibited slight amplification of the QQS 1 peak, as expected.

Species	Sample type	Swab type	Volume	No. of alleles detected	QQS 1 PH	QQL 1 PH
Cow	Blood	HFM	35 µL	0	0	0
Dog	Blood	HFM	35 µL	0	0	0
Dog	Buccal	COT	—	0	0	0
Horse	Blood	HFM	35 µL	0	0	0
Rabbit	Blood	HFM	35 µL	0	0	0
Sheep	Blood	HFM	35 µL	0	0	0
Pig	Blood	HFM	35 µL	0	0	0
Rhesus monkey	Blood	HFM	35 µL	7	203 (116 pg)	0
Human	Blood	HFM	5 µL	43	7,031	4,530
Negative control	No DNA template	—	—	0	0	0

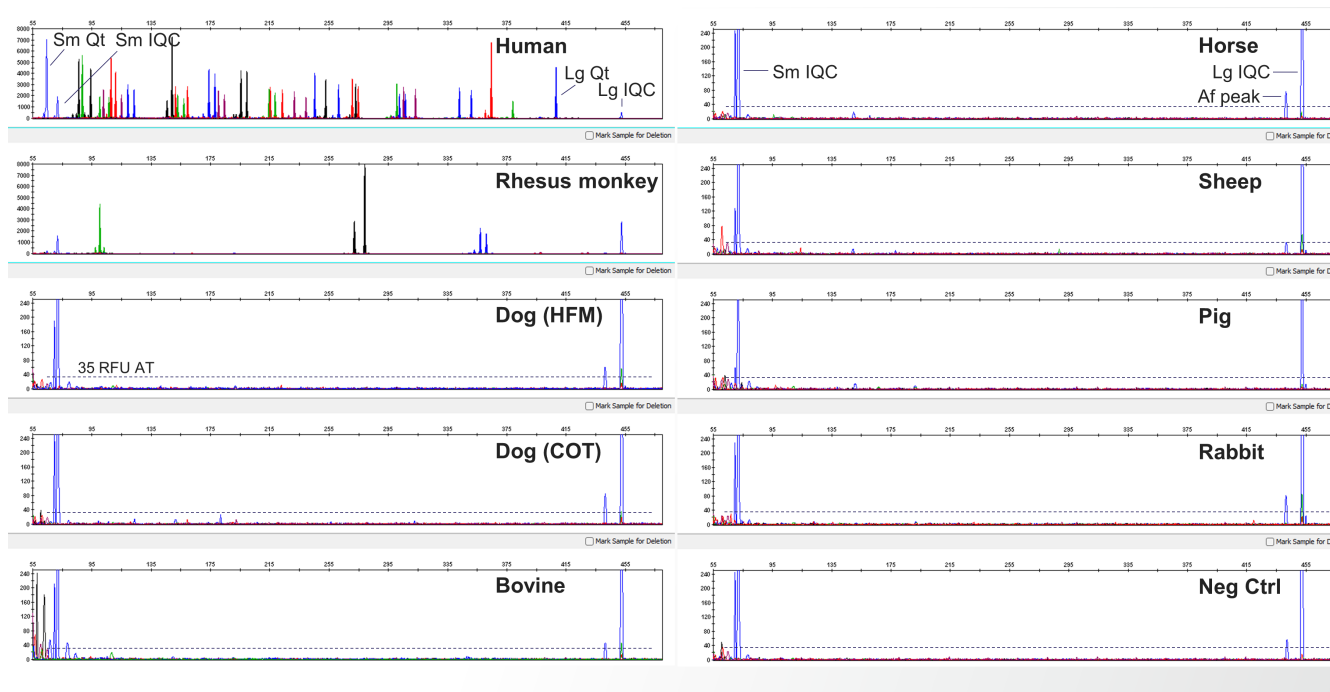


Figure 21 Results: Species specificity study Electropherograms are shown for 7 non-human animal species samples, 1 human male sample, and 1 negative control. None of the profiles except human and rhesus monkey had STR peaks above the 35 RFU PAT. Human and rhesus monkey Y-axes are set at 8,000 RFU; the remaining Y-axes are set at 250 RFU. FAM™ dye-labeled peaks from the RapidINTEL™ Plus IQC system (small IQC and large IQC) are indicated. Because small and large Quant targets are human DNA sequences, they are mainly seen in the human profile, although some weak amplification of the small Quant target is visible in the rhesus monkey DNA profile. Some profiles have a small artifact peak near the large IQC peak (labeled “Af peak” in the horse profile); this is not related to cross-reactivity with sample DNA, and may appear in negative controls. The artifact is outside of any marker region.

Subsampling study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Blood and buccal samples were obtained from human male and human female donors with COT swabs.
- The COT swabs were subsampled onto HFM swabs. See “Initial subsampling process” on page 55.

Initial subsampling process

The initial subsampling process was as follows:

- COT swabs loaded with 35 μ L of blood were subsampled onto HFM swabs. Each HFM swab was visually inspected to confirm blood transfer.
- COT buccal swabs were subsampled onto HFM swabs by pressing and holding a buccal swab against a HFM swab 10 times.
- After subsampling, all HFM swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.
- Each dried HFM swab was placed in a RapidINTEL™ Plus sample cartridge. 39 sample cartridge runs were performed on 5 RapidHIT™ ID instruments.

The amount of sample transferred by the initial subsampling process was greater than the optimal suggested load. See Figure 22.

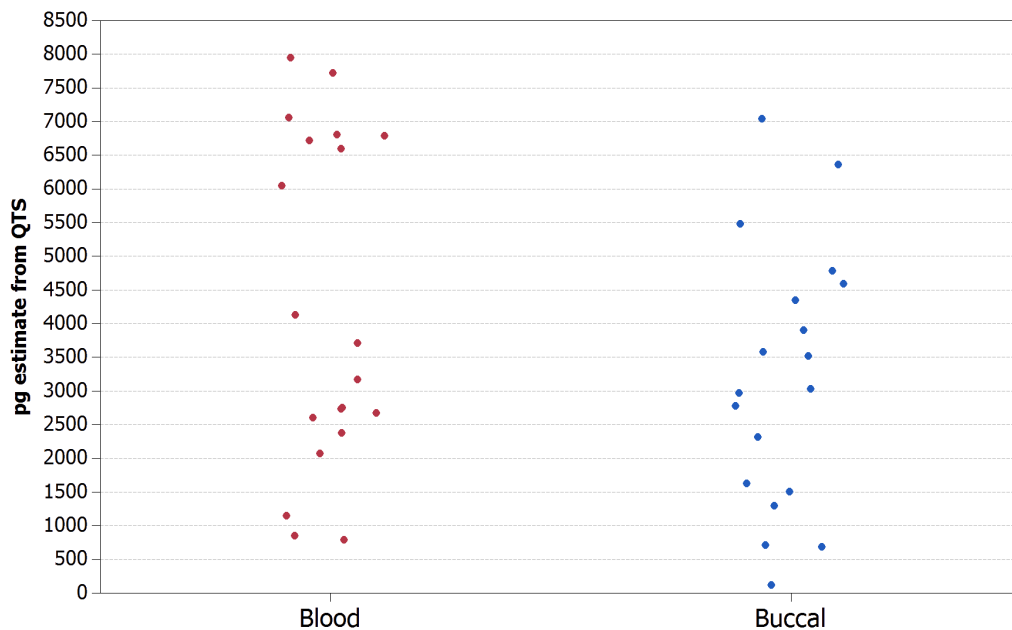


Figure 22 Subsampling blood and saliva swab samples

Optimization of the subsampling process

To further investigate the ideal subsampling process, the transfer step was repeated until observable transfer was seen:

- Blood (1 μ L) was applied to a HFM swab and allowed to dry.
- The dry HFM swab was subsampled by pressing it against a wetted HFM swab. The process was repeated 3 times. The transfer of a portion of blood is visible in the color change of the wetted, subsampling swab. Only a small portion of the original sample is transferred, as illustrated in the "Before" and "After" images shown in Figure 23.

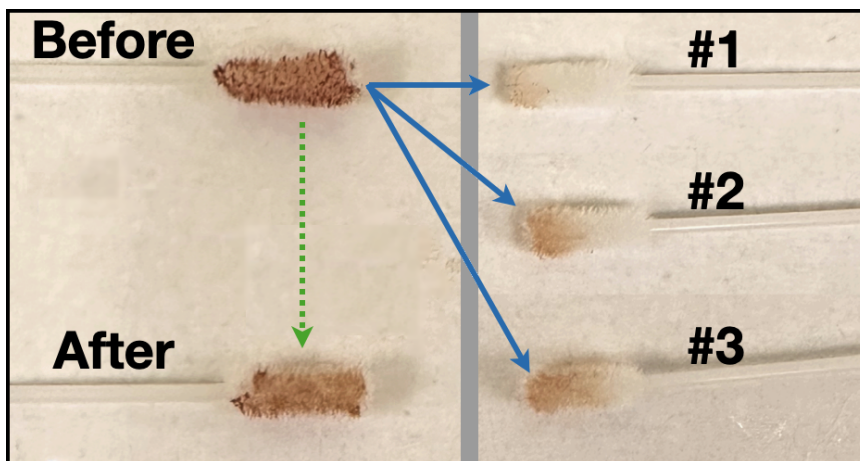


Figure 23 Subsequent subsampling process

The 3 subsampled swabs were placed in RapidINTEL™ Plus sample cartridges and run on 1 RapidHIT™ ID instrument.

- The **Specialized** (100- μ L) protocol was used.
- All 3 transfer runs generated full profiles.
- Two (2) runs passed with no quality flags (✅)
- One (1) run generated a quality flag (⚠️), but passed with review in RapidLINK™ Software v2.0.
- The electropherograms from the 3 separate subsampling transfers show the success of subsampling, and how little material is needed in the transfer to produce a full profile result. See Figure 24, Figure 25, and Figure 26. These figures correspond to the 3 transfers shown in Figure 23.



Figure 24 Results: Subsampling study—transfer 1



Figure 25 Results: Subsampling study—transfer 2



Figure 26 Results: Subsampling study—transfer 3

Case-type samples study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Multiple samples were prepared or collected to simulate the kind of biological evidence found at crime scenes. For preparation details, see Table 19. For a complete list of samples and input amounts, see Table 21.
- A few samples were measured DNA input amounts; many samples were unmeasured input amounts from stains, cast-off blood, and used or worn items.
- Of the 193 samples, 184 were single-source samples. Due to collection issues, the remaining 9 were mixture samples.

Table 19 Case-type study—sample preparation

Collection method	Preparation
Source: blood	
Purchased, collected from volunteers, or collected from various substrates; measured and unmeasured input amounts	1. Measured samples were deposited on various substrates at known input amounts. 2. Measured samples were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis. Unmeasured samples were allowed to air dry on the existing substrate. 3. Each of the dried samples were prepared and loaded in one of the following ways: • Hard surfaces and some fabrics: The dried sample was swabbed with a wetted HFM swab, then the swab was placed in a sample cartridge. • Some fabrics: A 4-mm punch was used to cut a piece of the fabric that contained the dried sample, then the piece was directly loaded into the sample cartridge lysis chamber (no swabs were used).
Source: saliva	
Collected from chewing gum samples; unmeasured input amounts	1. The gum was allowed to air dry. 2. The dried gum was prepared and loaded in one of the following ways: • The gum was swabbed with a wetted HFM swab, then the swab was placed in a sample cartridge. • A sterile scalpel was used to cut off 1/3 of the gum, then the piece of gum was directly loaded into the sample cartridge lysis chamber (no swabs were used). Note: Before cutting, we recommend that you chill the gum in the freezer for 15 minutes to let the gum harden.

Table 19 Case-type study—sample preparation *(continued)*

Collection method	Preparation
Collected from cigarette butt samples; unmeasured input amounts	1. The cigarette butts were allowed to air dry. 2. The dried cigarette butts were prepared and loaded in one of the following ways: • The cigarette butt was swabbed with a wetted HFM swab, then the swab was placed in a sample cartridge. • The cigarette paper (whole and half pieces, excluding the filter) was directly loaded into the sample cartridge lysis chamber, with the mouth end at the bottom of the chamber. The stick end of a COT swab was used to hold the paper in place at the bottom of the chamber.
Collected from various substrates; measured and unmeasured input amounts	1. The samples were allowed to air dry on the existing substrate. 2. Each of the dried samples were prepared and loaded in one of the following ways: • Hard surfaces and some fabrics: The dried sample was swabbed with a wetted COT or HFM swab, then the swab was placed in a sample cartridge. • Some fabrics: A 4-mm punch was used to cut a piece of the fabric that contained the dried sample, then the piece was directly loaded into the sample cartridge lysis chamber (no swabs were used).
Source: touch	
Collected from various substrates; unmeasured input amounts	1. Some of the touch substrates were cleaned before handling to ensure a single-source sample. 2. The samples were swabbed with wetted COT or HFM swabs. 3. The swabs were placed in the sample cartridges.
Source: semen	
Collected from various substrates; unmeasured input amounts	1. The samples were allowed to air dry on the existing substrate. 2. The dried samples were swabbed with wetted COT or HFM swabs. 3. The swabs were placed in the sample cartridges.
Source: bone, fingernail, hair, or tooth	
Collected from volunteers or purchased; measured input amounts	1. Hair samples were inspected for the presence of a hair root. 2. Each sample was directly loaded into the sample cartridge lysis chamber (no swabs were used).

Run and analysis summary

There were 193 RapidINTEL™ Plus sample cartridge runs performed on 10 RapidHIT™ ID instruments.

- The **Specialized** (100-µL) protocol or **General** (500-µL) protocol was used (indicated by "S" or "G" in the "Protocol" column of Table 21).
- The run results were assessed for pass/fail status, percent alleles recovered, dropout, STO flag indicator, and whether MIX, DEG, or INH quality flags (▲) were triggered.

In the case-type samples study, the identity of the main contributor is known, allowing the percent alleles recovered and the number of alleles dropping out to be calculated. These values allow review of the STO flag indicator—of the 184 single-source samples, the results break down as follows:

- There were 74 samples with a red STO indicator; 67 (90.5%) had dropouts.
- There were 31 samples with a yellow STO indicator; 8 (26%) had dropouts. Of the 8 dropout samples, 7 had flags for inhibition, degradation, or both.
- There were 79 samples with a green STO indicator; 1 (1.3%) had dropout. In this instance, the dropout was the complete loss of the TPOX locus, which generated a red CGQ flag for the sample.

See Table 21, Figure 27, and Figure 28.

Table 20 Table and figure notations

The notations indicate the loading method and protocol used for the sample. The notations are shown Table 21, Figure 27, and Figure 28.

Notation	Sample type	Loading method	Protocol
*	Bone	DL	General (500 µL)
^	Fingernail or tooth	DL	Specialized (100 µL)
**	Cigarette or gum	DL	General (500 µL)
		DL or HFM	Specialized (100 µL)
***	Hair	DL	General (500 µL)
		DL	Specialized (100 µL)
None	All other sample types	HFM	Specialized (100 µL)
		COT	General (500 µL)

Table 21 Case-type study—mock casework sample set and results

Samples are sorted by the body fluid or tissue source of the gDNA detected with the assay. For a description of the table notations, see Table 20.

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
Source: blood														
1	Fabric	Carpet	S	HFM	5 µL	Pass	100	2,319	>1,600	No	No	No	0	2,244
2	Fabric	Carpet, low pile	S	HFM	5 µL	Pass	100	762	746	No	No	No	0	1,072
3	Fabric	Colored cotton	S	HFM	2.5 µL	Quality flag	100	162	30	No	No	No	0	157.9
4	Fabric	Colored cotton, 4-mm cutting	S	DL	2.5 µL	Pass	100	2,056	>1,600	No	No	No	0	1,497
5	Fabric	Colored cotton, 4-mm cutting	S	DL	2.5 µL	Pass	100	3,660	>1,600	No	No	No	0	1,880
6	Fabric	Cotton swab, 2 mos. old	S	HFM	Cast-off	Pass	100	841	874	No	No	No	0	1,187
7	Fabric	Cotton swab, 2 mos. old	S	HFM	Cast-off	Pass	100	180	158	No	No	No	0	255.9
8	Fabric	Cotton swab, 4 mos. old	S	HFM	Cast-off	Pass	100	1,601	>1,600	No	No	No	0	1,383
9	Fabric	Cotton swab, 4 mos. old	S	HFM	Cast-off	Quality flag	98	318	56	No	Yes	No	1	240.5
10	Fabric	Denim	S	HFM	20 µL	Pass w. review	100	ND	292	No	No	Yes	0	471.3
11	Fabric	Denim	S	HFM	10 µL	Pass	100	692	1,032	No	No	No	0	929.0
12	Fabric	Denim	S	HFM	5 µL	Pass	100	576	141	No	No	No	0	334.3

Table 21 Case-type study—mock casework sample set and results *(continued)*

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) [5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
13	Fabric	Denim	S	HFM	10 µL	Pass	100	511	>1,600	No	No	No	0	1,067
14	Fabric	Denim, 3.7 years old	S	HFM	Cast-off	Pass	100	1,106	676	No	No	No	0	1,163
15	Fabric	Denim, 4-mm cutting	S	DL	10 µL	Pass w. review	100	1,671	60	No	Yes	No	0	1,566
16	Fabric	Denim, 4-mm cutting	S	DL	10 µL	Quality flag	95	957	ND	No	Yes	No	2	1,001
17	Fabric	Electrical tape	S	HFM	Cast-off	Pass	100	252	96	No	No	No	0	236.3
18	Fabric	Fabric, 4-mm cutting	G	DL	7.5 µL	Pass	100	1,969	>1,600	No	No	No	0	2,095
19	Fabric	Fabric, 4-mm cutting	G	DL	7.5 µL	Pass	100	2,045	>1,600	No	No	No	0	1,670
20	Fabric	White cotton	S	HFM	5 µL	Pass	100	610	487	No	No	No	0	765.0
21	Fabric	White cotton	S	HFM	5 µL	Pass	100	284	203	No	No	No	0	323.0
22	Fabric	White cotton, 4-mm cutting	S	DL	5 µL	Pass	100	5,518	>1,600	No	No	No	0	2,783
23	Fabric	White cotton, 4-mm cutting	S	DL	5 µL	Pass	100	3,608	>1,600	No	No	Yes	0	2,011
24	Fabric	Wool, 2 mos. old	S	HFM	Cast-off	Pass w. review	100	552	563	No	No	No	0	672.6
25	Fabric	Wool, 2 mos. old	S	HFM	Cast-off	Pass	100	171	190	No	No	No	0	359.8
26	Fabric	Wool, 2 mos. old	S	HFM	Cast-off	Pass	100	347	208	No	No	No	0	304.7

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
27	Hard surface	Cement	S	HFM	5 µL	Pass	100	999	691	No	No	No	0	867.5
28	Hard surface	Cement	S	HFM	5 µL	Pass	100	2,047	>1,600	No	No	No	0	1,768
29	Hard surface	Cement	S	HFM	5 µL	Pass	100	4,734	>1,600	No	No	Yes	0	2,838
30	Hard surface	Cement	S	HFM	5 µL	Pass	100	3,073	1,112	No	No	No	0	1,703
31	Hard surface	Drywall	S	HFM	5 µL	Pass	100	3,673	>1,600	No	No	No	0	2,223
32	Hard surface	Glass slide	S	HFM	1 µL	Pass	100	641	>1,600	No	No	No	0	1,385
33	Hard surface	Glass slide	S	HFM	1 µL	Pass	100	1,232	1,176	No	No	No	0	755.7
34	Hard surface	Glass slide	S	HFM	1 µL	Pass w. review	100	652	410	No	No	No	0	618.7
35	Hard surface	Glass slide	S	HFM	1 µL	Pass w. review	100	1,098	>1,600	No	No	No	0	1,418
36	Hard surface	Glass slide	S	HFM	1 µL	Pass	100	2,824	>1,600	No	No	No	0	2,319
37	Hard surface	Plywood, 2 mos. old	S	HFM	Cast-off	Quality flag	55	51	17	No	No	No	20	31.0
38	Hard surface	Plywood, 2 mos. old	S	HFM	Cast-off	Pass	100	211	66	No	No	No	0	204.5
39	Hard surface	Tile	S	HFM	1 µL	Pass	100	108	115	No	No	No	0	232.1
40	Hard surface	Tile	S	HFM	1 µL	Pass	100	1,032	>1,600	No	No	No	0	1,855
41	Hard surface	Tile	S	HFM	1 µL	Pass	100	556	486	No	No	No	0	524.8
42	Hard surface	Tile	S	HFM	1 µL	Pass	100	2,840	>1,600	No	No	No	0	2,078
Source: saliva														
1	Hard surface	Beer can	S	HFM	Used	Pass	100	1,065	ND	Yes	Yes	No	0	479.5

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
2	Hard surface	Beer can, 4 years old	S	HFM	Used	Quality flag	80	582	ND	No	Yes	No	9	192.6
3	Hard surface	Coffee cup, ceramic	S	HFM	Used	Pass	100	767	234	No	No	No	0	704.4
4	Hard surface	Coffee cup, ceramic	S	HFM	Used	Quality flag	S	172	178	No	No	No	0	204.3
5	Hard surface	Coffee cup, ceramic	S	HFM	Used	Pass	100	1,071	>1,600	No	No	No	0	1,201
6	Hard surface	Coffee cup, ceramic	G	COT	Used	Quality flag	97	91	55	No	No	No	1	109.9
7	Hard surface	Coffee cup, ceramic	S	HFM	Used	Quality flag	95	138	74	No	No	No	2	157.3
8	Hard surface	Coffee cup, ceramic	S	HFM	Used	Pass	100	1,739	537	No	No	No	0	1,074
9	Hard surface	Coffee cup, plastic	S	HFM	Used	Quality flag	100	80	94	No	No	No	0	136.2
10	Hard surface	Coffee cup, plastic	G	COT	Used	Pass	100	197	257	No	No	No	0	336.9
11	Hard surface	Glass slide	S	HFM	8 µL	Pass	100	2,541	>1,600	No	No	No	0	2,037
12	Hard surface	Glass slide	S	HFM	8 µL	Pass	100	2,673	>1,600	No	No	No	0	1,496
13	Hard surface	Plastic Straw	S	HFM	Used	Pass	100	385	242	No	No	No	0	378.5
14	Hard surface	Plastic Straw	S	HFM	Used	Quality flag	43	198	18	No	Yes	No	21	32.4
15	Hard surface	Plastic water bottle	G	COT	Used	Quality flag	98	118	60	No	No	No	1	132.8

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
16	Hard surface	Plastic water bottle	S	HFM	Used	Pass	100	825	>1,600	No	No	No	0	989.2
17	Hard surface	Plastic water bottle	S	HFM	Used	Quality flag	98	195	146	No	No	No	1	229.1
18	Hard surface	Plastic water bottle	S	HFM	Used	Pass w. review	100	2,583	>1,600	No	No	No	0	2,132
19	Hard surface	Soda can	G	COT	Used	Pass w. review	100	1,743	>1,600	No	No	No	0	2,186
20	Hard surface	Soda can	S	HFM	Used	Pass	100	898	366	No	No	No	0	1,036
21	Hard surface	Soda can	S	HFM	Used	Pass	100	1,692	>1,600	No	No	No	0	2,383
22	Hard surface	Soda can	S	HFM	Used	Pass	100	666	620	No	No	No	0	976.7
23	Hard surface	Soda can	G	COT	Used	Quality flag	24	ND	16	No	No	No	31	20.0
24	Hard surface	Toothbrush	S	HFM	Used	Pass	100	517	>1,600	No	No	No	0	1,610
25	Hard surface	Toothbrush	S	HFM	Used	Pass	100	1,556	>1,600	No	No	No	0	1,707
26	Hard surface	Toothbrush	S	HFM	Used	Quality flag	95	1,911	>1,600	Yes	No	No	2	1,786
27	Hard surface	Toothbrush, electric	G	COT	Used	Quality flag	16	ND	ND	No	No	No	32	7.5
28	Hard surface	Toothbrush, electric	S	HFM	Used	Pass	100	608	671	No	No	No	0	961.3
29	Hard surface	Toothbrush, electric	S	HFM	Used	Pass	100	1,709	58	No	Yes	No	0	546.6
30	Cigarette**	Cigarette paper	S	HFM	Smoked	Pass	100	299	141	No	No	No	0	647.6

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
31	Cigarette**	Cigarette paper	S	HFM	Smoked	Pass	100	420	171	No	No	No	0	629.8
32	Cigarette**	Cigarette paper	S	HFM	Smoked	Pass	100	866	320	No	No	No	0	999.9
33	Cigarette**	Cigarette paper	S	HFM	Smoked	Pass	100	1,675	569	No	No	No	0	1,582
34	Cigarette**	Cigarette paper half	S	DL	Smoked	Pass	100	1,186	245	No	No	Yes	0	693.2
35	Cigarette**	Cigarette paper half	G	DL	Smoked	Quality flag	75	139	ND	No	Yes	No	10	77.8
36	Cigarette**	Cigarette paper half	G	DL	Smoked	Quality flag	100	218	49	No	No	No	0	256.8
37	Cigarette**	Cigarette paper whole	G	DL	Smoked	Pass	100	203	77	No	No	No	0	277.3
38	Cigarette**	Cigarette paper whole	G	DL	Smoked	Quality flag	93	217	98	No	No	No	3	190.9
39	Cigarette**	Cigarette paper whole	G	DL	Smoked	Quality flag	93	562	17	No	Yes	No	3	251.9
40	Cigarette**	Cigarette paper whole	G	DL	Smoked	Quality flag	95	747	ND	No	Yes	Yes	2	349.4
41	Fabric	White cotton	S	DL	20 µL	Pass	100	2,018	>1,600	No	No	No	0	1,787
42	Fabric	White cotton	S	HFM	20 µL	Quality flag	88	60	23	No	No	No	5	77.7
43	Fabric	White cotton	S	HFM	20 µL	Pass	100	127	91	No	No	No	0	159.9
44	Fabric	White cotton	S	DL	20 µL	Pass	100	3,115	>1,600	No	No	No	0	2,136

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
45	Gum**	Fruit gum	G	DL	Chewed	Pass	100	3,483	284	No	Yes	Yes	0	1,404
46	Gum**	Fruit gum	S	HFM	Chewed	Pass	100	3,144	891	No	No	No	0	2,088
47	Gum**	Fruit gum	S	HFM	Chewed	Quality flag	97	246	50	No	No	No	1	130.3
48	Gum**	Fruit gum	S	DL	Chewed	Quality flag	76	2,673	ND	No	Yes	Yes	9	486.1
49	Gum**	Fruit gum	S	DL	Chewed	Quality flag	97	1,601	27	No	Yes	Yes	1	426.9
50	Gum**	Fruit gum	G	DL	Chewed	Pass	100	473	77	No	Yes	No	0	301.1
51	Gum**	Fruit gum	S	HFM	Chewed	Pass w. review	100	144	117	No	No	No	0	205.5
52	Gum**	Mint gum	G	DL	Chewed	Pass	100	439	1,098	No	No	No	0	925.0
53	Gum**	Mint gum	G	DL	Chewed	Quality flag	98	122	143	No	No	No	1	217.0
54	Gum**	Mint gum	S	HFM	Chewed	Pass w. review	100	3,405	>1,600	No	No	No	0	3,070
55	Gum**	Mint gum	S	HFM	Chewed	Pass	100	691	211	No	No	No	0	401.4
Source: touch														
1	Hard surface	Axe, wood handle	S	HFM	Used	Quality flag	18	45	ND	No	Yes	No	36	10.7
2	Hard surface	Bar, Metal	S	HFM	Used	Quality flag	2	ND	ND	No	No	No	43	2.3
3	Hard surface	Bar, metal	S	HFM	Used	Pass	100	734	213	No	No	No	0	668.2
4	Hard surface	Bar, metal	S	HFM	Used	Quality flag	90	2,256	ND	No	Yes	No	4	461.1
5	Hard surface	Cell phone	S	HFM	Used	Quality flag	100	234	99	No	No	No	0	269.7
6	Hard surface	Cell phone	S	HFM	Used	Quality flag	37	29	ND	No	Yes	No	24	27.4

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
7	Hard surface	Cell phone	S	HFM	Used	Quality flag	88	32	60	No	No	No	5	95.3
8	Hard surface	Cell phone	S	HFM	Used	Quality flag	93	94	107	No	No	No	3	131.5
9	Hard surface	Cell phone	G	COT	Used	Quality flag	29	ND	ND	No	No	No	29	15.7
10	Hard surface	Cell phone	G	COT	Used	Quality flag	29	ND	33	No	No	No	30	20.3
11	Hard surface	Copper wire, twisted	S	HFM	Used	Quality flag	40	218	ND	No	Yes	No	24	60.3
12	Hard surface	Copper wire, twisted	G	COT	Used	Quality flag	33	44	ND	No	Yes	No	27	17.5
13	Hard surface	Copper wire, twisted	S	HFM	Used	Quality flag	13	33	ND	No	Yes	No	35	5.0
14	Hard surface	Crush gripper, metal	S	HFM	Used	Quality flag	70	1,767	ND	No	Yes	Yes	13	190.5
15	Hard surface	Duct tape	S	HFM	Used	Quality flag	30	36	ND	No	Yes	No	28	18.4
16	Hard surface	Duct tape	S	HFM	Used	Quality flag	90	250	ND	No	Yes	No	4	153.3
17	Hard surface	Eyeglasses, plastic frame	S	HFM	Worn	Quality flag	93	276	ND	No	Yes	No	3	176.0
18	Hard surface	Eyeglasses, wire frame	S	HFM	Worn	Pass w. review	100	5,129	>1,600	No	No	Yes	0	2,874
19	Hard surface	Eyeglasses, wire frame	G	COT	Worn	Quality flag	15	ND	ND	No	No	No	34	8.7
20	Hard surface	Flick knife, metal thumb stud	S	HFM	Used	Quality flag	86	433	ND	No	Yes	No	6	192.7

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
21	Hard surface	Flick knife, plastic handle	S	HFM	Used	Quality flag	89	179	ND	No	Yes	No	5	93.0
22	Hard surface	Flick knife, wood handle	S	HFM	Used	Quality flag	85	266	ND	No	Yes	No	6	117.2
23	Hard surface	Garden tool, rubber handle	S	HFM	Used	Quality flag	48	35	ND	No	Yes	No	21	42.7
24	Hard surface	Garden tool, rubber handle	S	HFM	Used	Quality flag	3	ND	ND	No	No	No	37	1.4
25	Hard surface	Hammer, wood handle	S	HFM	Used	Quality flag	90	189	ND	No	Yes	No	4	137.8
26	Hard surface	Keyboard	S	HFM	Used	Pass	100	675	456	No	No	No	0	920.8
27	Hard surface	Keyboard	G	COT	Used	Quality flag	12	ND	ND	No	No	No	36	5.0
28	Hard surface	Keyboard	S	HFM	Used	Pass	100	1,287	312	No	No	No	0	813.5
29	Hard surface	Keyboard	S	HFM	Used	Quality flag	93	1,214	281	Yes	No	No	3	822.1
30	Hard surface	Kitchen knife, plastic handle	S	HFM	Used	Quality flag	85	146	ND	No	Yes	No	6	108.3
31	Hard surface	Kitchen knife, plastic handle	S	HFM	Used	Quality flag	13	ND	ND	No	No	No	33	8.3
32	Hard surface	Screwdriver, plastic handle	S	HFM	Used	Quality flag	45	ND	ND	No	No	No	22	36.1
33	Hard surface	Screwdriver, plastic handle	S	HFM	Used	Pass	100	381	150	No	No	No	0	246.5
34	Hard surface	Screwdriver, plastic handle	S	HFM	Used	Quality flag	98	819	65	No	Yes	No	1	413.0

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
35	Hard surface	Steering wheel, plastic	S	HFM	Used	Quality flag	79	118	18	No	Yes	No	8	70.2
36	Hard surface	Sunglasses, plastic frame	S	HFM	Worn	Quality flag	25	ND	ND	No	No	No	30	16.6
37	Hard surface	Sunglasses, plastic frame	G	COT	Worn	Quality flag	5	ND	ND	No	No	No	38	3.8
38	Hard surface	Sunglasses, wire frame	S	HFM	Worn	Pass	100	2,949	>1,600	No	No	No	0	2,083
39	Razor blade	Razor blade	S	HFM	Used	Quality flag	83	93	52	Yes	No	No	7	63.1
40	Razor blade	Razor blade	G	COT	Used	Quality flag	8	ND	ND	No	No	No	37	5.9
41	Razor blade	Razor blade	S	HFM	Used	Quality flag	7	ND	ND	No	No	No	41	3.2
42	Razor blade	Razor blade	G	COT	Used	Quality flag	31	ND	ND	No	No	No	27	21.4
43	Razor blade	Razor blade	S	HFM	Used	Quality flag	12	35	ND	No	Yes	No	36	7.8
44	Razor blade	Razor blade	S	HFM	Used	Quality flag	100	235	98	No	No	No	0	144.0
45	Razor blade	Razor blade	G	COT	Used	Quality flag	41	45	45	No	No	No	23	44.1
46	Razor blade	Razor blade	S	HFM	Used	Quality flag	91	229	ND	Yes	Yes	No	4	227.2
47	Fabric	Baseball bat, cloth wrap	S	HFM	Used	Quality flag	98	317	52	No	Yes	No	1	169.9
48	Fabric	Baseball cap, last worn 1 year ago	S	HFM	Worn	Pass	100	3,417	>1,600	No	No	No	0	2,623
49	Fabric	Baseball cap, last worn 1 year ago	S	HFM	Worn	Pass	100	814	238	No	No	No	0	657.0

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
50	Fabric	Baseball cap, recently worn	S	HFM	Worn	Pass	100	1,049	442	No	No	No	0	922.0
51	Fabric	Baseball cap, recently worn	G	COT	Worn	Quality flag	60	104	ND	No	Yes	No	17	54.2
52	Fabric	Baseball cap, recently worn	S	HFM	Worn	Pass	100	2,526	>1,600	No	No	No	0	2,451
53	Fabric	Baseball cap, recently worn	S	HFM	Worn	Quality flag	98	247	130	No	No	No	1	135.9
54	Fabric	Collar, T-shirt	S	HFM	Worn	Quality flag	15	27	ND	No	Yes	No	33	10.0
55	Fabric	Collar, T-shirt	G	COT	Worn	Quality flag	29	ND	ND	No	No	No	30	19.3
56	Fabric	Collar, T-shirt	S	HFM	Worn	Quality flag	31	ND	ND	No	No	No	29	15.3
57	Fabric	Collar, T-shirt	G	COT	Worn	Quality flag	62	56	37	No	No	No	15	40.0
58	Fabric	Face mask, disposable	S	HFM	Worn	Pass	100	405	619	No	No	No	0	743.4
59	Fabric	Face mask, disposable	G	COT	Worn	Quality flag	98	99	104	No	No	No	1	171.9
60	Fabric	Glove leather	S	HFM	Worn	Quality flag	2	ND	ND	No	No	No	43	2.0
61	Fabric	Glove leather	S	HFM	Worn	Quality flag	9	ND	ND	No	No	No	40	4.0
62	Fabric	Ski mask, extended wear	S	HFM	Worn	Quality flag	49	53	76	No	No	No	20	39.3
63	Fabric	Ski mask, extended wear	G	COT	Worn	Quality flag	72	44	27	No	No	No	11	47.7
64	Fabric	Steering wheel, leather	S	HFM	Used	Quality flag	57	141	ND	No	Yes	No	19	52.1

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
65	Fabric	Steering wheel, leather	G	COT	Used	Pass	100	327	356	No	No	No	0	428.6
66	Fabric	Steering wheel, leather	S	HFM	Used	Pass w. review	100	549	216	No	No	No	0	407.9
67	Firearm	Gun grip, hard rubber	G	COT	Used	Quality flag	20	ND	ND	No	No	No	32	14.0
68	Firearm	Gun grip, hard rubber	S	HFM	Used	Quality flag	93	419	26	No	Yes	No	3	152.2
69	Firearm	Gun magazine	S	HFM	Used	Quality flag	49	59	ND	No	Yes	No	21	39.4
70	Firearm	Gun magazine	S	HFM	Used	Quality flag	20	89	ND	No	Yes	No	35	12.9
71	Firearm	Gun trigger	S	HFM	Used	Quality flag	71	324	20	No	Yes	No	12	82.5
72	Firearm	Gun trigger	S	HFM	Used	Quality flag	27	83	ND	No	Yes	No	32	22.1
73	Firearm	Shell casing, fired	S	HFM	Used	Quality flag	5	ND	ND	No	No	No	40	4.8
74	Firearm	Shell casing, not fired	S	HFM	Used	Quality flag	15	1,686	ND	No	Yes	No	35	75.0
Source: semen														
1	Fabric	Colored cotton, 7 days old	G	COT	Stain	Quality flag	100	3,426	>1,600	Yes	No	No	0	1,681
2	Fabric	Colored cotton, 7 days old	S	HFM	Stain	Quality flag	100	1,221	>1,600	Yes	No	No	0	1,186

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
3	Fabric	Colored cotton, 7 days old	G	COT	Stain	Quality flag	100	4,522	>1,600	Yes	No	No	0	2,729
4	Fabric	Colored cotton, 7 days old	S	HFM	Stain	Quality flag	67	958	1,062	Yes	No	No	0	1,082
5	Fabric	Colored felt	S	HFM	Stain	Pass w. review	100	5,643	>1,600	No	Yes	Yes	0	3,207
6	Fabric	Colored felt	S	HFM	Stain	Pass w. review	100	4,964	>1,600	No	No	No	0	2,839
7	Fabric	Denim	S	HFM	Stain	Pass w. review	100	1,043	902	No	No	No	0	1,218
8	Fabric	Denim	S	HFM	Stain	Pass w. review	100	2,809	862	No	No	No	0	2,067
9	Fabric	Denim	G	COT	Stain	Pass	100	1,472	416	No	No	No	0	1,340
Source: bone, fingernail, hair, or tooth														
1	Bone*	Tibia, powdered	G	DL	10 mg	Pass	100	3,284	>1,600	No	No	No	0	2,048
2	Fingernail^	Fingernail, clipping	S	DL	Clipped	Pass	100	2,462	1089	No	No	No	0	1,647
3	Fingernail^	Fingernail, clipping cleaned with EtOH	S	DL	Clipped	Pass	100	1,097	422	No	No	No	0	863.3

Table 21 Case-type study—mock casework sample set and results (continued)

No.	Substrate category	Detailed substrate	Protocol ^[1]	Loading method ^[2]	Input amount	RL software ^[3]	% AR ^[4]	QTS (pg) ^[5]	QTL (pg)	Quality flag			Allele dropouts	STO PH
										MIX	DEG	INH		
4	Fingernail^	Fingernail, clipping cleaned with EtOH	S	DL	Clipped	Pass	100	667	126	No	Yes	No	0	497.3
5	Hair***	Hair beard	S	DL	Plucked	Pass w. review	100	7,850	>1,600	No	No	Yes	0	4,297
6	Hair***	Hair eyebrow	S	DL	Plucked	Pass w. review	100	8,442	>1,600	No	Yes	Yes	0	3,786
7	Hair***	Hair head	G	DL	Plucked	Pass w. review	100	120	209	No	No	No	0	280.5
8	Hair***	Hair head	G	DL	Plucked	Pass	100	1,498	>1,600	No	No	No	0	2,373
9	Hair***	Hair head	S	DL	Plucked	Pass w. review	100	6,552	>1,600	No	Yes	Yes	0	3,237
10	Hair***	Hair head	S	DL	Plucked	Pass	100	983	>1,600	No	No	No	0	1,732
11	Hair***	Hair head	S	DL	Plucked	Pass	100	292	644	No	No	No	0	463.7
12	Tooth^	Whole tooth ground	S	DL	Extracted	Pass w. review	100	772	193	No	No	Yes	0	617.2
13	Tooth^	Whole tooth ground	S	DL	Extracted	Pass	100	10,054	789	No	Yes	Yes	0	1,878

^[1] G=General (500-µL) protocol. S=Specialized (100-µL) protocol.

^[2] See “Sample loading” on page 24.

^[3] Indicates our internal review of the sample, using RapidLINK™ Software v2.0: Pass=The software passed the sample without review. Quality flag=The software generated a flag for one or more of the markers in the sample. Pass w. review=Flags that were generated by the software for the following reasons were manually reviewed and passed: spikes, pull-ups, known dye artifacts, D19S433 high stutter peaks and/or TH01 n-5 stutter peaks, and PHR ≤40% when QQS 1 was >3,800 RFU.

^[4] The percent of single-source or (for mixtures) main contributor alleles recovered.

^[5] Boldfaced values indicate that the sample load was >2 ng of gDNA in the PCR and the sample may display some high input generated artifacts.

The case-type study results are illustrated in Figure 27 and Figure 28.

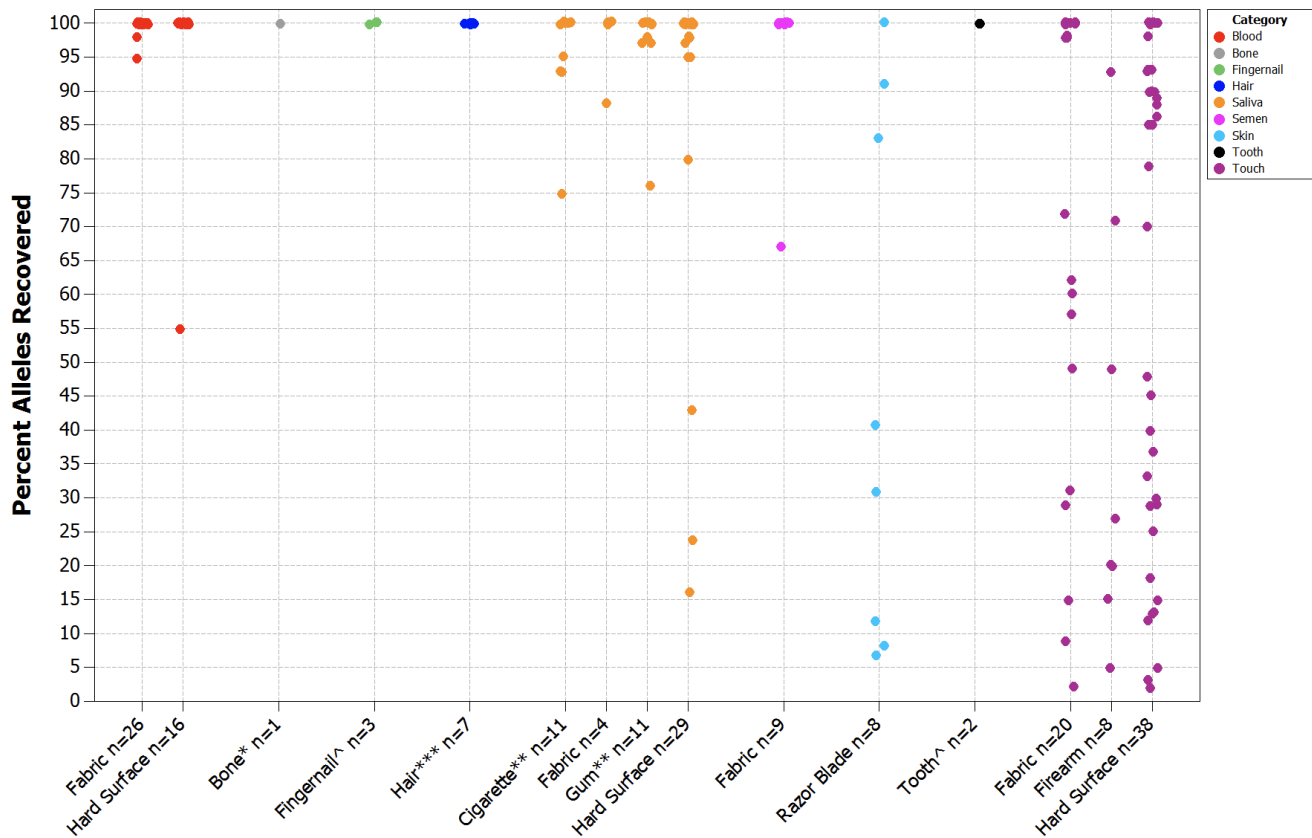


Figure 27 Results: Case-type study—percent of major contributor alleles recovered The percent of alleles recovered for the various mock casework sample types are displayed by the sample source and, where appropriate, the substrate on which the source was located. The results show good recovery from various body fluids and tissues, but as expected, highly variable results from touch samples.

For detailed substrate information, see Table 21. For a description of the figure notations, see Table 20.

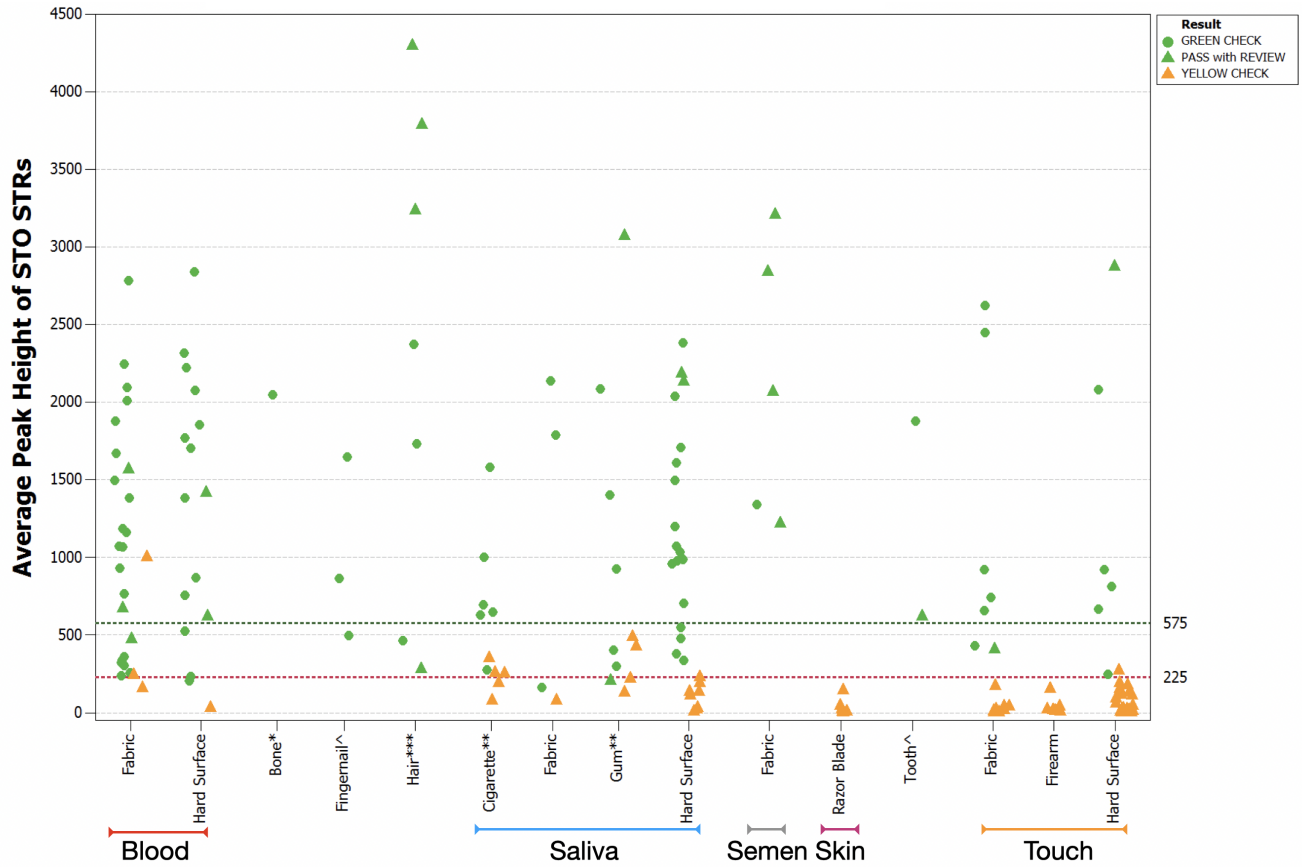


Figure 28 Results: Case-type study—profile results A STO flag indicator of ■ (pass) or ▲ (quality flag) strongly correlates with the ability of RapidHIT™ ID System v2.0 to produce a first pass success profile, or a profile that passes with review. For detailed substrate information, see Table 21. For a description of the figure notations, see Table 20.



Equivalency and validation studies (one-punch cartridge)

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■ Objective of the equivalency and validation studies	78
■ Materials and methods	79
■ Equivalency studies (one-punch cartridge)	81
■ Validation studies (one-punch cartridge)	84

Product update

To prevent the lysate collection punches from moving in the RapidINTEL™ Plus cartridge PCR chamber, the two circular punches were replaced with one oblong punch. The oblong punch is approximately the same size as the two circular punches combined.

Note: No changes were made to the RapidHIT™ ID System Software or RapidLINK™ Software v2.0.

Objective of the equivalency and validation studies

Equivalency and validation studies were performed to assess the performance of the one-punch RapidINTEL™ Plus Sample Cartridge compared to the two-punch RapidINTEL™ Plus Sample Cartridge on RapidHIT™ ID System v2.0

The following equivalency studies were performed:

- Positive control
- Measurement systems analysis (MSA)
- Sensitivity

The following validation studies were performed:

- Positive control
- Contamination
- Population
- Sensitivity

The equivalency and validation studies assessed:

- Overall performance comparison between the one-punch and two-punch RapidINTEL™ Plus cartridges
- Accurate detection and flagging of peaks that do not comply with the system thresholds
- Ability to detect DNA profiles at risk of PCR stochastic effects
- Primary analysis pass rate (the percentage of samples that meet all system thresholds and generate full, single-source profiles without manual review)
- Secondary analysis pass with review recovery rate (the percentage of samples that meet all system thresholds and generate full, single-source profiles after manual review)
- Sample input amounts to determine the optimal sample type and amounts required to provide accurate results

Materials and methods

Instruments, cartridges, and software

Eleven (11) RapidHIT™ ID System v2.0 instruments were used for the equivalency and validation studies. Each instrument was used in at least one study; however, not all instruments were used in every study.

Before the studies were run, a RapidINTEL™ Plus positive control cartridge and a RapidINTEL™ Plus allelic ladder cartridge were run on all instruments.

Note: Data presented in this chapter was obtained using RapidINTEL™ Plus cartridges run on RapidHIT™ ID System v2.0 and analyzed on RapidLINK™ Software v2.0 or GeneMapper™ ID-X Software v1.7. Capillary electrophoresis sizing analysis was done using GeneScan™ 600 LIZ™ Size Standard v2.0.

Instruments, cartridges, and software

Item	Catalog number
RapidHIT™ ID System v2.0	A41810
RapidHIT™ ID Primary Cartridge GlobalFiler™ Express 150 Kit	A53084
RapidINTEL™ Plus 50 Sample Cartridge Kit	A54338
RapidINTEL™ Plus Positive Control Cartridge	A54342
RapidINTEL™ Plus Negative Control Cartridge	A54343
RapidINTEL™ Plus Ladder Cartridge Kit (allelic ladder)	A54344

Sample collection, preparation, and loading

Sample collection

Samples were prepared with standard swabs or with micro swabs, as described in Table 22. Not all swab types were used in every study.

Table 22 Swab types used

"MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Swab type	Tip size	Item	Cat. no.	Swab type abbreviations used in this document	Use cases
Spun cotton tip	Standard	Puritan™ 3" Sterile Standard Cotton Swab with Semi-Flexible Polystyrene Handle	25-803 2PC (MLS)	COT	Routine sampling; used with the General (500-μL) protocol
Nylon or polyester tip (flocked tip)	Micro	Puritan™ HydraFlock™ 6" Sterile Micro Ultrafine Flock Swab with Polystyrene Handle, with 100-mm Breakpoint	25-3318-H (MLS)	HFM	Specialized sampling of smaller sample amounts that require detection sensitivity; used with the Specialized (100-μL) protocol or the General (500-μL) protocol

Sample preparation

Samples were prepared as described in each study section, under "Cartridge and sample summary".

Whether obtained from commercial suppliers or from known individuals, all human biological samples (blood, saliva, semen, hair, bone, fingernails, and tooth) were obtained with informed consent.

Sample loading

Samples were loaded into the RapidINTEL™ Plus cartridges in one of the following ways:

- Inserting a swab with sample into a cartridge. Swab types used were COT or HFM.
- Directly loading a sample into a cartridge (no swab used). In this document, this method may be referred to as "direct load" or "DL".

Equivalency studies (one-punch cartridge)

Equivalency data set

Before performing the validation studies, an equivalency data set was used to establish that comparable results were obtained from the updated RapidINTEL™ Plus cartridges. The equivalency data set includes 54 samples processed with RapidINTEL™ Plus cartridges on RapidHIT™ ID System v2.0. The equivalency data set is provided in Table 23.

Table 23 Equivalency data set: Studies and samples

Study	No. of runs		No. of instruments	Protocol ^[1]	Sample type	Swab type/sample amount
	Two circular punches	One oblong punch				
Positive control	9	7	7	General	Positive control	COT swabs preloaded in the positive control cartridges: 330 ng
Measurement systems analysis (MSA)	56	33	7	Specialized	gDNA: Male (DNA Control 007)	HFM swabs: 60, 120, 180, 240, 300, 500, and 1,000 pg
Sensitivity	24	21	7	Specialized	Blood sample (male)	HFM swabs: 0.1, 0.5, and 1.0 µL

^[1] The protocol was read from the cartridge RFID tag.

Positive control study

Cartridge and sample summary

RapidINTEL™ Plus positive control cartridges were used.

- The cartridges are preloaded with COT swabs.
- Each swab contains 330 ng total human male gDNA (DNA Control 007).

Run and analysis summary

There were 7 one-punch RapidINTEL™ Plus positive control cartridge runs performed on 7 RapidHIT™ ID instruments (1 run per instrument).

- The **General** (500-µL) protocol was used.
- All 7 runs passed with no quality flags (✓).
- The run results were consistent with the following:
 - Validation and verification studies performed with the two-punch RapidINTEL™ Plus positive control cartridges; see “Positive control study” on page 29
 - Nine (9) concurrent runs performed with the two-punch RapidINTEL™ Plus positive control cartridges

Measurement systems analysis (MSA) study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Each sample cartridge was directly loaded with diluted human male gDNA (DNA Control 007).
- The samples were deposited on HFM swabs at 60, 120, 180, 240, 300, 500, and 1,000 pg.

Run and analysis summary

There were 33 one-punch RapidINTEL™ Plus sample cartridge runs performed on 7 RapidHIT™ ID instruments.

- The **Specialized** (100-μL) protocol was used.
- The run results were assessed for the average QQS-1 peak heights.
- All 7 input amounts produced results that were consistent with the following:
 - MSA testing performed with the two-punch RapidINTEL™ Plus sample cartridges; see “Quantification markers” on page 98
 - Fifty-six (56) concurrent runs performed with the two-punch RapidINTEL™ Plus sample cartridges; see Figure 29 and Figure 30.

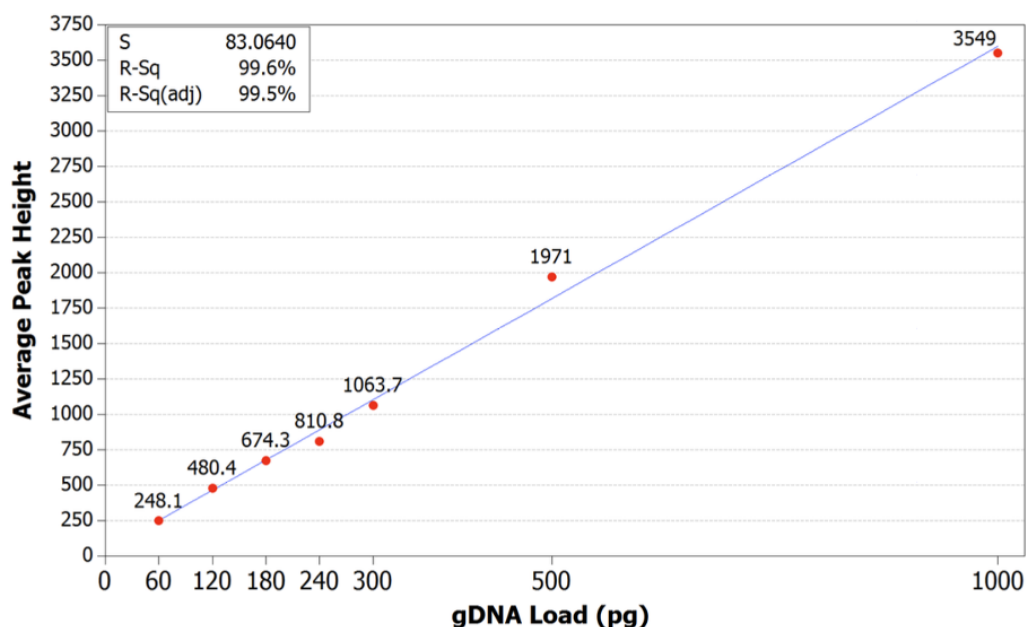


Figure 29 Results: MSA study—One-punch RapidINTEL™ Plus sample cartridges (gDNA dilutions, direct load) Fitted line plot. Average QQS-1 peak heights vs. gDNA input from 33 runs using one-punch RapidINTEL™ Plus sample cartridges.

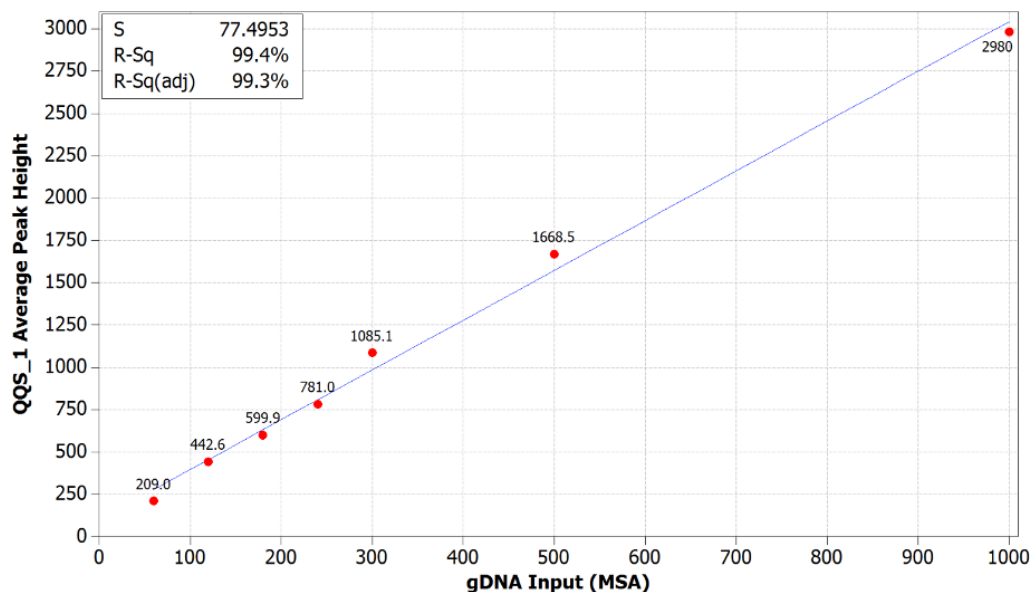


Figure 30 Results: MSA study—Two-punch RapidINTEL™ Plus sample cartridges (gDNA dilutions, direct load) Fitted line plot. Average QQS-1 peak heights vs. gDNA input from 56 concurrent runs using two-punch RapidINTEL™ Plus sample cartridges.

Sensitivity study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Blood samples were provided by 1 unique human donor (male).
- Blood samples were deposited on HFM swabs at 0.1, 0.5, and 1.0 µL.
- All swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.

Run and analysis summary

There were 21 one-punch RapidINTEL™ Plus sample cartridge runs performed on 7 RapidHIT™ ID instruments.

- The **Specialized** (100-µL) protocol was used.
- The run results were assessed for the percent of alleles recovered. 100% allele recovery was obtained for all 3 input amounts.
- All 3 input amounts produced results that were consistent with the following:
 - Validation and verification studies performed with the two-punch RapidINTEL™ Plus sample cartridges; see “Sensitivity study” on page 36
 - Twenty-four (24) concurrent runs performed with the two-punch RapidINTEL™ Plus sample cartridges

Validation studies (one-punch cartridge)

Validation data set

Table 24 Validation data set: Studies and samples

Study	No. of runs	No. of instruments	Protocol ^[1]	Sample type	Swab type/sample amount
Positive control	5	5	General	Positive control	COT swabs preloaded in the positive control cartridges: 330 ng
Contamination	25	2	Specialized	Negative control	Each run was performed after a 35- μ L blood sample run that used the General protocol.
Sensitivity	147	10	General or Specialized	Blood samples (male and female)	HFM or COT swabs: 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, 10, 20, and 35 μ L
				Saliva samples (male)	HFM or COT swabs: 1.0, 4.0, 8.0, 35 μ L
Population	309	11	Specialized	Blood samples (male and female)	HFM swabs: 0.5 and 0.05 μ L

^[1] The protocol was read from the cartridge RFID tag.

Positive control study

Cartridge and sample summary

RapidINTEL™ Plus positive control cartridges were used.

- The cartridges are preloaded with COT swabs.
- Each swab contains 330 ng total human male gDNA (DNA Control 007).

Run and analysis summary

There were 5 one-punch RapidINTEL™ Plus positive control cartridge runs performed on 5 RapidHIT™ ID instruments (1 run per instrument).

- The **General** (500- μ L) protocol was used.
- All 5 runs passed with no quality flags (✓).

Contamination study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges and RapidINTEL™ Plus negative control cartridges were used.

For the RapidINTEL™ Plus sample cartridges:

- Two (2) unique human donors (one male and one female) provided blood samples.
- Blood samples (35 µL) were deposited on COT swabs.
- All swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.

Run and analysis summary

There were 20 one-punch RapidINTEL™ Plus sample (blood) cartridge runs performed on 10 RapidHIT™ ID instruments. The **General** (500-µL) protocol was used.

There were 22 one-punch RapidINTEL™ Plus negative control cartridge runs performed on 10 RapidHIT™ ID instruments.

- The **Specialized** (100-µL) protocol was used.
- Each negative control run was performed immediately after a 35-µL blood sample run. For example:
 - **Run 1**—35-µL blood sample 1 (male)
 - **Run 2**—Negative control
 - **Run 3**—35-µL blood sample 2 (female)
 - **Run 4**—Negative control

Note: Peaks were detected in 2 negative control runs. An additional negative control run was consecutively performed for each of these 2 runs, for a total of 22 negative control runs.

- The run results were assessed for reproducible peaks in the assay read region that were above the peak detection threshold of 35 RFU:

Sample	Number of negative control runs		Electropherograms
	Peaks >35 RFU	Peaks ≤35 RFU	
Blood sample 1 (male)	0	10	See Figure 31.
Blood sample 2 (female)	2	8	See Figure 32. The 2 peaks that were >35 RFU were not reproducible in the consecutive negative control runs on either of the 2 RapidHIT™ ID instruments. See Figure 33 and Figure 34.

These results are consistent with capillary electrophoresis DNA carryover from an injection of high-quantity sample DNA to an injection of no-or low-quantity sample DNA.

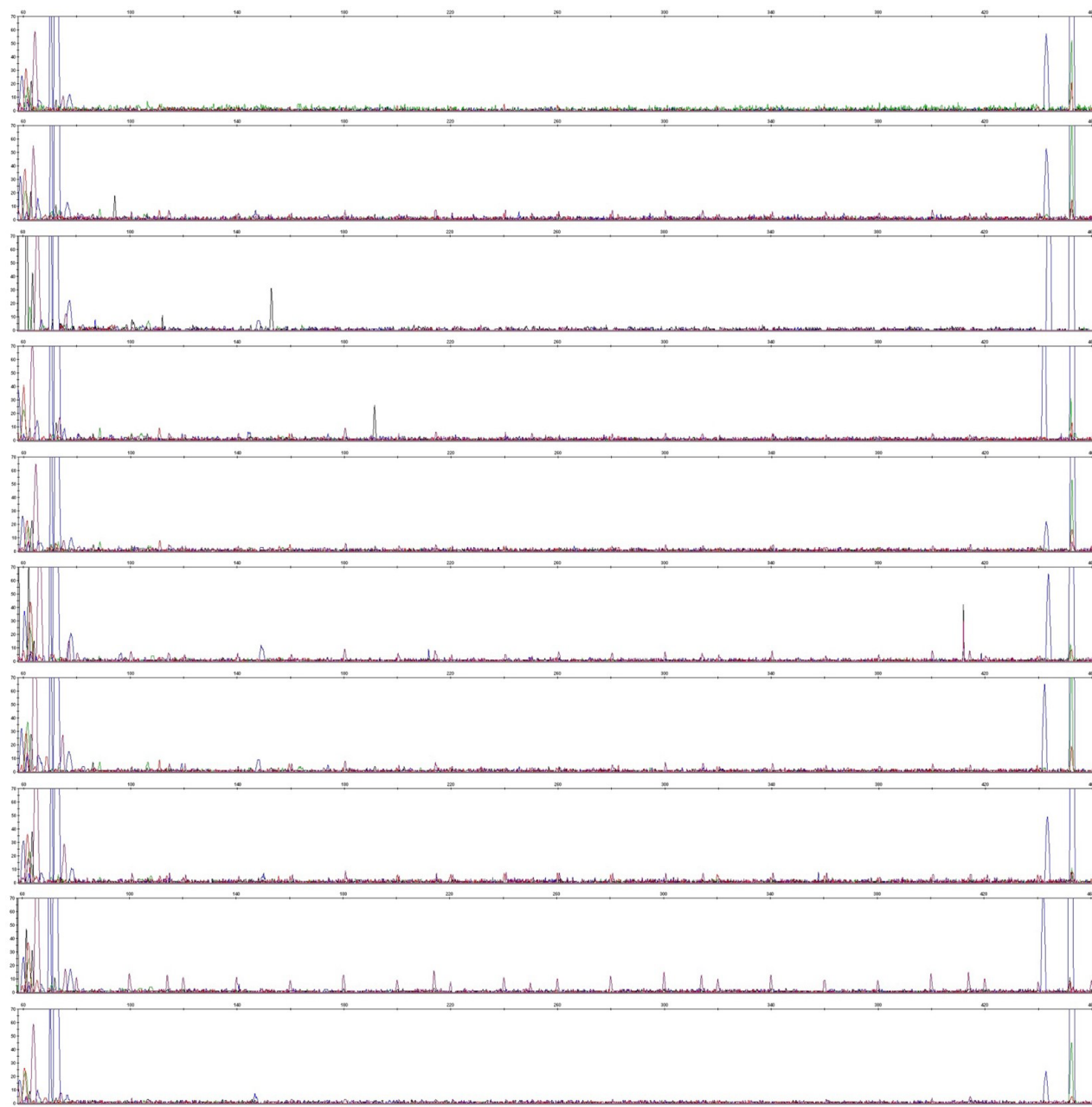


Figure 31 Results: Contamination study—Negative control runs for blood sample 1 (n=10) from RapidHIT™ ID instruments 1–10. Electropherograms are shown for 10 negative control runs from 10 instruments. Before each negative control run, a 35- μ L blood sample (male source, COT swab) was processed using the **General** protocol. The STR dyes are overlaid; the Y-axis is 70 RFU.

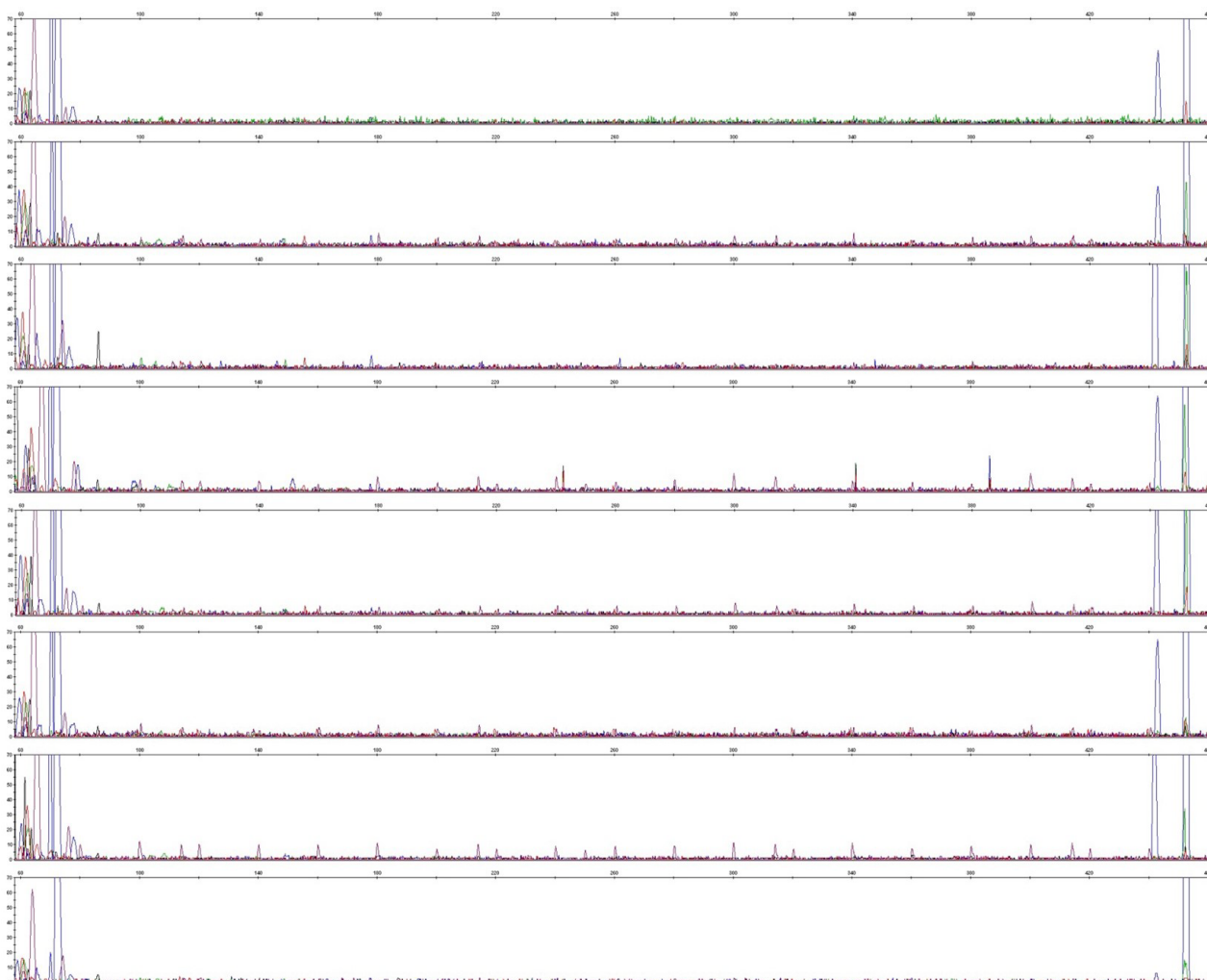


Figure 32 Results: Contamination study—Negative control runs for blood sample 2 (n=8) from RapidHIT™ ID instruments 1–8. Electropherograms are shown for 8 negative control runs from 8 instruments. Before each negative control run, a 35- μ L blood sample (female source, COT swab) was processed using the **General** protocol. The STR dyes are overlaid; the Y-axis is 70 RFU.

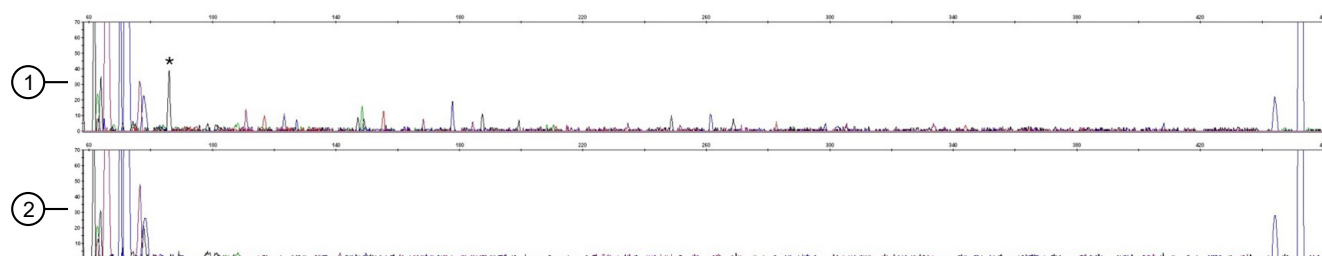


Figure 33 Results: Contamination study—Negative control runs for blood sample 2 (n=2) from RapidHIT™ ID instrument 9. Electropherograms are shown for 2 consecutive negative control runs from 1 instrument. The **General** protocol was used. The STR dyes are overlaid; the Y-axis is 70 RFU.

- ① **Top electropherogram**—Negative control run following a 35- μ L blood sample (female source, COT swab) showing 1 peak above the 35 RFU threshold (marked with an asterisk).
- ② **Bottom electropherogram**—Additional negative control run immediately following the previous negative control run (top electropherogram).

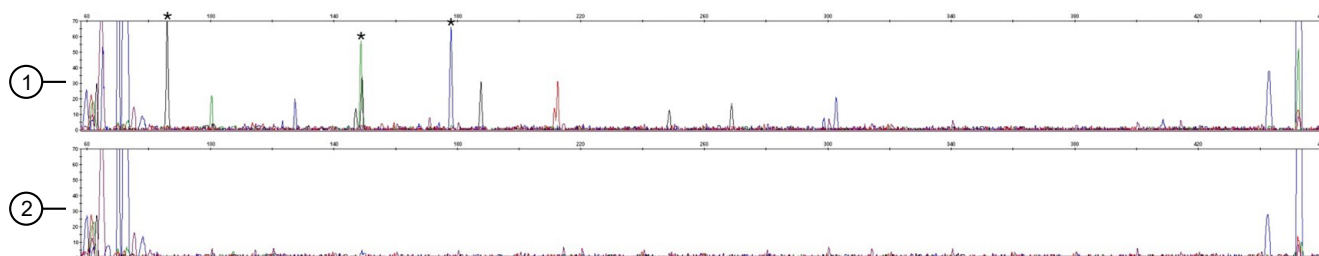


Figure 34 Results: Contamination study—Negative control runs for blood sample 2 (n=2) from RapidHIT™ ID instrument 10. Electropherograms are shown for 2 consecutive negative control runs from 1 instrument. The **General** protocol was used. The STR dyes are overlaid; the Y-axis is 70 RFU.

- ① **Top electropherogram**—Negative control run following a 35- μ L blood sample (female source, COT swab) showing peaks above the 35 RFU threshold (marked with asterisks).
- ② **Bottom electropherogram**—Additional negative control run immediately following the previous negative control run (top electropherogram).

Sensitivity study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- Blood samples were provided by 249 unique human donors (male and female).
- Saliva samples were provided by 1 unique human donor (male).
- Blood samples were deposited on HFM swabs and COT swabs at varying input amounts.
- Saliva samples were deposited on HFM swabs and COT swabs at varying input amounts.
- All swabs were dried in a biosafety cabinet overnight for better stability, then stored at 4°C until analysis.

Table 25 Sensitivity study—sample details

Swab type	No. of runs	Input amount	Protocol
Blood (249 unique donors)			
HFM swabs	353	0.05, 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, 10, 20, and 35 μ L	Specialized (100- μ L)
HFM swabs	30	1.0, 2.5, 5.0, 10, 20, and 35 μ L	General (500- μ L)
COT swabs	29	1.0, 2.5, 5.0, 10, 20, and 35 μ L	General (500- μ L)
Saliva (1 unique donor)			
HFM swabs	20	1.0, 4.0, 8.0, and 35 μ L	Specialized (100- μ L)
HFM swabs	9	8.0 and 35 μ L	General (500- μ L)
COT swabs	15	4.0, 8.0, and 35 μ L	General (500- μ L)

Run and analysis summary

There were 147 one-punch RapidINTEL™ Plus sample cartridge runs performed on 10 RapidHIT™ ID instruments.

- The **Specialized** (100-μL) protocol or the **General** (500-μL) protocol was used, as indicated in Table 25.
- The run results were assessed for the percent of alleles recovered. See Figure 35 through Figure 37.

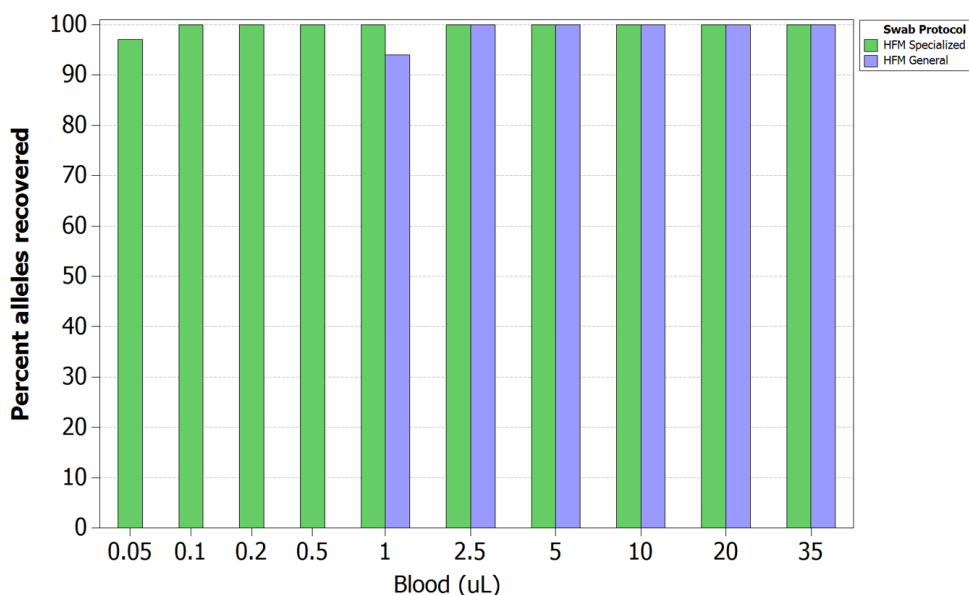


Figure 35 Results: Sensitivity study—blood samples, HFM, General and Specialized protocols The percent of alleles recovered on HFM swabs (**General** and **Specialized** protocols) is shown for 61 blood donors and 6 or 10 input amounts.

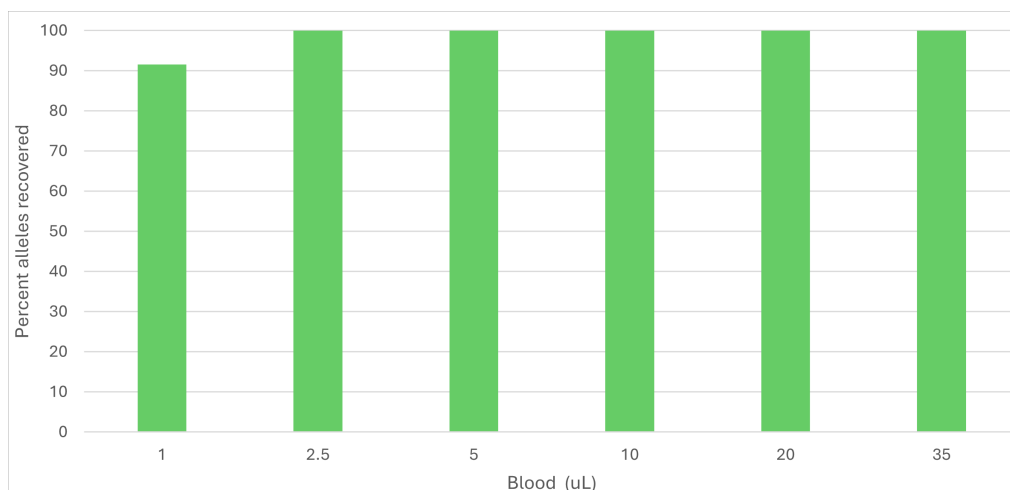


Figure 36 Results: Sensitivity study—blood samples, COT, General protocol The percent of alleles recovered on COT swabs (**General** protocol) is shown for 1 blood donor and 6 input amounts.

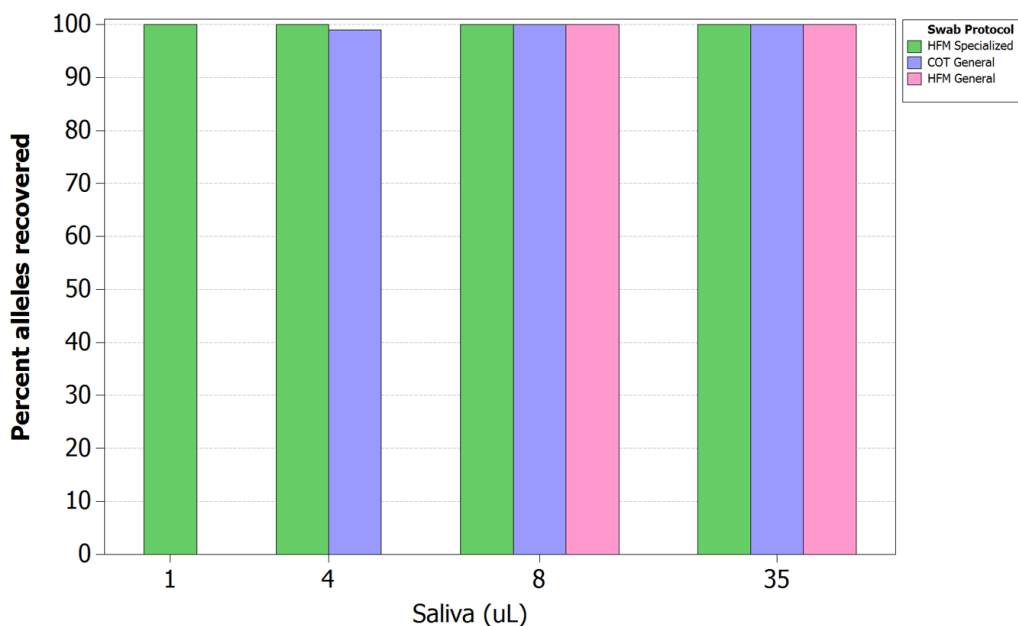


Figure 37 Results: Sensitivity study—saliva samples, HFM and COT swabs, General and Specialized protocols The percent of alleles recovered on HFM (**General** and **Specialized** protocols) and COT swabs (**General** protocol) is shown for 1 saliva donor and 2, 3, or 4 input amounts.

Population study

Cartridge and sample summary

RapidINTEL™ Plus sample cartridges were used.

- A total of 309 single-source blood samples from individuals of various ethnicities was used.
- For 249 blood samples, the blood was deposited on HFM swabs at 0.5 µL.
- For 60 blood samples, the blood was deposited on HFM swabs at 0.05 µL.
- All swabs were dried in a safety cabinet overnight for better stability, then stored at 4°C until analysis.

Run and analysis summary

There were 309 one-punch RapidINTEL™ Plus sample cartridge runs performed on 11 RapidHIT™ ID instruments.

- The **Specialized** (100-µL) protocol was used.
- For the 249 0.5-µL samples, the run results were assessed for first pass success rate, allele detection sensitivity, and genotype concordance:
 - First pass success rate was 95.5%
 - Allele detection sensitivity was 99.98% (1 null allele)
 - Genotype concordance was 99.2%; see Figure 38
- For the 60 0.05-µL samples, the run results were assessed for STO flag indicators. See Figure 39.

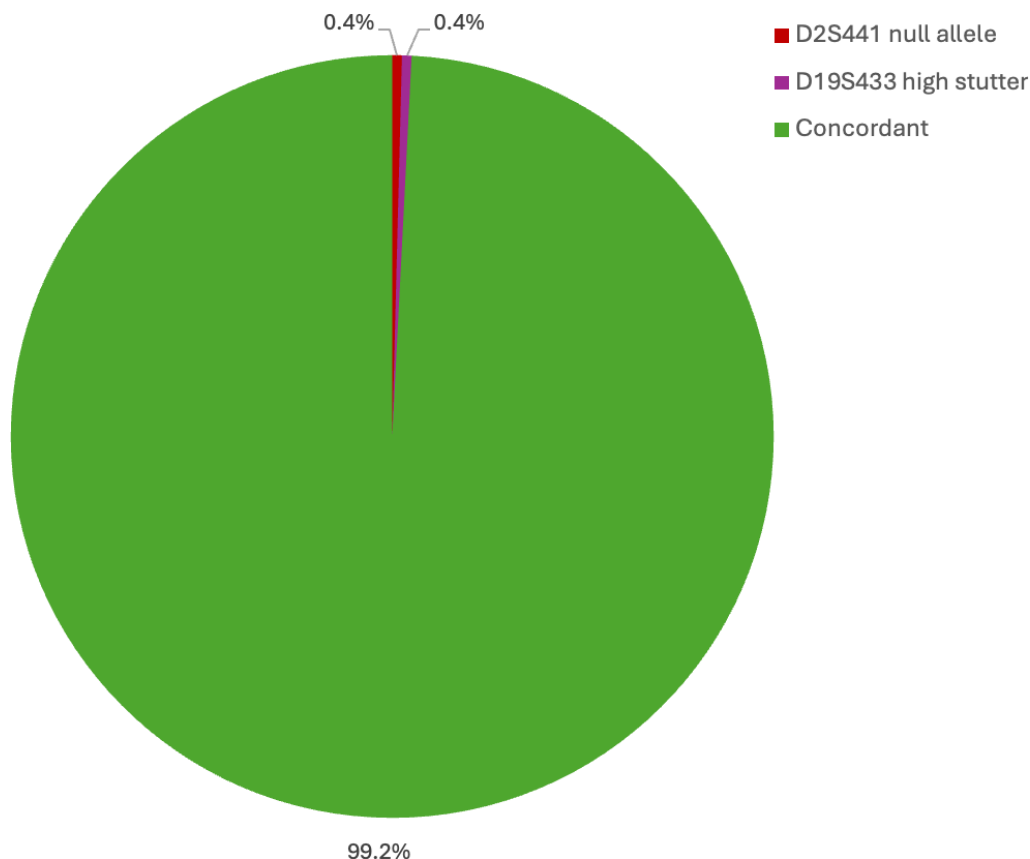


Figure 38 Results: Population study—genotype concordance Concordance is shown for 249 population sample runs. 247 out of 249 runs (99.2%) gave completely concordant genotypes. Known artifacts, flagged stutter, low-level single spurious peaks, and flagged OL (off-ladder) were removed during analysis of the data set.

STO Flag	Sample Count	First-Pass Success	Average STR Peak Height (RFU)	Samples with Drop-Out	STO Flag Confidence
 ≤ 225 RFU Average Large STR PH	64	20.3% (13)	135	25	60.9% (14/40)
 > 225 RFU, ≤ 575 RFU Average Large STR PH	70	90.0% (63)	369	1	98.6% (69/70)
 > 575 RFU Average Large STR PH	354	93.5% (331)	1469	0	100%

Figure 39 Results: Population study—STO flag indicators Comparison of first pass success rate, allele recovery, average STR peak height, number of samples displaying allelic drop-out, STO flag confidence, and allelic drop-out confidence for each STO flag indicator.



Thresholds, flags, and artifacts

■ Development of the RapidINTEL™ Plus system thresholds	92
■ Stochastic (STO) quality flag	97
■ Quantification markers	98
■ Amplification artifacts	100
■ System artifact thresholds and filters	102

Development of the RapidINTEL™ Plus system thresholds

Specific system thresholds are required to address the sensitivity of the RapidINTEL™ Plus Sample Cartridge and primary analysis performed on RapidHIT™ ID System v2.0.

RapidINTEL™ Plus thresholds are shown in “RapidINTEL™ Plus peak detection and filtering thresholds” on page 92 and “RapidINTEL™ Plus stutter thresholds” on page 93. For more information on thresholds, see the *RapidHIT™ ID System v2.0 User Guide* (Pub. No. [MAN0026593](#)).

RapidINTEL™ Plus peak detection and filtering thresholds

Marker	Peak amplitude threshold	Local cutoff	Minimum peak height ratio	Heterozygous minimum peak height	Homozygous minimum peak height
Amelogenin	35	0.10	0.20	35	153
CSF1PO	35	0.10	0.20	50	219
D10S1248	35	0.12	0.20	50	215
D12S391	35	0.11	0.20	50	210
D13S317	35	0.10	0.20	50	213
D16S539	35	0.11	0.20	50	186
D18S51	35	0.10	0.20	50	244
D19S433	35	0.20	0.20	50	187
D1S1656	35	0.10	0.20	50	265
D21S11	35	0.10	0.20	50	210
D22S1045	35	0.11	0.20	50	269
D2S1338	35	0.13	0.20	50	299

(continued)

Marker	Peak amplitude threshold	Local cutoff	Minimum peak height ratio	Heterozygous minimum peak height	Homozygous minimum peak height
D2S441	35	0.11	0.20	50	300
D3S1358	35	0.10	0.20	50	180
D5S818	35	0.11	0.20	50	235
D7S820	35	0.10	0.20	50	299
D8S1179	35	0.10	0.20	50	141
DYS391	35	0.10	0.00	35	35
FGA	35	0.10	0.20	50	150
SE33	35	0.10	0.20	60	311
TH01	35	0.13	0.20	50	209
TPOX	35	0.10	0.20	50	164
vWA	35	0.13	0.20	50	287
Y indel	60	0.10	0.00	60	60

RapidINTEL™ Plus stutter thresholds

Marker	Reverse (minus 1 repeat)	Forward (plus 1 repeat)	N-1	N-2
Amelogenin	N/A	N/A	N/A	N/A
CSF1PO	0.19	0.03	N/A	N/A
D10S1248	0.22	0.03	N/A	N/A
D12S391	0.27	0.03	N/A	N/A
D13S317	0.25	0.03	N/A	N/A
D16S539	0.17	0.04	N/A	N/A
D18S51	0.25	0.05	N/A	N/A
D19S433	0.22	0.03	N/A	N/A
D1S1656	0.29	0.03	N/A	0.03
D21S11	0.25	0.05	N/A	N/A
D22S1045	0.29	0.12	N/A	N/A
D2S1338	0.26	0.03	N/A	N/A
D2S441	0.20	0.03	0.30	N/A

(continued)

Marker	Reverse (minus 1 repeat)	Forward (plus 1 repeat)	N-1	N-2
D3S1358	0.22	0.03	N/A	N/A
D5S818	0.17	0.03	N/A	N/A
D7S820	0.19	0.03	N/A	N/A
D8S1179	0.19	0.03	N/A	N/A
DYS391	0.18	0.03	N/A	N/A
FGA	0.26	0.03	N/A	N/A
SE33	0.33	0.06	N/A	0.20
TH01	0.14	0.03	N/A	N/A
TPOX	0.14	0.03	N/A	N/A
vWA	0.22	0.03	N/A	N/A
Y indel	N/A	N/A	N/A	N/A

Stutter percentages for RapidINTEL™ Plus STR markers

The calculated stutter percentage for STR markers, grouped by dye channel, are shown in Figure 40 through Figure 44. Stutter values were calculated from capillary electrophoresis peak height data from studies of 2,095 samples. Allele numbers on the X-axis correspond to the *parent* allele identity.

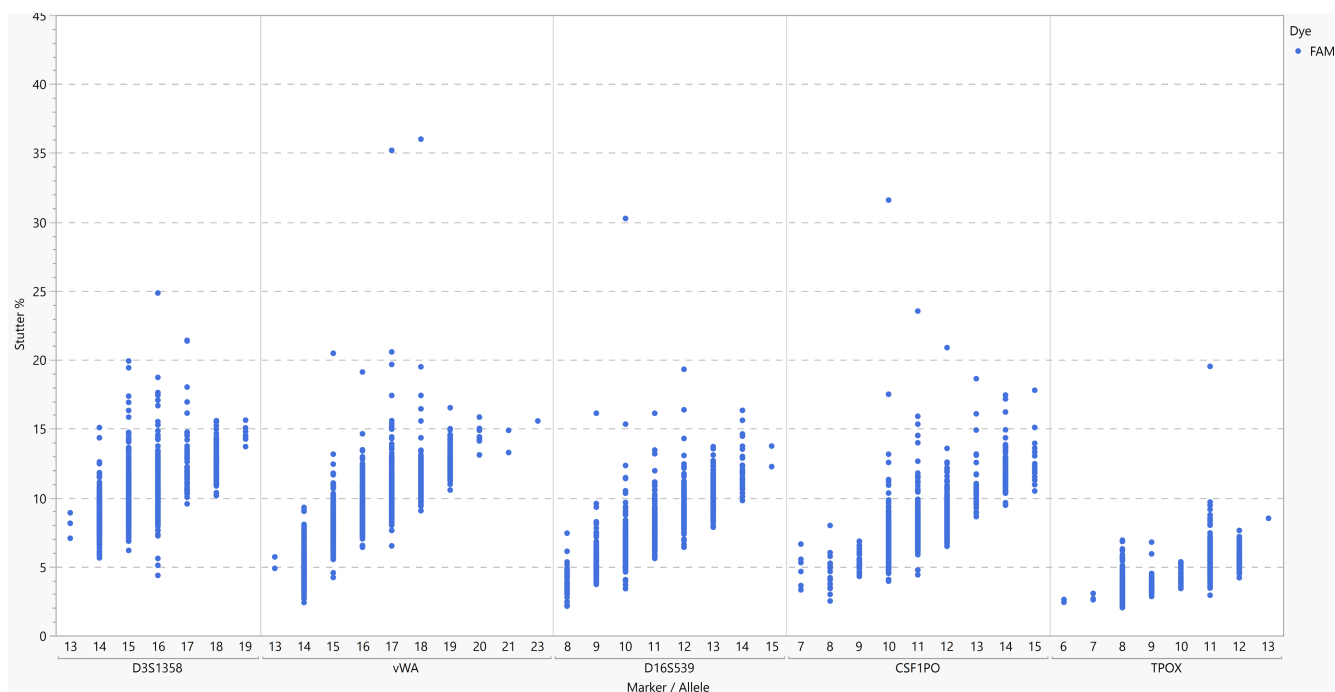


Figure 40 FAM™ dye: Stutter percent vs. marker and allele

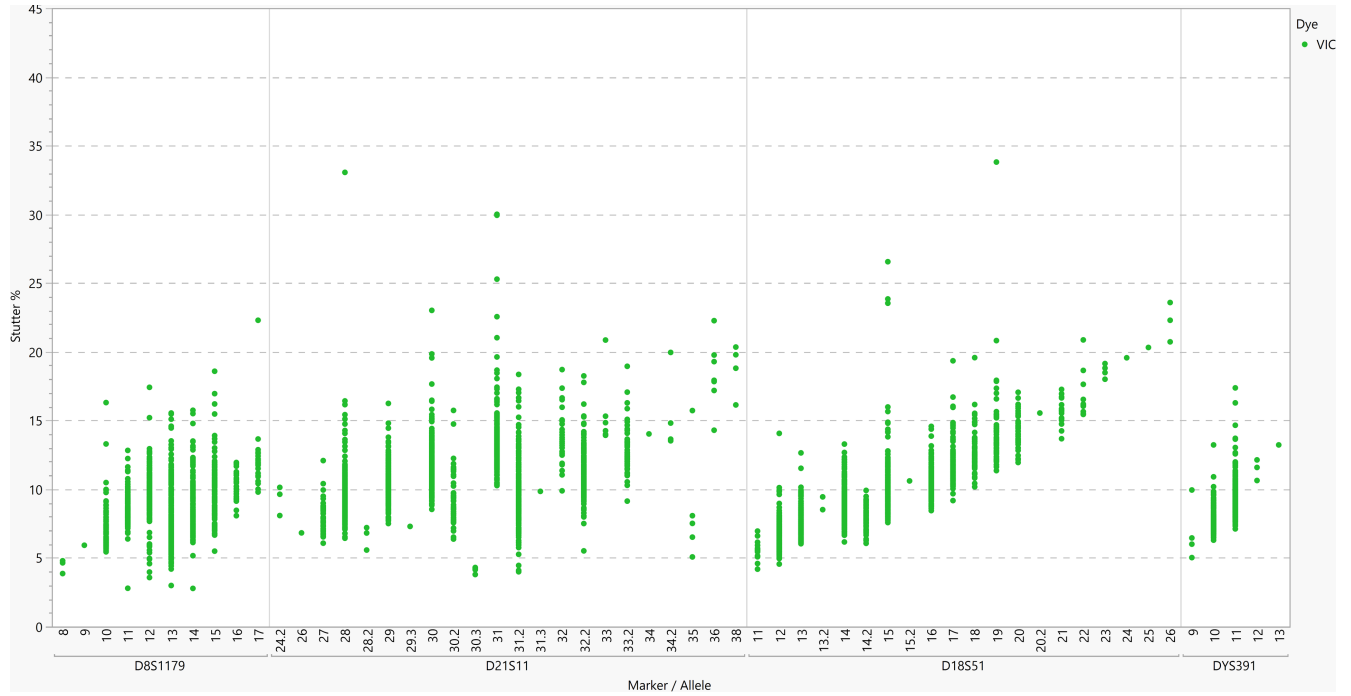


Figure 41 VIC™ dye: Stutter percent vs. marker and allele

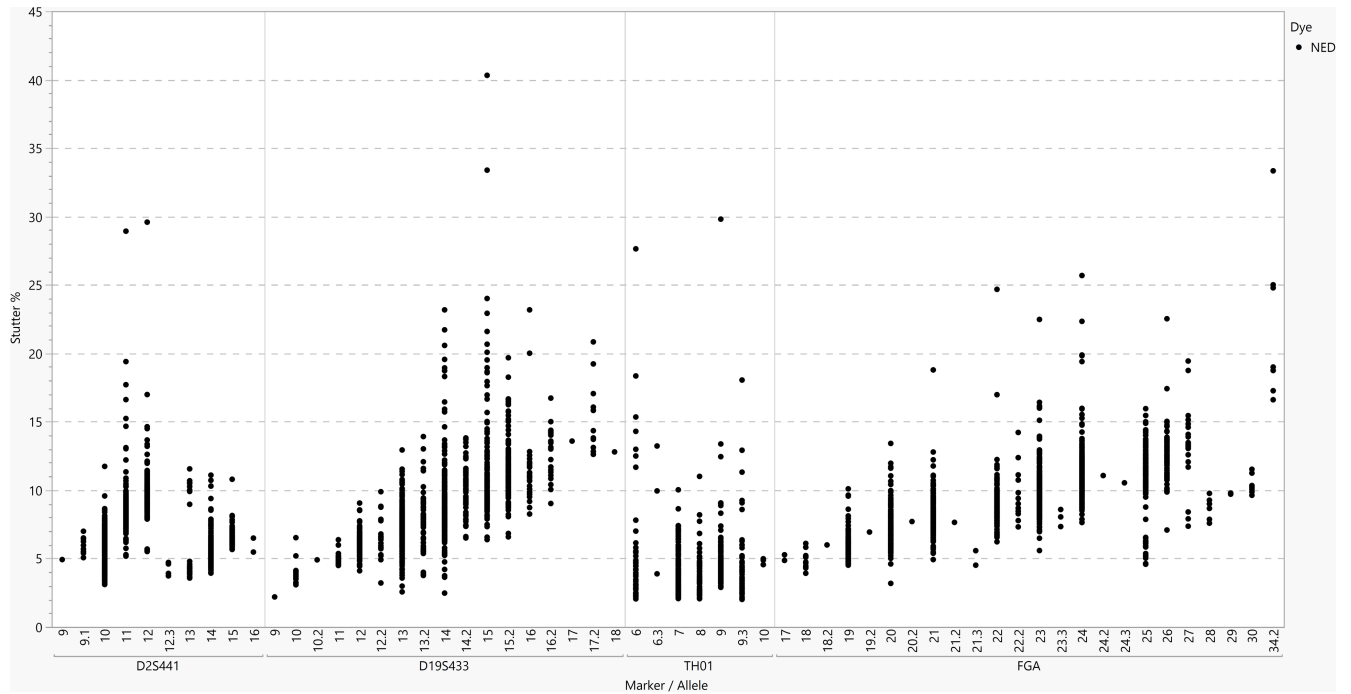


Figure 42 NED™ dye: Stutter percent vs. marker and allele



Appendix A Thresholds, flags, and artifacts

Development of the RapidINTEL™ Plus system thresholds

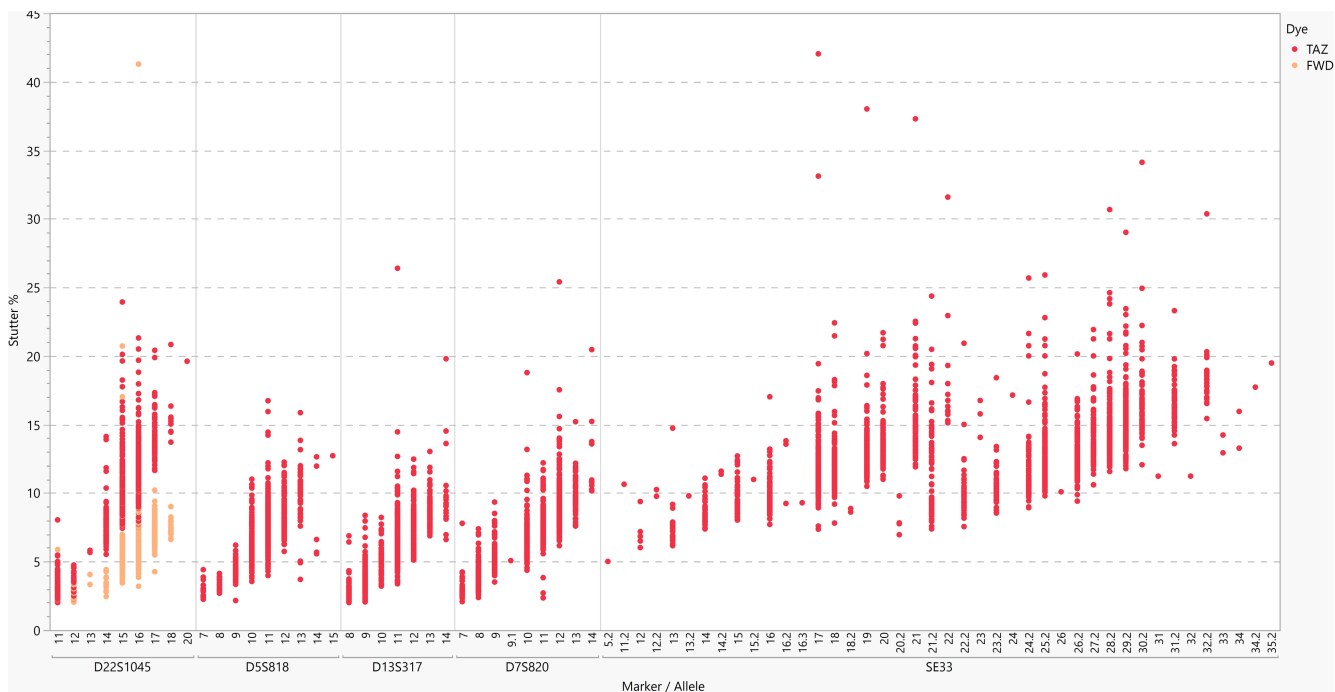


Figure 43 TAZ™ dye: Stutter percent vs. marker and allele The lighter-colored (orange-pink) data points in the D22S1045 locus indicate plus stutter.

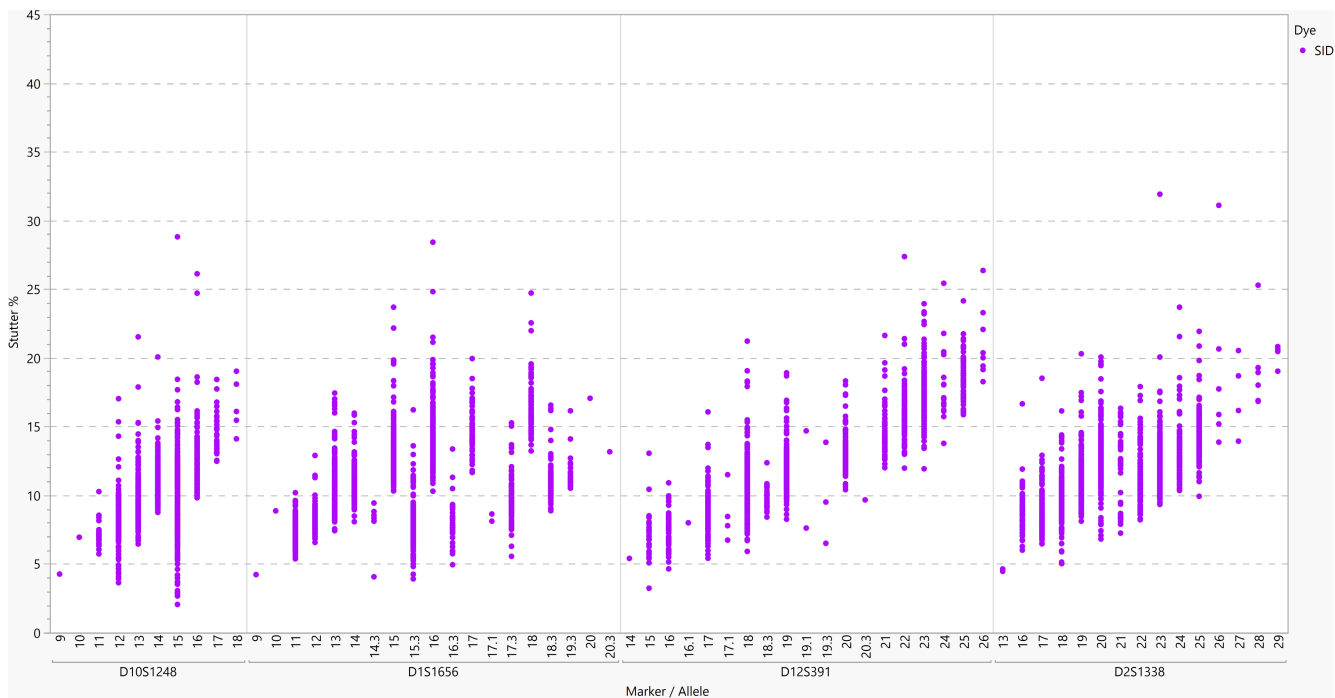


Figure 44 SID™ dye: Stutter percent vs. marker and allele

Stochastic (STO) quality flag

In RapidLINK™ Software v2.0, the STO quality flag assesses the likelihood of a sample having peak height imbalances <40% or allelic dropout. This assessment is based on the combined average peak height of a subset of larger STR loci (CSF1PO, D18S51, FGA, SE33, and D2S1338).

In RapidLINK™ Software v2.0, the STO flag is displayed as follows:

RapidHIT™ ID System v2.0 and RapidLINK™ Software v2.0 provide confidence estimates for sample profiles by monitoring the peak heights of several STR markers in the RapidINTEL™ Plus multiplex. This is communicated to the user via the STO quality flag and indicates the likelihood of allele dropouts due to stochastic effects in low-DNA quantity samples. Based on the average peak height of CSF1PO (FAM™ dye), D18S51 (VIC™ dye), FGA (NED™ dye), SE33 (TAZ™ dye), and D2S1338 (SID™ dye) allele peaks, the classification of profiles is made according to the rules shown in Table 26.

Note: Data from the 1,513 sample calibration data set were used to determine the RFU cutoff values for the three STO flag indicators. These RFU cutoff values remained accurate, even after including an additional 829 samples in the validation and verification studies. In the case-type study (page 59), the samples slightly deviated from the percentages shown in Table 26, due to challenging sample types that are known to exhibit strong inhibition (for example, cigarettes and chewing gum).

Table 26 STO flag indicators

STO flag indicator	Average peak height (loci subset)	Description ^[1]	Internal testing ^[2]	
			No. of flagged samples	% of samples with dropout
	>575 RFU	The combined average peak height of these loci is >575 RFU, which indicates that the sample is unlikely to have peak height imbalances or allelic dropout.	1,379 samples	0.15%
	>225 RFU and ≤575 RFU	The combined average peak height of these loci is between 226 and 575 RFU, which indicates that the sample is likely to have peak height imbalances but unlikely to have allelic dropout.	432 samples	1.4%
	≤225 RFU	The combined average peak height of these loci is ≤225 RFU, which indicates that the sample is likely to have allelic dropout.	531 samples	75.3%

^[1] The two highest peaks from each locus were used for the average peak height calculation.

^[2] During internal testing, we tested a total of 2,342 samples. These two columns indicate: 1) the number of samples that produced the flag and 2) the percent of samples with allelic dropout.

Quantification markers

RapidINTEL™ Plus multiplex contains two quant markers, QQS 1 (small quant marker) and QQL 1 (large quant marker), that amplify highly conserved (non-STR) regions of human gDNA. The peak heights of these markers increase or decrease with the amount of DNA in a sample. Estimations (QTS, QTL) of human gDNA in the PCR reaction are based on the amplification of the two quantification targets.

- **QQS 1 (small quant marker)**—The small target at 64 bp estimates the total load of human gDNA in the PCR reaction.
- **QQL 1 (large quant marker)**—The large target at 408 bp estimates the amount of human gDNA present at the size of the larger STRs in the assay.

A measurement systems analysis (MSA) was performed using a range of purified gDNA inputs, from 60 pg to 1,000 pg. Peak heights for the small target plotted to a linear equation, while peak heights for the large target plotted to a quadratic equation (see Figure 45). Due to the large target's response to human gDNA load, the system can only estimate $\leq 1,600$ pg at the large target.

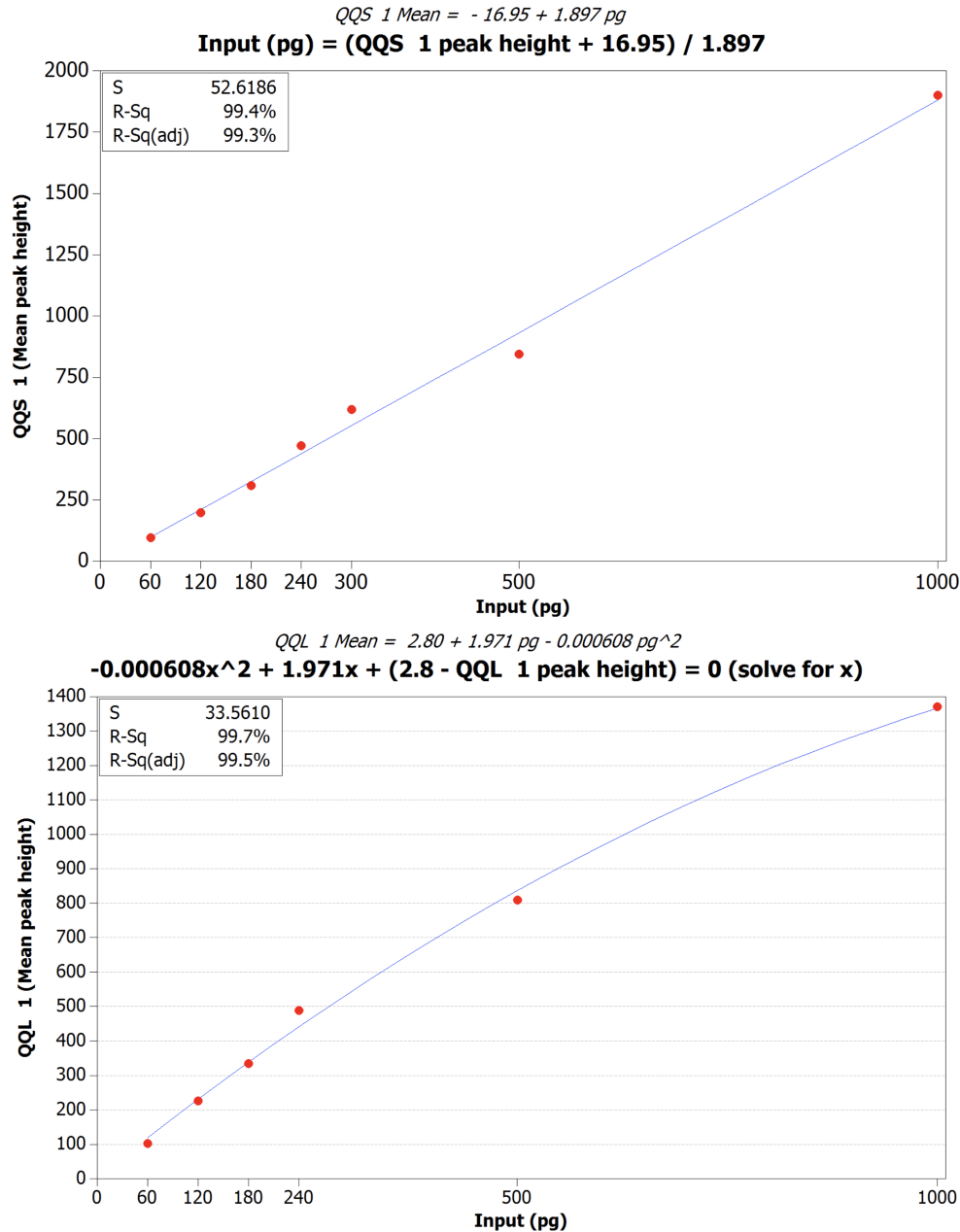


Figure 45 Small and large quant markers QQS 1 and QQL 1 The top (QQS 1) and bottom (QQL 1) graphs show the results of a MSA of quant marker peak height response across a range of input DNA amounts. Analysis of the MSA data was used to develop regression formulas that were incorporated into RapidHIT™ ID System v2.0 that allow the system to estimate input DNA amounts from QQS 1 and QQL 1 peak heights.

Amplification artifacts

Artifacts seen with the RapidINTEL™ Plus assay are commonly the result of a high sample load. When the QTS value is >2,000 pg (QQS 1 peak height is >3,800 RFU), the sample runs the risk of producing one or more artifacts.

The D19S433 locus is susceptible to producing extreme stutter amplification of 30–45%, which exceeds the stutter threshold of 22% set for the D19S433 locus. The D19S443 artifact is of concern for homozygote samples where the peak may not trigger a flag at the locus when the sample input level far exceeds the optimal range. TH01 may show a –5 bp amplification artifact of 10–30% of the main peak. See Figure 46.



Figure 46 Potential artifacts seen in the NED™ dye channel at D19S433 and TH01 when sample loads are excessive. The QQS 1 peak is >3,800 RFU and the QTS value in RapidLINK™ Software v2.0 is >2 ng. D19S433 displays extremely high stutter (30–45%) and TH01 shows a peak 5 bp in front of a main allele peak. The inset shows a closer view of the D19S433 and TH01 artifact peaks circled in red. The QQS loci (outlined by the red rectangle in the FAM™ dye channel) shows a QQS 1 quantification peak of 14,875, an estimated sample load of 7.85 ng.

Single-source female samples may see pull-up into the male-only loci in the assay. Pull-up peaks may be seen in the YINDEL and DYS391 loci when peaks in D2S441 are greater than ~3,000 RFU (YINDEL) or peaks in TPOX are greater than ~1,750 RFU (D2S441). Pull-up peaks <2% are expected to be removed by the system optics. The common alleles in D2S441 that overlap with the YINDEL allele sizes are D2S441 10 and D2S441 11. Both produce an OL allele call in the YINDEL when present and generate a flag for the locus. The only D2S441 allele to overlap with a YINDEL allele, YINDEL-1, is the rare microvariant D2S441 9.1. See Figure 47.

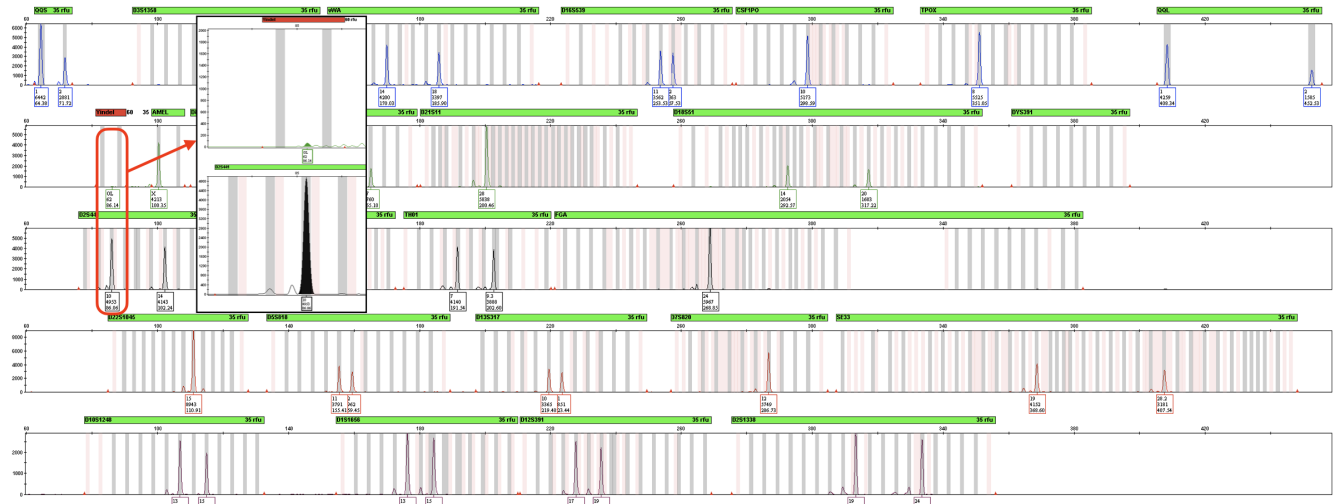


Figure 47 YINDEL pull-up Female samples with high input may show pull-up at the YINDEL locus because of significant amplification of allele D2S441 10. Pull-up from D2S441 10 into the VIC™ dye channel at ~86 bp produces an OL allele call for YINDEL when no true YINDEL allele is present to generate a local peak height threshold. The inset shows the 1.3% D2S441 10 pull-up producing an OL peak of 62 RFU at the YINDEL locus.

The common alleles in TPOX that may cause pull-ups in DYS391 are TPOX 11 and TPOX 12. TPOX 11 pull-up produces a DYS391 6 allele while TPOX 12 may produce a DYS391 7 allele. Most observed incidents of pull-up in the male loci of single-source female samples are from homozygote peaks. See Figure 48.

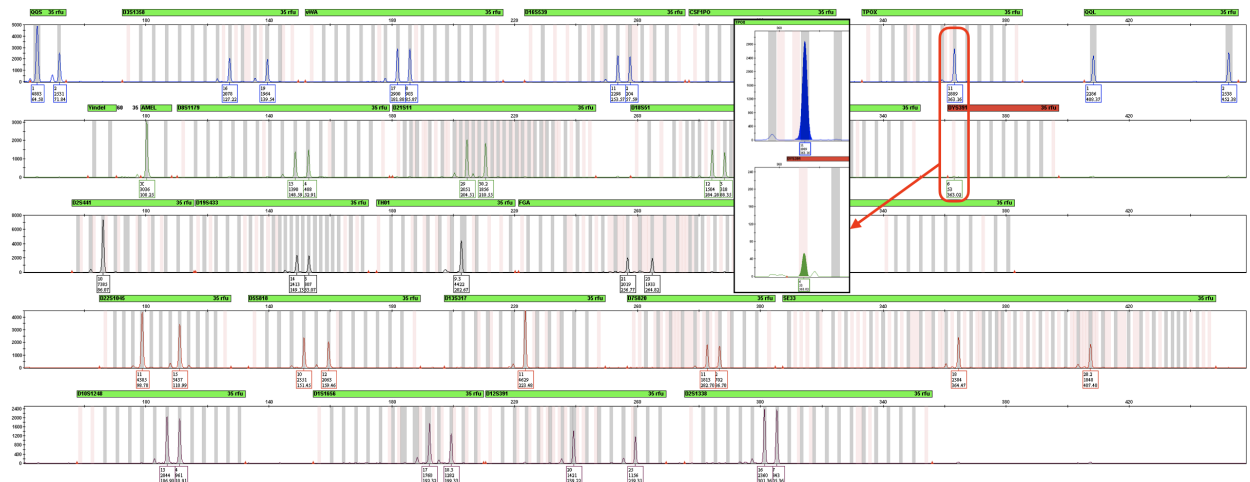


Figure 48 DYS391 pull-up

Female samples with high input may show pull-up at the DYS391 locus because of significant amplification of alleles in TPOX. Pull-up from TPOX alleles 11 and 12 into the VIC™ dye channel produce allele calls for DYS391 6 (TPOX 11) or DYS391 7 (TPOX 12) when no true DYS391 allele is present to generate a local peak height threshold. The inset shows the 1.8% TPOX 11 pull-up producing a DYS391 6 peak of 53 RFU.

System artifact thresholds and filters

The addition of the quantification (QQS 1 and QQL 1) and amplification (QQS 2 and QQL 2) markers to the RapidINTEL™ Plus assay required analysis filters for potential amplification artifacts caused by their incorporation.

Using the stutter filter function, the –0.25 to –4.8 bp artifact seen with QQS 2 is filtered at 90% of the main peak. The filter is tuned to avoid filtering the QQS 1 peak by the cascading function of the stutter. In addition, a plus stutter threshold of 90% for the range of +0.25 to +4.0 bp is implemented to filter possible noise peaks generated when the QQS 2 peak reaches saturation levels. Filtering within the QQS locus is difficult because the two targets are unrelated and can have exponentially different amplification.

The QQL 2 target may produce an artifact at –10.5 bp. A minus stutter filter threshold of 40% for the range of –0.5 to –12.5 bp is implemented to filter this artifact. A plus stutter threshold of 40% for the range of +0.5 to +5.0 bp is implemented to filter possible noise peaks generated when the QQL 2 peak reaches saturation levels.

Amelogenin and the Y indel loci are non-STR loci and have no microvariants. To avoid noise generated from saturated alleles or high pull-ups in off ladder (OL) locations of these loci, stutter filter functions were implemented. Amelogenin uses stutter thresholds of 30% from –0.5 to –4.0 bp and +0.5 to +4.0 bp. Y indel uses stutter thresholds of 20% from –1.0 to –3.0 bp and +1.0 to +3.0 bp.

To mitigate noise peaks in non-STR regions following the QQS and Y indel loci, the blackout region filter is used. The 6-FAM™ dye channel blackout range is 76.0–91.0 bp. The VIC™ dye channel blackout range is 89.7–98.0 bp.



Supplemental information

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Contents and storage

Table 27 RapidINTEL™ Plus sample cartridge kits

Kit	Cat. No.	Contents	Amount	Storage
RapidINTEL™ Plus 50 Sample Cartridge Kit	A54338	RapidINTEL™ Plus Sample Cartridge	50	15–25°C for ≤2 months, or 4–10°C until the expiration date on the label
		RapidINTEL™ Plus Negative Control Cartridge	2	
		RapidINTEL™ Plus Positive Control Cartridge	2	
RapidINTEL™ Plus Sample Cartridge Evaluation Kit	A55598	RapidINTEL™ Plus Sample Cartridge	10	

Table 28 RapidHIT™ ID Primary Cartridge GlobalFiler™ Express 150 Kit (Cat. No. [A53084](#))

Contents	Amount	Storage
Box 1		
RapidHIT™ ID Primary Cartridge— 150 runs	1 (for 150 runs)	Room temperature (15–30°C)
RapidHIT™ ID Utility Cartridge (for installation or primary cartridge replacement)	2	
Box 2		
RapidHIT™ ID Gel Cartridge (for installation or primary cartridge replacement)	1	2–8°C
RapidHIT™ ID ACE GlobalFiler™ Express Control (Ladder) Cartridge (allelic ladder for installation or primary cartridge replacement)	1	4–10°C
RapidHIT™ ID ACE GlobalFiler™ Express Positive Control Cartridge (for installation or primary cartridge replacement)	1	

Table 29 RapidINTEL™ Plus allelic ladder, control, and utility cartridges

Contents	Cat. No.	Amount	Storage
RapidINTEL™ Plus Ladder Cartridge Kit (allelic ladder)	A54344	5	4–10°C
RapidINTEL™ Plus Negative Control Cartridge	A54343	1	
RapidINTEL™ Plus Positive Control Cartridge	A54342	1	
RapidHIT™ ID Utility Cartridge	A42471	1	Room temperature (15–30°C)

RapidINTEL™ Plus assay markers and dye labels

Dye	Color	Markers by dye
6-FAM™	Blue	QQS, D3S1358, vWA, D16S539, CSF1PO, TPOX, QQL
VIC™	Green	Y indel, Amelogenin, D8S1179, D21S11, D18S51, DYS391
NED™	Yellow	D2S441, D19S433, TH01, FGA
TAZ™	Red	D22S1045, D5S818, D13S317, D7S820, SE33
SID™	Purple	D10S1248, D1S1656, D12S391, D2S1338
LIZ™	Orange	GeneScan™ 600 LIZ™ Size Standard v2.0



Documentation and support

Related documentation

Document	Publication number
<i>RapidHIT™ ID System v2.0 User Guide</i>	MAN0026593
<i>RapidINTEL™ Plus Sample Cartridge User Bulletin</i>	MAN0026595
<i>RapidLINK™ Matching Suite Software v1.0 User Guide</i>	MAN0030052
<i>RapidLINK™ Software v2.0 User Guide</i>	MAN0030010
<i>SmallPond™ DNA Matching System Installation and Setup Guide</i>	MAN0025091
<i>Technical Note: Artifacts Identified Post-Developmental Validation: RapidHIT™ ID ACE GlobalFiler™ Express and RapidINTEL™ Sample Cartridges</i>	TCF0000734
<i>Technical Note: Impact on Genotyping Results when a Native Allelic Ladder is not Available in the Applied Biosystems™ RapidHIT™ ID Allelic Ladder Library</i>	TCF1000105

Customer and technical support

For support, use one of the contact methods listed below, depending on your location.

Location	Contact method
In North America	Send an email to: HIDTechSupport@thermofisher.com
	Call 888-821-4443; select option 2, say "Application Support", then say "HID" or "Human Identification".
Outside North America	Contact your local support office.

For the latest services and support information for all locations, go to [thermofisher.com/support](https://www.thermofisher.com/support) to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.



Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have questions, contact Life Technologies at www.thermofisher.com/support.



Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).

- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020

- [cdc.gov/labs/bmbi](https://www.cdc.gov/labs/bmbi)

Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)

[who.int/publications/i/item/9789240011311](https://www.who.int/publications/i/item/9789240011311)

