Validation of Rapid DNA Methods

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Standards and Technology
U.S. Department of Commerce

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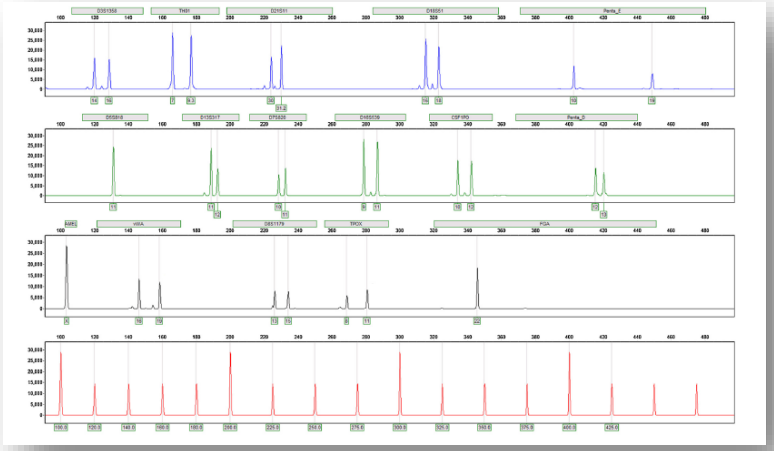
Rapid DNA (RDNA) Typing
Forensic DNA Typing

- Laboratory process: *DNA extraction, quantification, amplification (PCR), separation/detection, data analysis*

- Rapid DNA – fully integrated process

Sample = swab in



Answer = STR Profile out

Sampling of RDNA Instruments

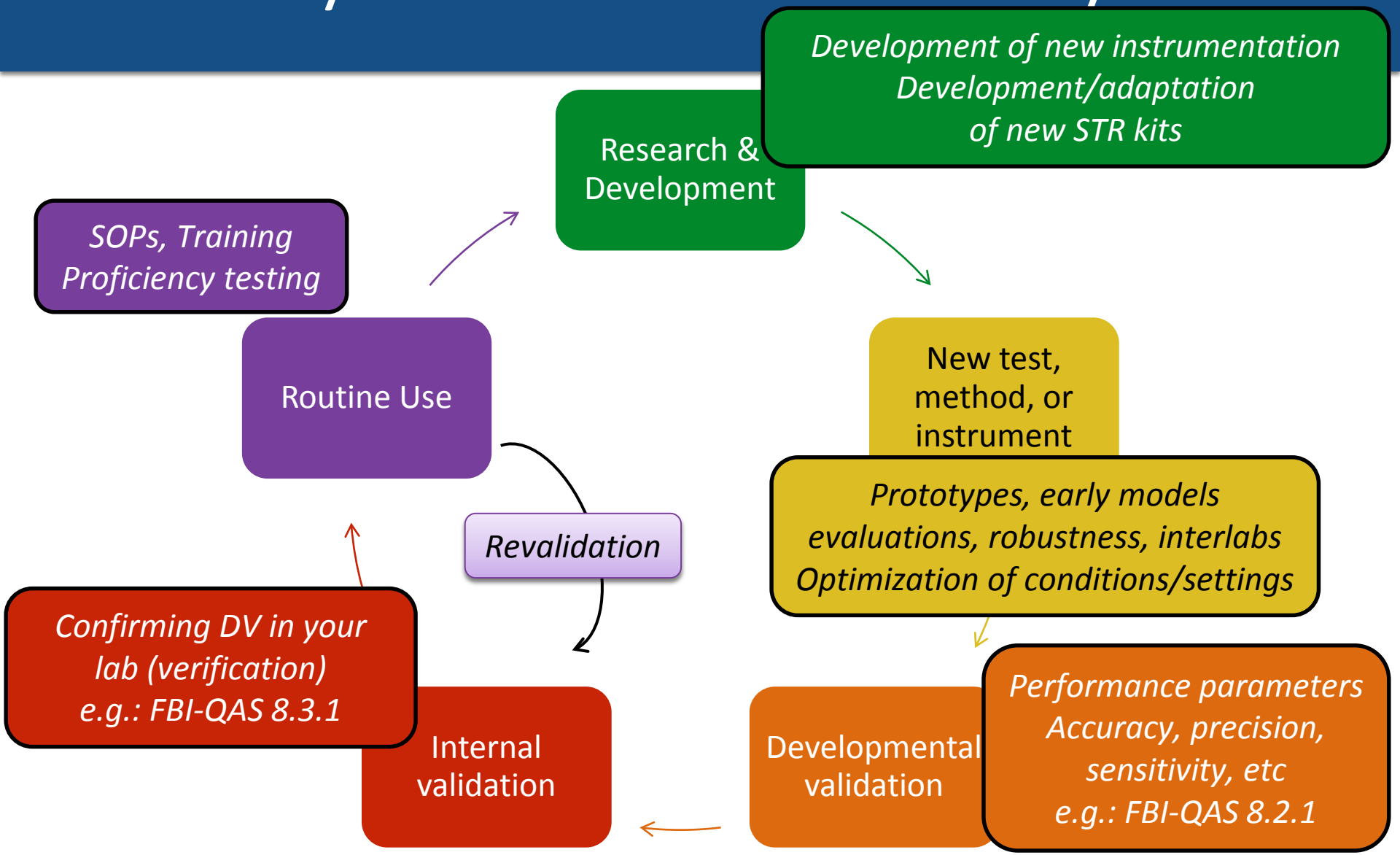
IntegenX

- RapidHIT 200
  - PowerPlex 16HS
  - Globalfiler
- RapidHIT ID
  - Globalfiler

NetBio/ANDE

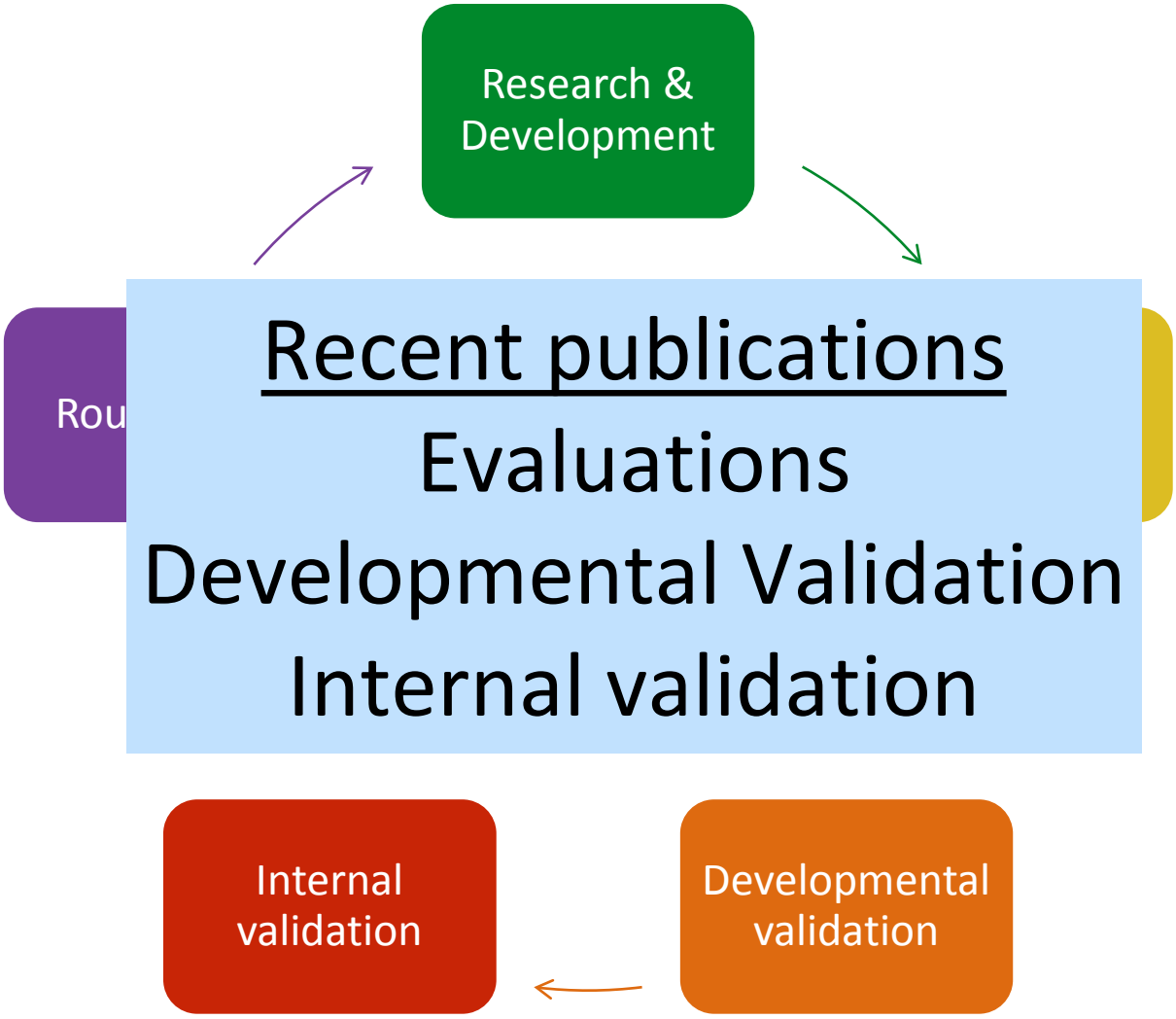
- ANDE/DNAScan
  - PowerPlex 16
- ANDE
  - FlexPlex (27)

Lifecycle of a method of analysis



Adapted from Feinberg et al. (2004) Anal. Bioanal. Chem. 380: 502-514

Lifecycle of a method of analysis



Adapted from Feinberg et al. (2004) Anal. Bioanal. Chem. 380: 502-514

Evaluation

- First pass check into how an instrument or method performs
- Might be performed on prototypes, early access equipment
  - Instrument and reagents might still be optimized by the developer
- Might be structured similar to a “formal” validation
  - Run a number of samples to check the accuracy, sensitivity
  - Assess performance over multiple cartridges
  - Anything you might be interested in testing

Could be somewhat subjective

Evaluations

Forensic Science International: Genetics 23 (2016) 1–8

Contents lists available at ScienceDirect

Forensic Science International: Genetic

journal homepage: www.elsevier.com/locate/fsig

Research paper

Evaluation of the RapidHIT™ 200 and RapidHIT GlobalFiler kit for fully automated STR genotyping

Mavis Date-Chong*, William R. Hudlow, Martin R. Buoncristiani

Jan Bashinski DNA Laboratory, Bureau of Forensic Services, California Department of Justice, Richmond, CA 94804, USA

Forensic Science International: Genetics 19 (2015) 22–27

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Evaluation of the RapidHIT™ 200 System: A comparative study of its performance with Maxwell® DNA IQ™/Identifiler® Plus/ABI 3500xL workflow

Zhonghui Thong [a,1,*], Yong Han Phua [a,1,**], Eileen Shuzhen Loo [a], Sze Kae Goh [a], Jiatian Ang [a], Woan Foon Looi [a], Christopher Kiu Choong Syn [a,b]

[a] *DNA Profiling Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, 169078, Singapore*
[b] *Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore*

Forensic Science International: Genetics Supplement Series 5 (2015) e1–e2

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

Rapid DNA maturity assessment

Erica L. Romsos [a,*], Sanae Lembirick [b], Peter M. Vallone [a]

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Forensic Science International: Genetics 13 (2014) 104–111

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

An evaluation of the RapidHIT® system for reliably genotyping reference samples

Bobby L. LaRue [a,*], Andrea Moore [a], Jonathan L. King [a], Pamela L. Marshall [a], Bruce Budowle [a,b]

[a] *Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, Fort Worth, TX, USA*
[b] *Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia*

Forensic Science International: Genetics 14 (2015) 76–85

Contents lists available at ScienceDirect

Forensic Science International: Genetics

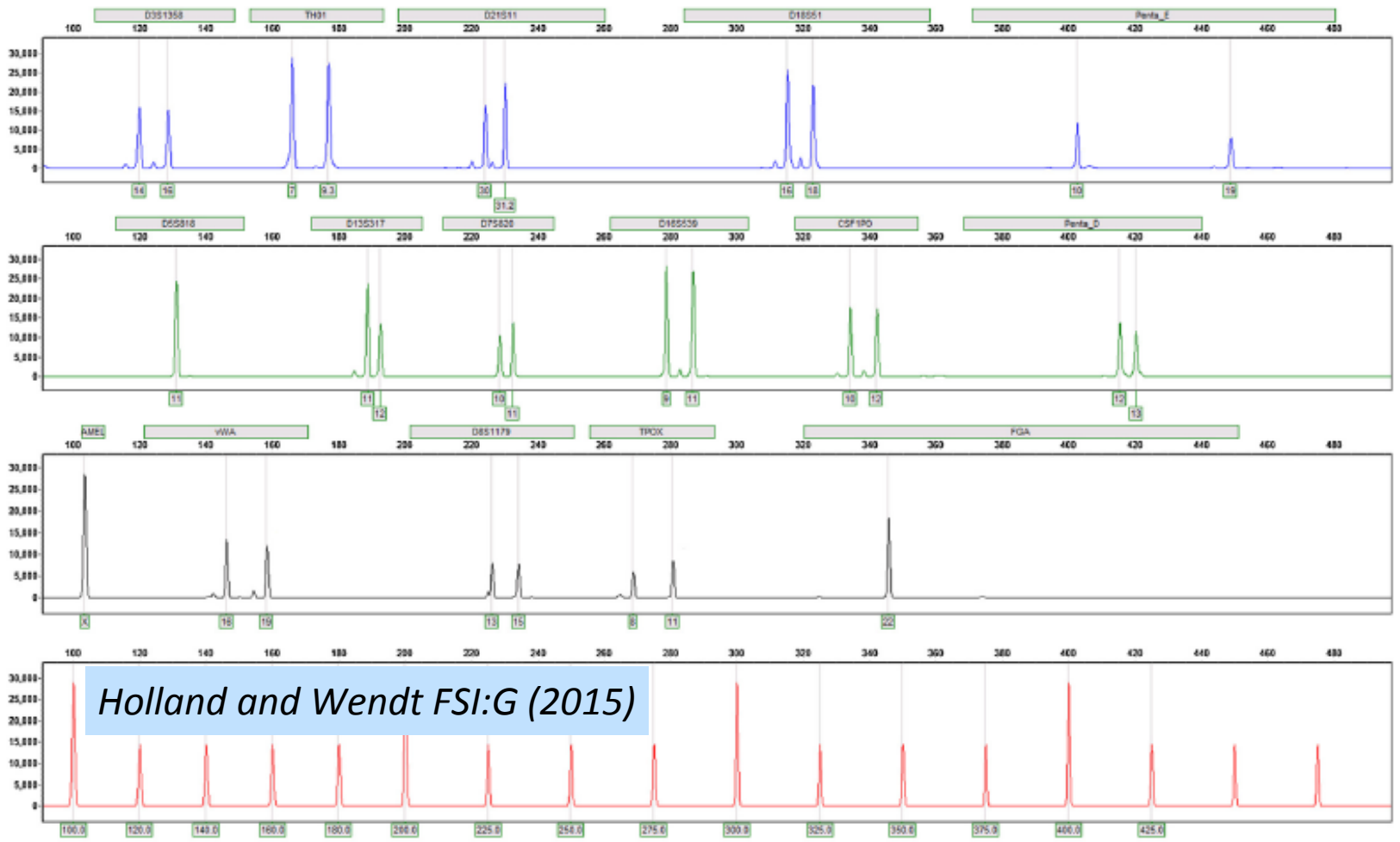
journal homepage: www.elsevier.com/locate/fsig

Evaluation of the RapidHIT™ 200, an automated human identification system for STR analysis of single source samples

Mitchell Holland *, Frank Wendt

Forensic Science Program, The Pennsylvania State University, 107 Whitmore Laboratory, University Park, PA 16802, USA

Evaluations
Generating a RDNA Profile



Holland and Wendt FSI:G (2015)

Evaluations - Sensitivity

Extracted DNA applied to swab

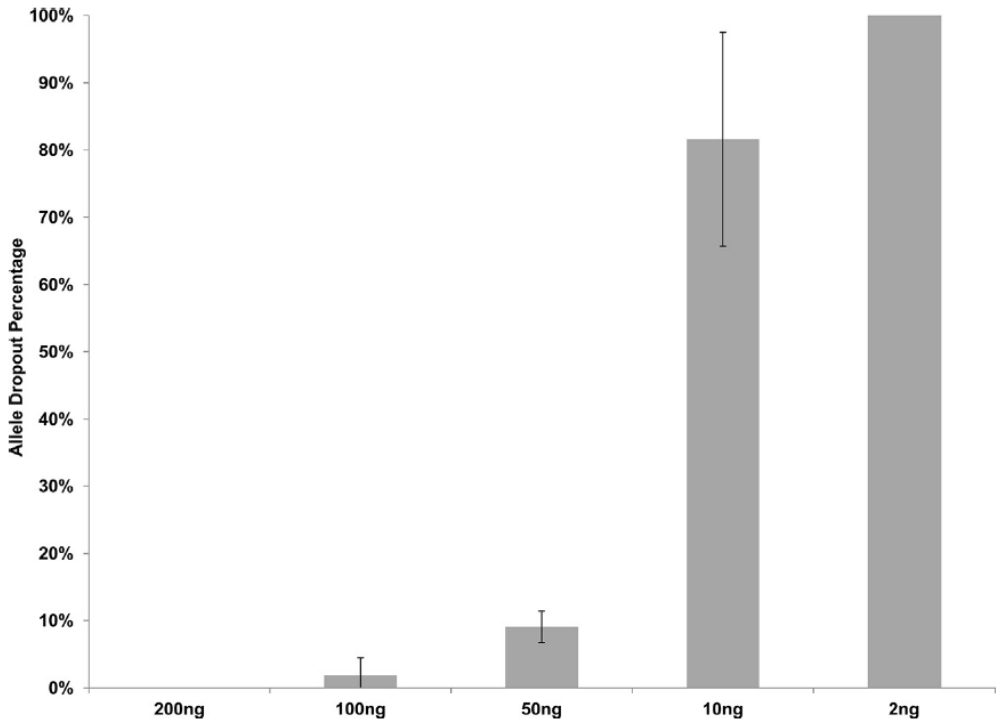


Fig. 4. Allele dropout as template DNA decreases. Percentage of alleles that "dropped out" with amount of DNA applied to sample swab. Error bars represent standard deviation.

LaRue et al. FSI:G (2014)

Evaluations - Sensitivity

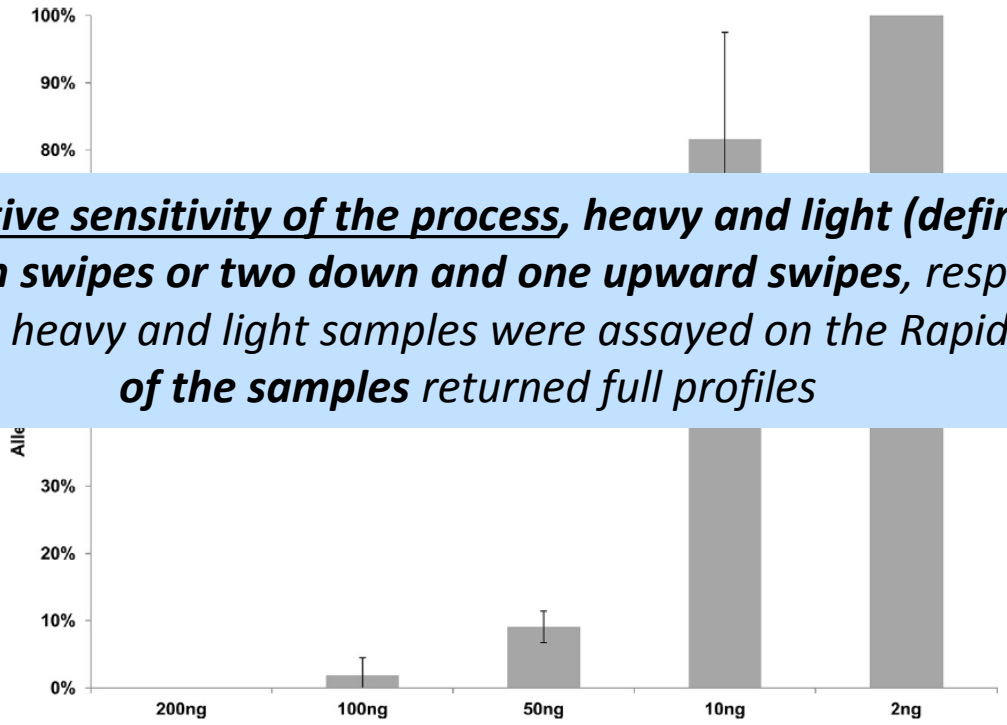


Fig. 4. Allele dropout as template DNA decreases. Percentage of alleles that "dropped out" with amount of DNA applied to sample swab. Error bars represent standard deviation.

***To test the relative sensitivity of the process, heavy and light (defined as either three up and down swipes or two down and one upward swipes**, respectively) buccal swabs were used. Five heavy and light samples were assayed on the RapidHIT system and **all of the samples** returned full profiles*

LaRue et al. FSI:G (2014)

Evaluations - Success

Thong et al. FSI:G (2015)

Table 1
Sensitivity study with different volumes of blood ranging from 50 μl to 0.125 μl. The samples were processed via RapidHIT™ 200 System and standard protocol. Data is presented as percentage of alleles called, mean peak height (in relative fluorescent units [RFU]) and peak height ratio (%).

	RapidHIT™			Standard protocol		
Volume of blood (μl)	Alleles called (%)	Mean peak height (RFU)	Peak height ratio (%)	Alleles called (%)	Mean peak height (RFU)	Peak height ratio (%)
50	100	8286.1	87.9–89.8	100	3821.1	87.2–91.7
1	93.2–100	918.6	59.1–74.4	100	5401.3	87.4–92.9
0.5	68.2–93.2	432.9	63.9–70.6	100	5096.0	79.7–92.2
0.33	38.6–86.4	209.7	56.0–73.8	100	3549.1	85.6–89.9
0.2	40.9–61.4	168.7	59.6–71.4	93.8–100	1586.9	79.5–86.4
0.125	11.4–52.3	120.2	55.8–73.9	78.1–100	1173.5	71.9–87.7

Based on these two criteria, first-pass genotyping success rates for our set of 34 buccal samples were determined. These rates are provided below using three scenarios, each with a different set of loci required to achieve a full genotype.

Scenario I = All GFE Loci (24 loci for males, 22 loci for females):
50% first-pass success rate (17 of the 34 buccal samples).

Scenario II = Expanded Core CODIS Loci (20 loci; GFE loci omitting analysis of Amel, SE33,Y-Indel, DYS391):
64.7% (22 of the 34 buccal samples).

Scenario III = Current Core CODIS Loci (13 loci):
88.2% (30 of the 34 buccal samples).

Date-Chong et al. FSI:G (2016)

Evaluations - Success

Romsos et al. FSI:G (2015)

E.L. Romsos et al./Forensic Science International: Genetics Supplement Series 5 (2015) e1–e2

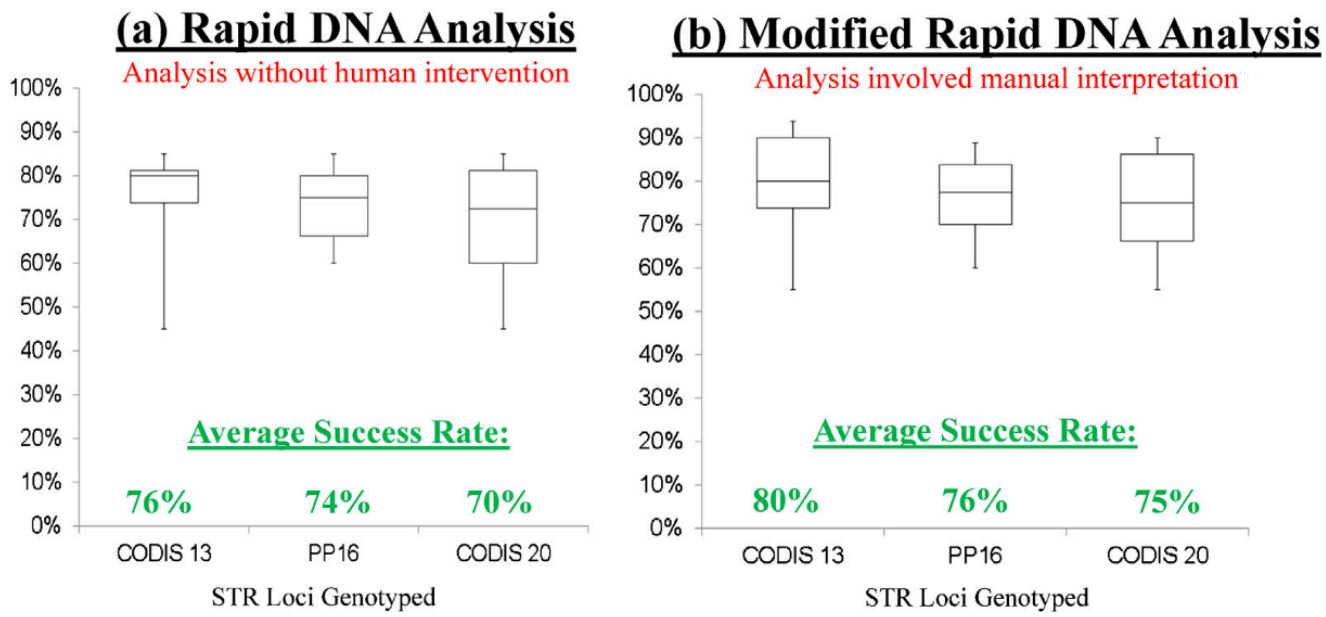


Fig. 1. Genotyping success for Rapid DNA Analysis (a), and Modified Rapid DNA Analysis (b). Success rates indicated the average success for each STR locus group genotyped. The minimum and maximum success rates observed within individual participating laboratories is represented by the whiskers of the boxplot.

Seven labs, two RDNA platforms,
11 independent instruments, 280 samples

Developmental Validation

- What is expected performance of an instrument?

- Often the developer or inventor of a method/instrument publishes this data
  - Following some standard

- Often called a ***developmental validation***

Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples.

https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view

Developmental Validation

Forensic Science International: Genetics 13 (2014) 247–258

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Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Developmental validation of the GlobalFiler® express kit, a 24-marker STR assay, on the RapidHIT® System

Lori K. Hennessy *, Neelima Mehendale, Kaiwan Chear, Stevan Jovanovich, Stephen Williams, Charles Park, Stefanie Gangano

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Forensic Science International: Genetics 25 (2016) 145–156

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Developmental validation of the DNAscan™ Rapid DNA Analysis™ instrument and expert system for reference sample processing

Angelo Della Manna[a], Jeffrey V. Nye[b], Christopher Carney[c], Jennifer S. Hammons[d], Michael Mann[d], Farida Al Shamali, PhD[e], Peter M. Vallone, PhD[f], Erica L. Romsos, PhD[f], Beth Ann Marne[g], Eugene Tan, PhD[h], Rosemary S. Turingan, PhD[h], Catherine Hogan[h], Richard F. Selden, MD PhD[h], Julie L. French[i,*]

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[c] *Florida Department of Law Enforcement DNA Investigative Support Database, 2331 Phillips Road, Tallahassee, FL 32308, USA*
[d] *Defense Forensic Science Center, Office of Chief Scientist, 4930 North 31st Street, Forest Park, GA 30297, USA*
[e] *Dubai Police GHQ, Gen. Dept. Forensic Sciences & Criminology, P.O. Box 1493, Dubai, UAE*
[f] *National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8314, USA*
[g] *Pennsylvania State Police, Forensic DNA Division, 80N. Westmoreland Avenue, Greensburg, PA 15601, USA*
[h] *NetBio, 830 Winter Street, Waltham, MA, USA*
[i] *GE Healthcare Life Sciences, 100 Results Way, Marlborough, MA 01752, USA*

Forensic Science International: Genetics 16 (2015) 181–194

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Developmental validation of a fully integrated sample-to-profile rapid human identification system for processing single-source reference buccal samples

Stevan Jovanovich[a,*], Greg Bogdan[a], Richard Belcinski[a], Jacklyn Buscaino[a], Dean Burgi[a], Erica L.R. Butts[b], Kaiwan Chear[a], Brian Ciopyk[a], David Eberhart[a], Omar El-Sissi[a], Helen Franklin[a], Stefanie Gangano[a], Jennifer Gass[a], Dennis Harris[a], Lori Hennessy[a], Alex Kindwall[a], David King[a], Jim Klevenberg[a], Yuan Li[a], Neelima Mehendale[a], Roger McIntosh[a], Bill Nielsen[a], Charles Park[a], Francesca Pearson[a], Robert Schueren[a], Nancy Stainton[a], Charles Troup[a], Peter M. Vallone[b], Mattias Vangbo[a], Timothy Woudenberg[a], David Wyrick[a], Stephen Williams[a]

[a] *IntegenX Inc. 5720 Stoneridge Drive, Suite 300, Pleasanton, CA 94588-2739, USA*
[b] *National Institute of Standards and Technology, Gaithersburg, MD 20899-8314, USA*

Forensic Science International: Genetics 28 (2017) 21–34

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Validation of a rapid DNA process with the RapidHIT® ID system using GlobalFiler® Express chemistry, a platform optimized for decentralized testing environments

Susana Salceda, Arnaldo Barican, Jacklyn Buscaino, Bruce Goldman, Jim Klevenberg, Melissa Kuhn, Dennis Lehto, Frank Lin, Phong Nguyen, Charles Park, Francesca Pearson, Rick Pittaro, Sayali Salodkar, Robert Schueren, Corey Smith, Charles Troup, Dean Tsou, Mattias Vangbo, Justus Wunderle, David King*

IntegenX Inc, 5720 Stoneridge Drive, Suite 300, Pleasanton, CA, 94588, USA

Dev Val - Extraction

Jovanovich et al. FSI:G (2015)

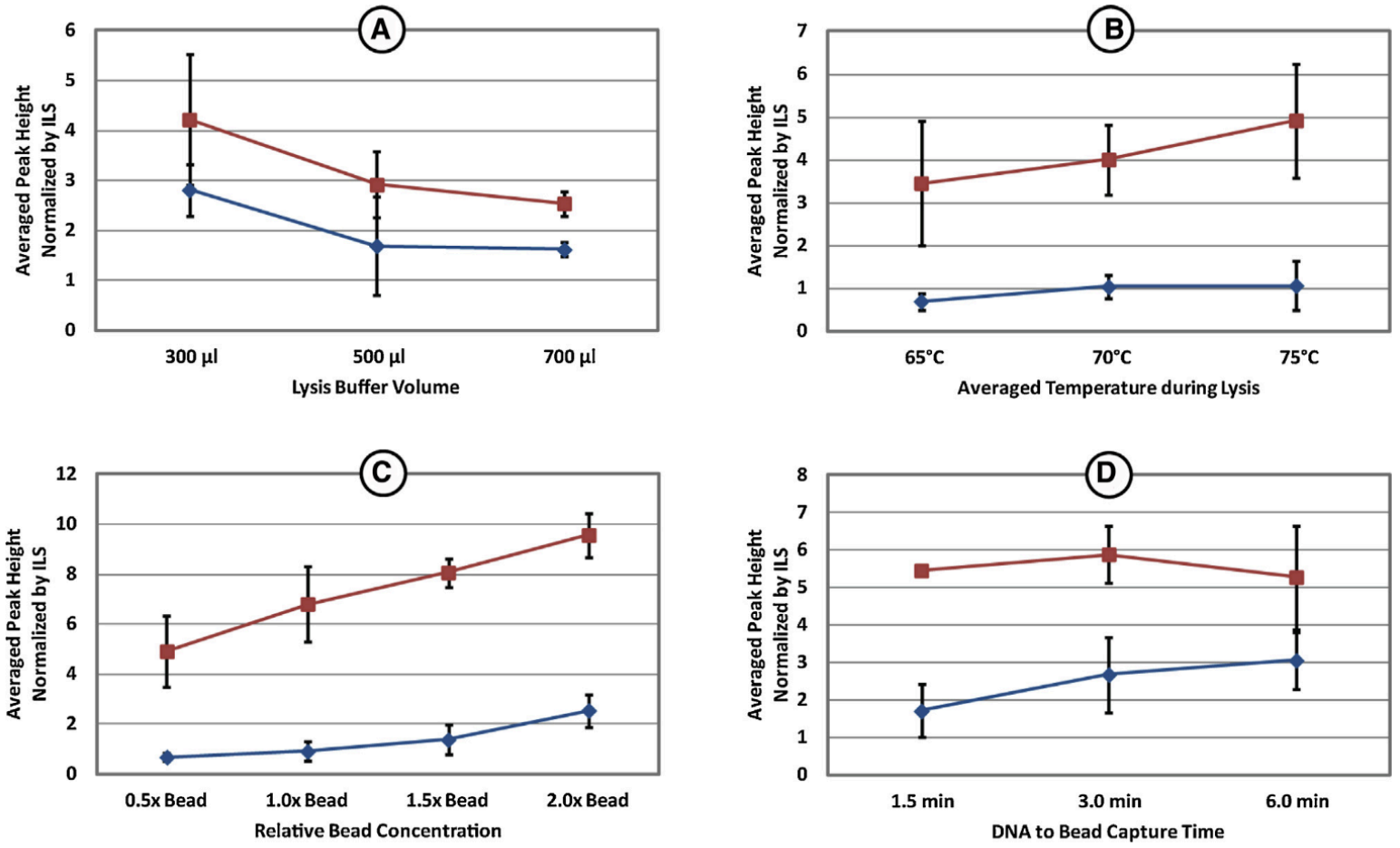


Fig. 3. Boundary testing of sensitivity of extraction to buffer volume (A), and lysis temperature (B) and of DNA purification to bead concentration (C) and capture time (D) were measured. Each data point was run in triplicate on a single instrument and is plotted as the mean ± standard deviation (S.D.) of the average STR peak height normalized by dividing the average peak height of the STR peaks by the average peak height of the ILS peaks from that sample (panels A, C, D: ◆10,000 1000F cells ■500,000 1000F cells; panel B: ◆10,000 1000F cells, ■50,000 1000F cells).

Dev Val – Precision and PCR-based studies

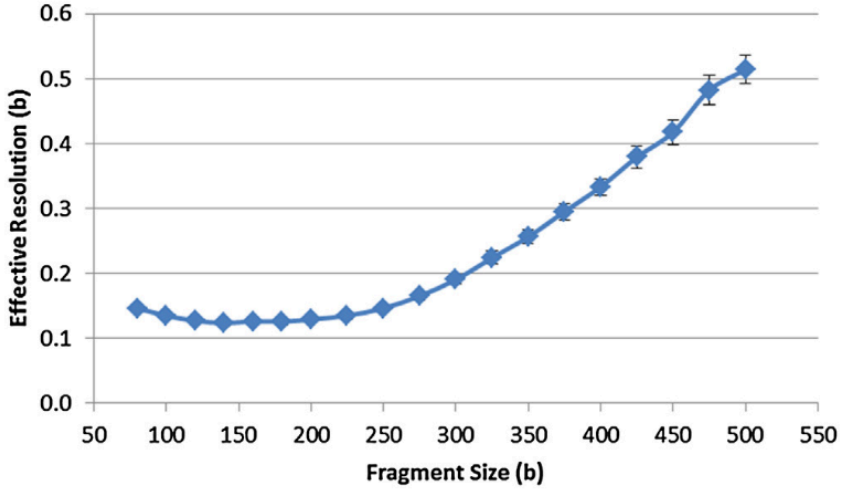


Fig. 12. Effective Resolution by fragment size in base pairs with standard deviation.

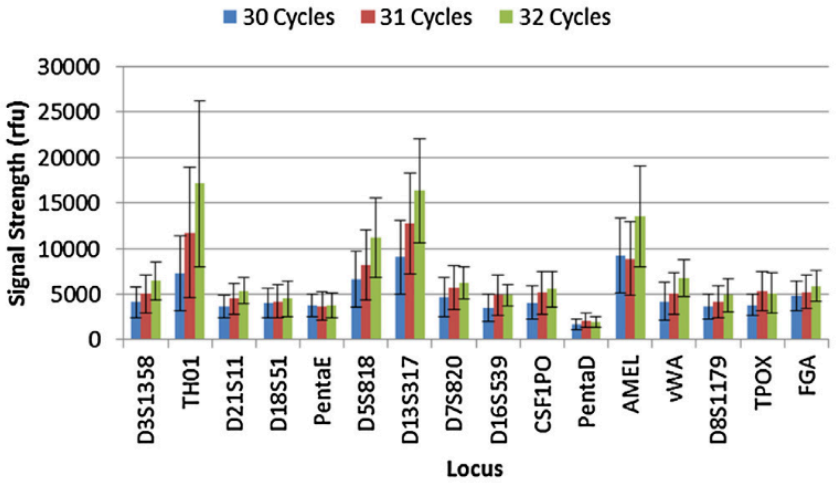


Fig. 13. Effect of 30, 31, and 32 cycles on signal strength and inter-locus signal strength balance.

Della Manna et al. FSI:G (2016)

Dev Val - Sensitivity

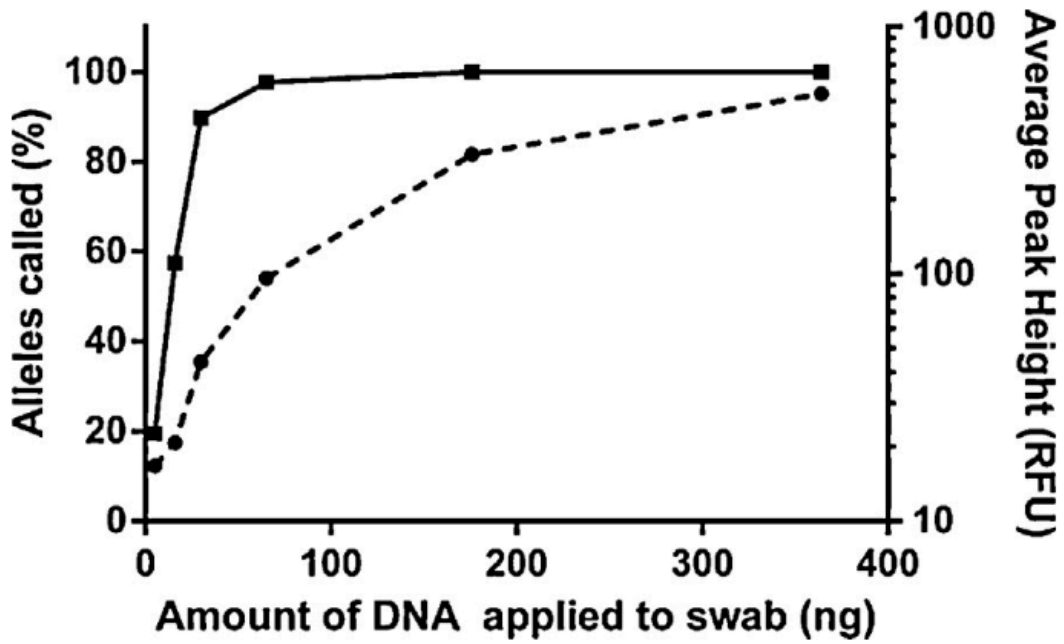


Fig. 10. Sensitivity of system detection for known DNA loads in saliva showing percentage of alleles called (■) and average peak heights (●). Average peak heights are scaled to reflect a maximum signal height of 29,000 RFU in the GeneMarker software. The average peak height for a profile is calculated from all detected alleles (average signal is used at heterozygous loci and signals are halved for homozygous loci).

Jovanovich et al. FSI:G (2015)

Dev Val - Sensitivity

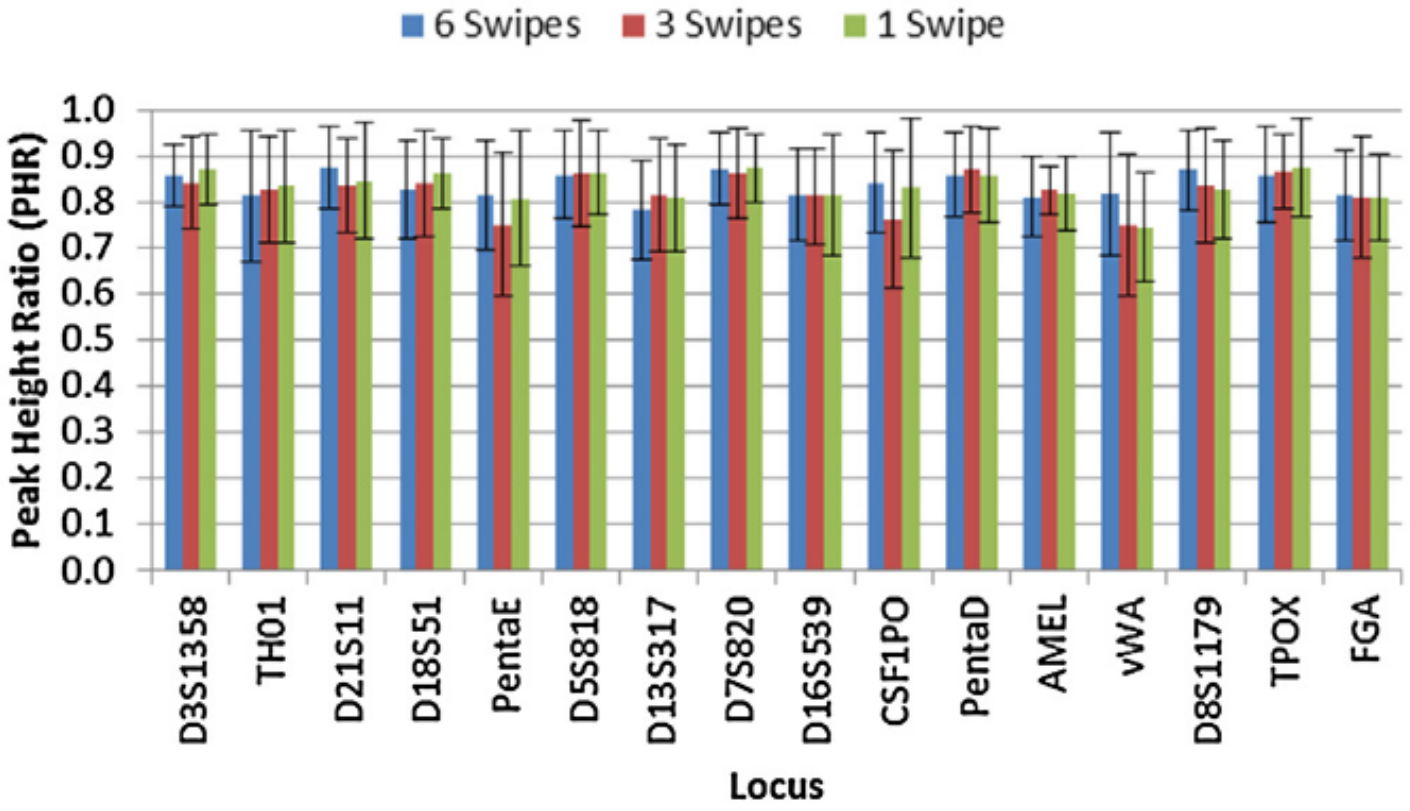
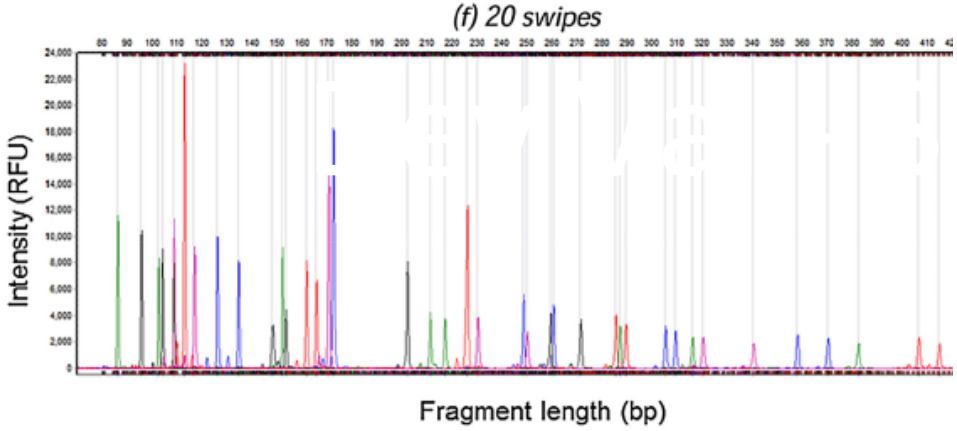
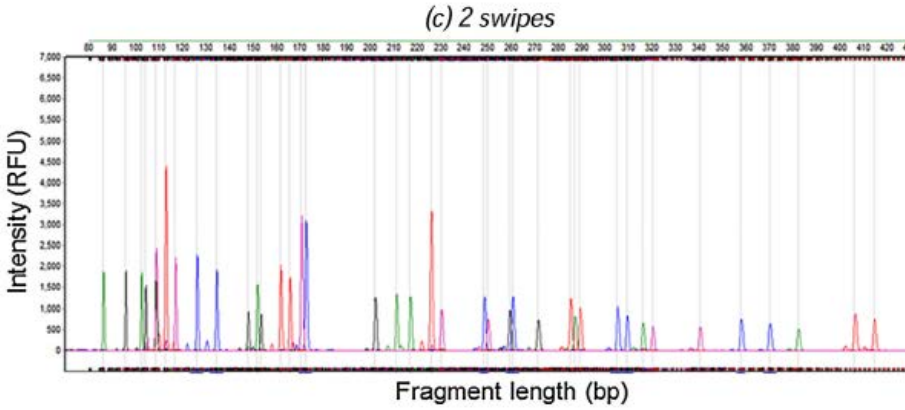
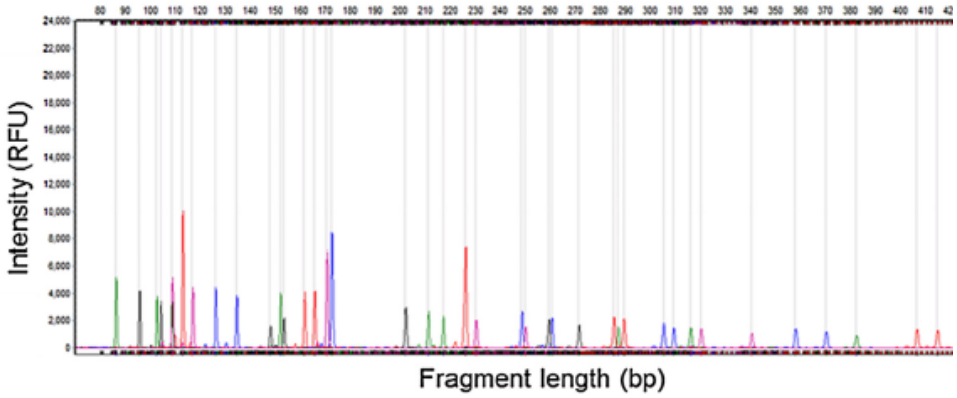


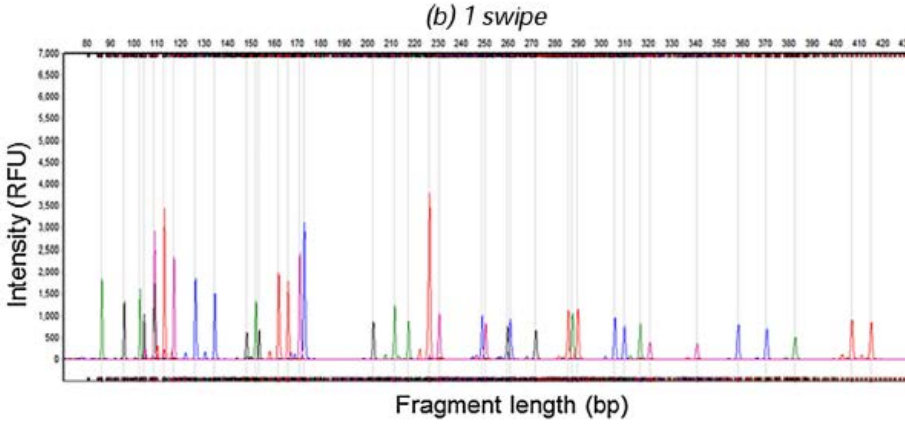
Fig. 3. Peak height ratio with standard deviation for buccal samples yielding a full profile for the CODIS core loci.

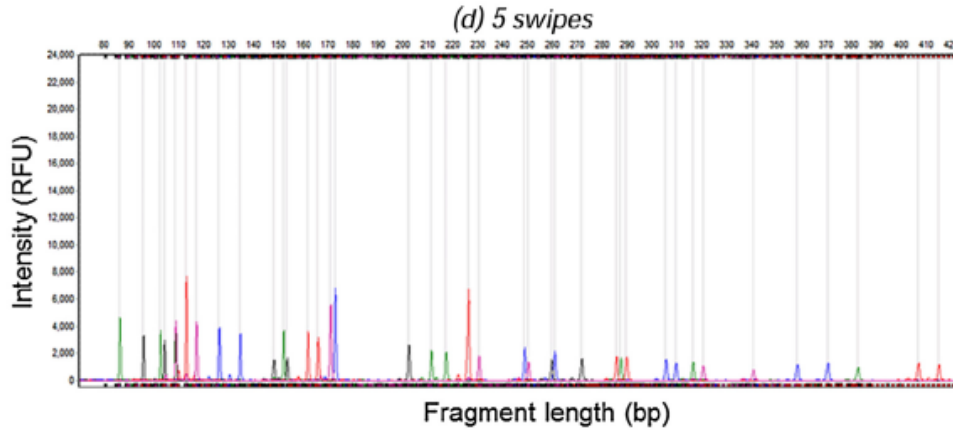
Della Manna et al. FSI:G (2016)

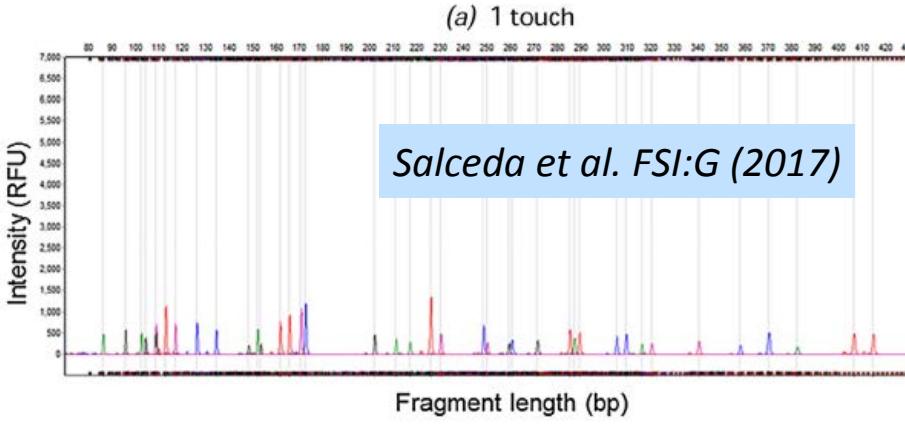












Salceda et al. FSI:G (2017)

8. Electropherograms from the sensitivity study for the male donor. Note the vertical axis scale is 0–24,000 RFU for (d)–(f) and 0–7,000 RFU from (c).

Fig. 8. (Continued)

Dev Val - Inhibitors

Jovanovich et al. FSI:G (2015)

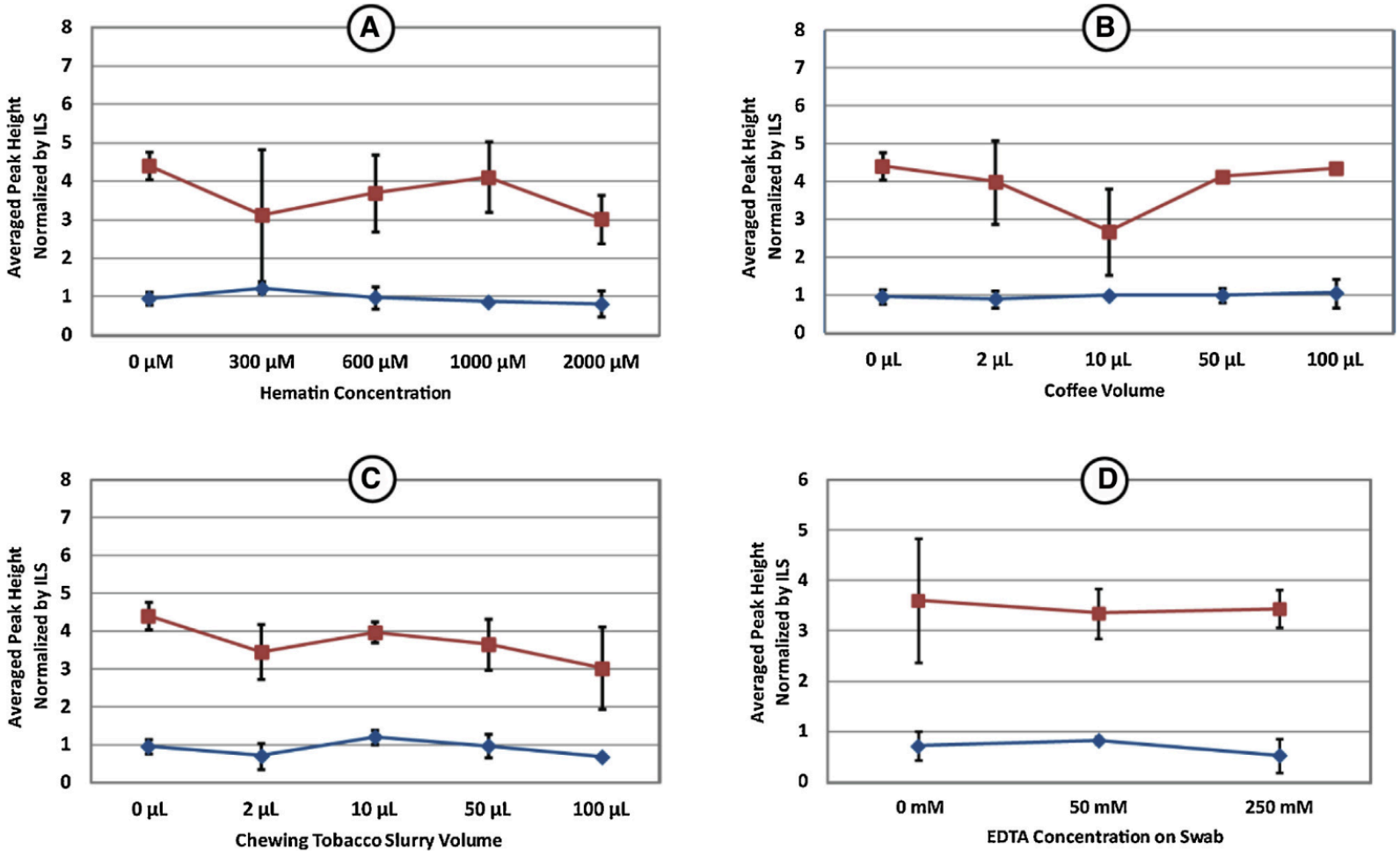


Fig. 4. Effect of inhibitors on peak heights. Hematin (A), coffee (B), mint tobacco slurry (C), and EDTA (D) were added to swabs and tested in the system. Each data point was run in triplicate on a single instrument and is plotted as the mean ± S.D. (◆10,000 1000F cells, ■50,000 1000F cells).

Dev Val - Inhibitors

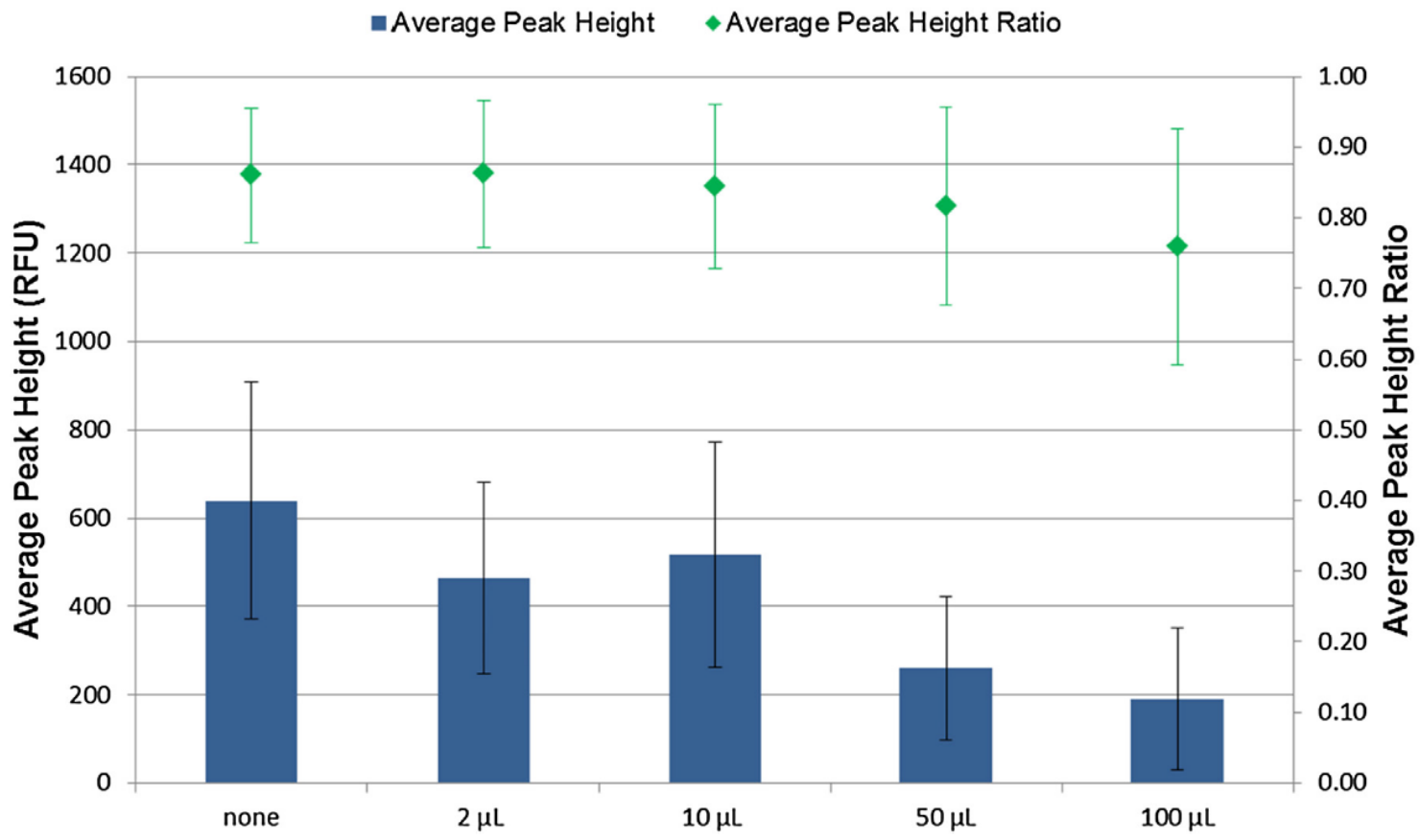


Fig. 6. Effect of tobacco on control swabs (n = 3 per condition) with 100,000 1000 M cells (average ± SD).

Salceda et al. FSI:G (2017)

Dev Val - Stability

Jovanovich et al. FSI:G (2015)

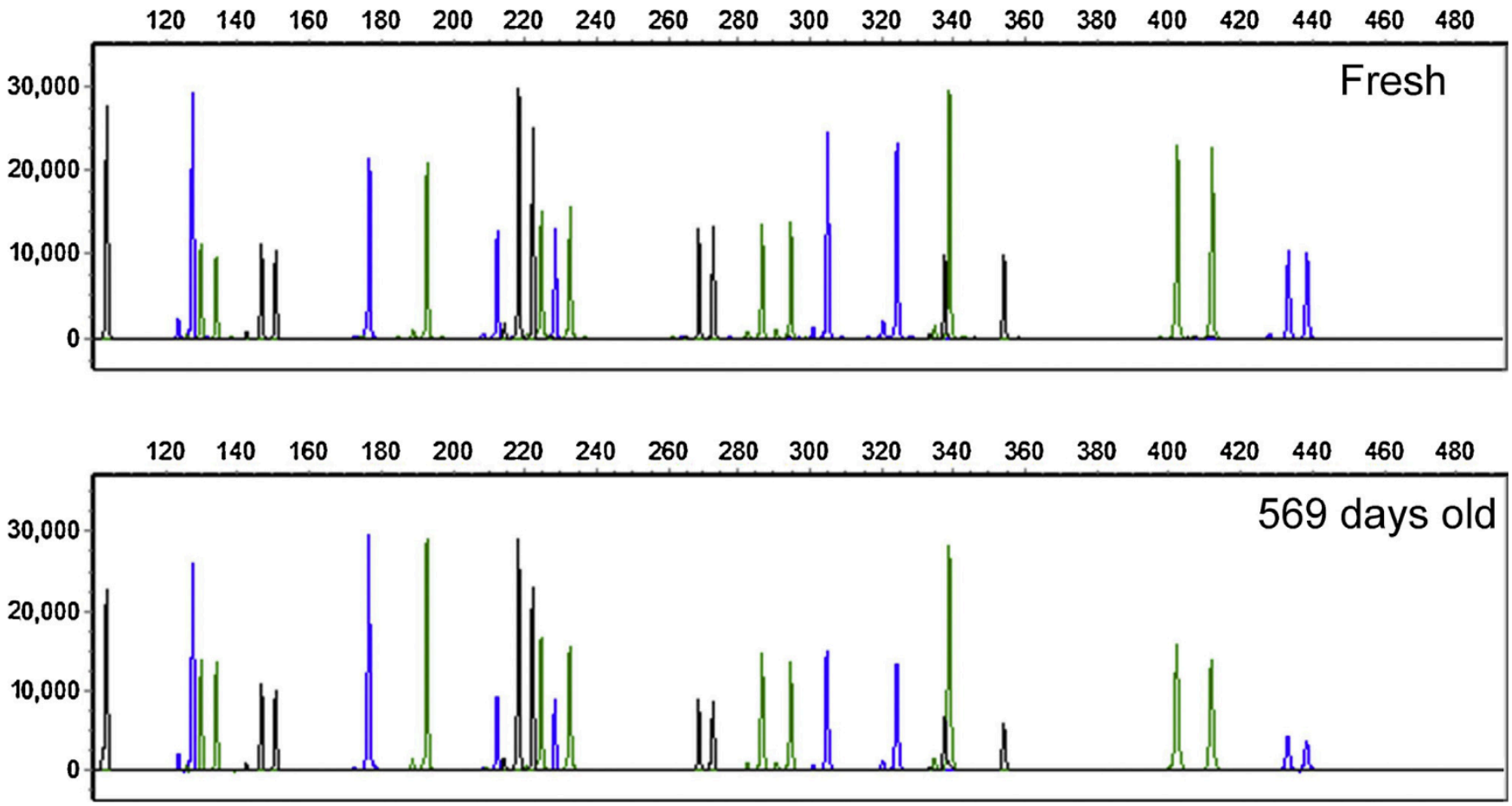


Fig. 11. Electropherograms of fresh and 569 day old swabs from the same donor yield the same profiles.

Dev Val - Precision

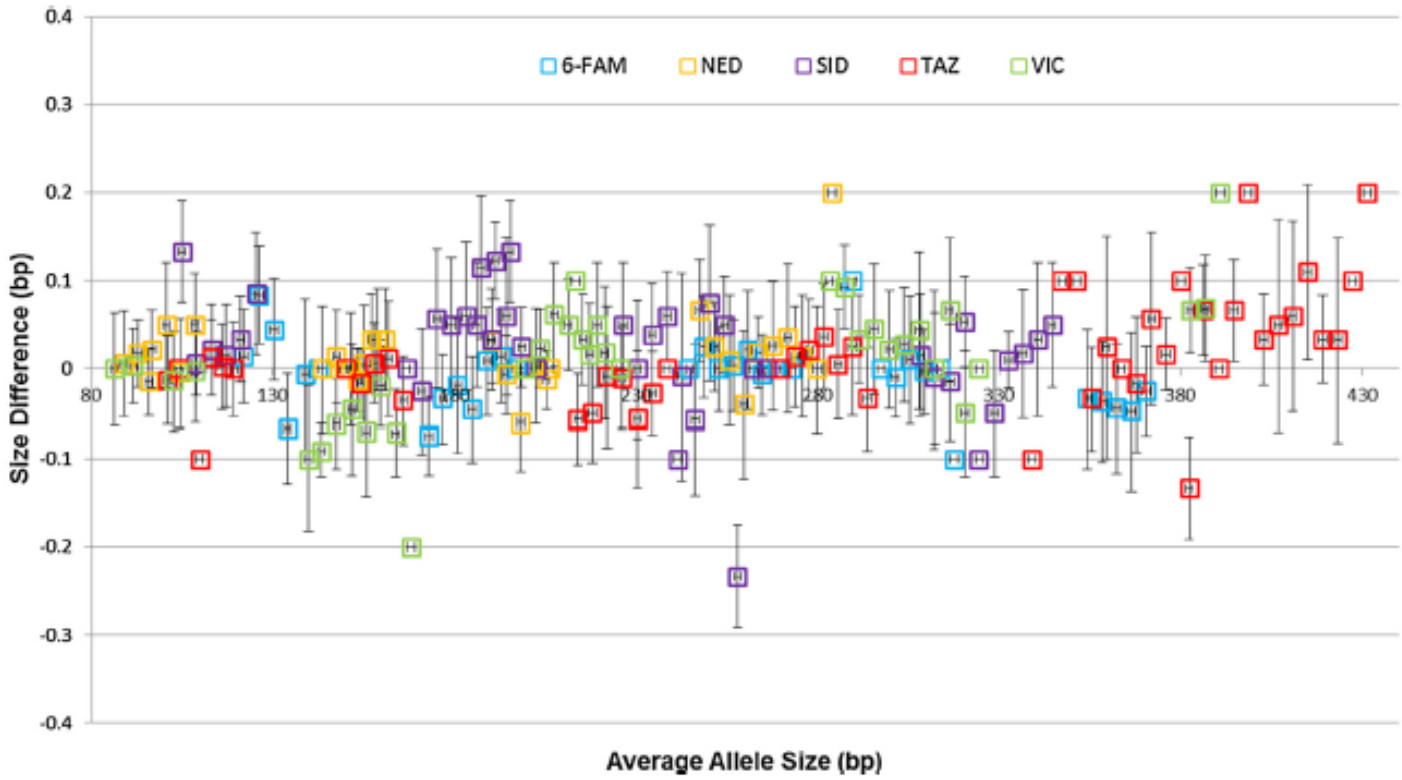
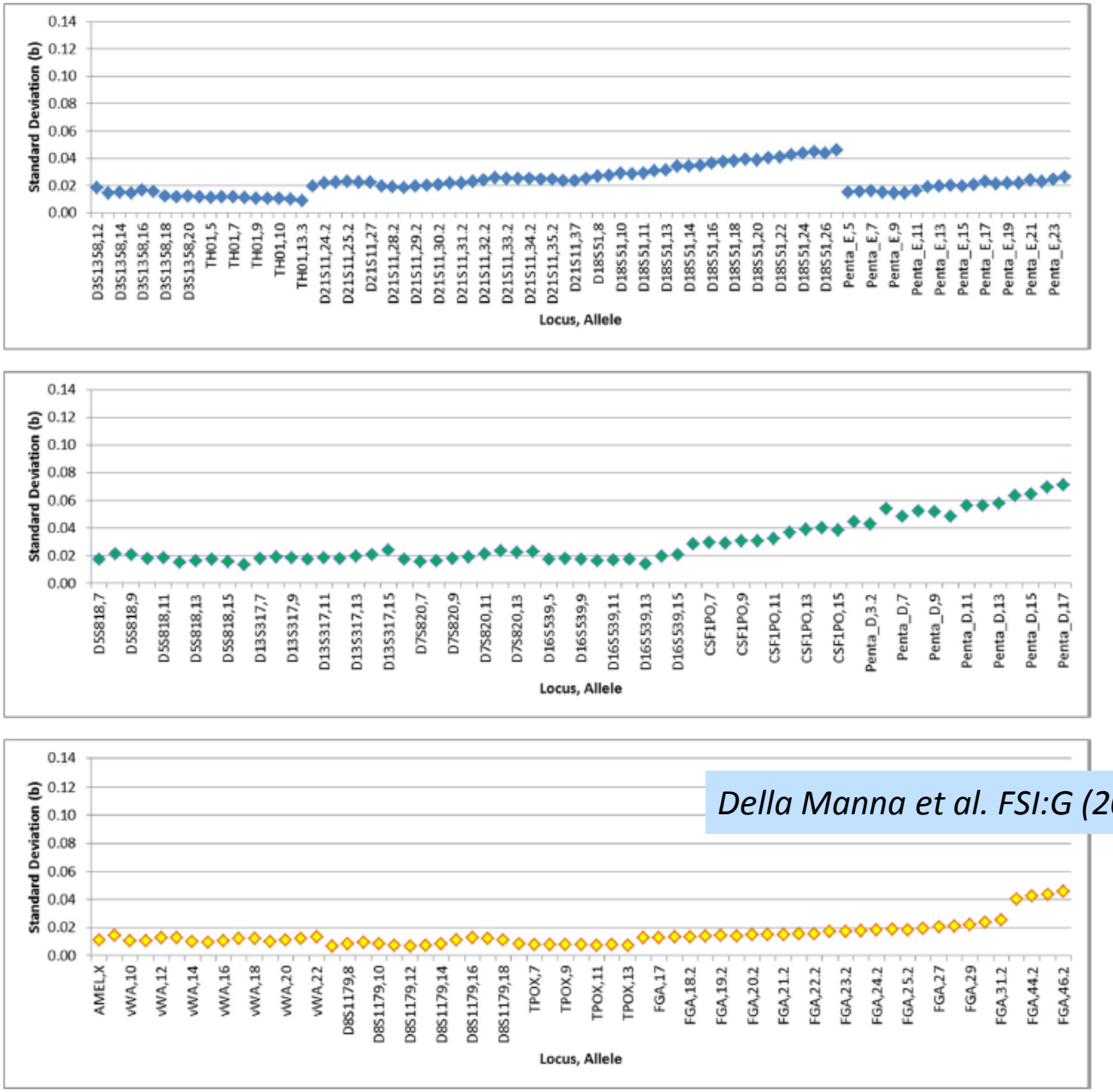


Fig. 9. Accuracy. Size difference (in base pairs) between an allele and its corresponding allele in the allelic ladder used to size the sample (53 samples, 2138 alleles). The color of each data point indicates the corresponding dye in the GlobalFiler® Express assay; FAM (blue), VIC (green), NED (yellow), TAZ (red) and SID (purple).

Salceda et al. FSI:G (2017)



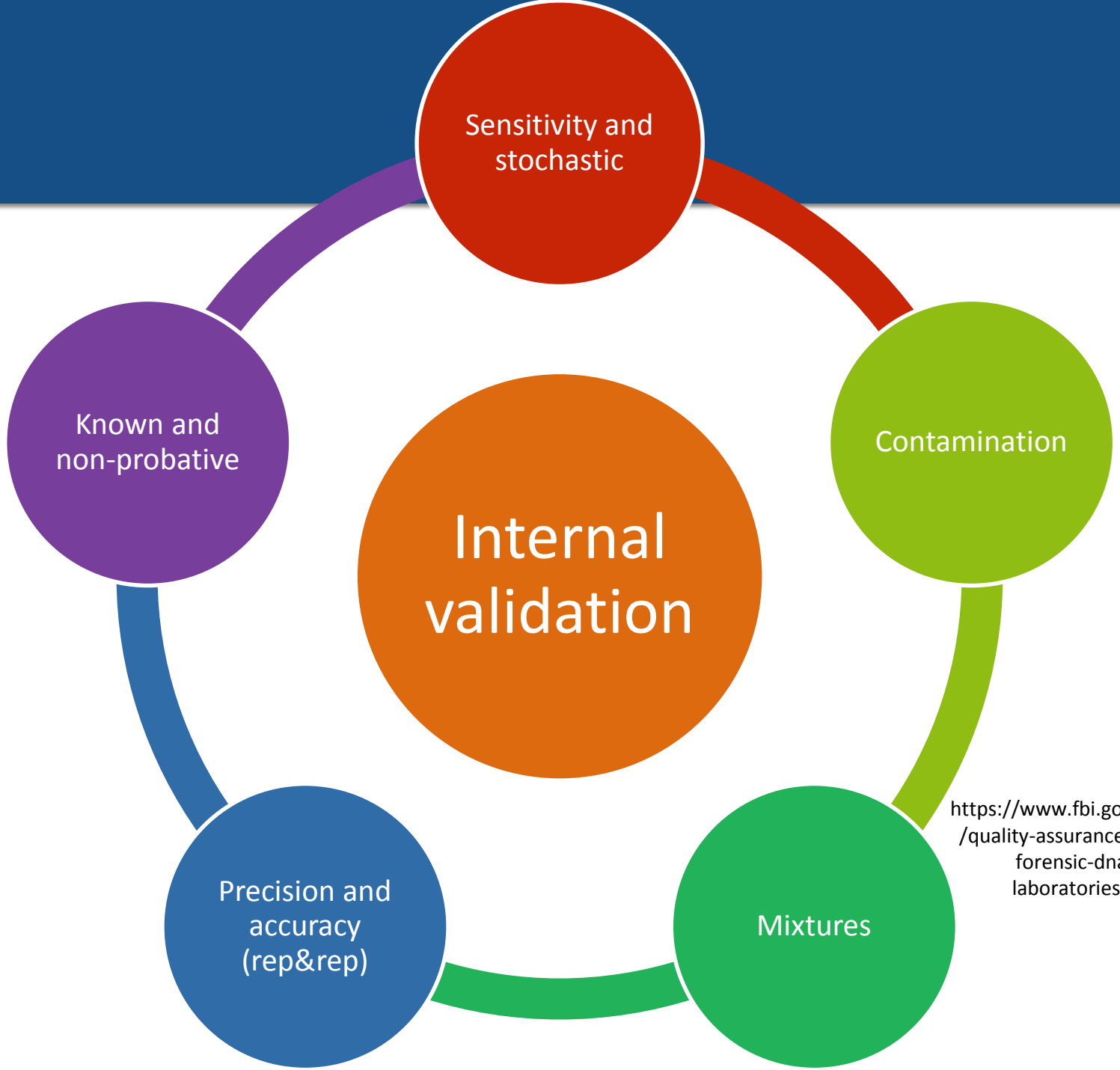
Della Manna et al. FSI:G (2016)

Fig. 6. Sizing variation at a single standard deviation for each allele in the allelic ladder calculated for 418 runs on 14 DNAscan instruments.

Internal Validation

- Why do we perform an internal validation study?

- To confirm that a method or instrument performs as expected.
  - A Verification



https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view

Internal Validation

Forensic Science International: Genetics 29 (2017) 100–108

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Internal validation of the DNAscan/ANDE™ Rapid DNA Analysis™ platform and its associated PowerPlex® 16 high content DNA biochip cassette for use as an expert system with reference buccal swabs☆

Lilliana I. Moreno[a,b,*], Alice L. Brown[a], Thomas F. Callaghan[b]

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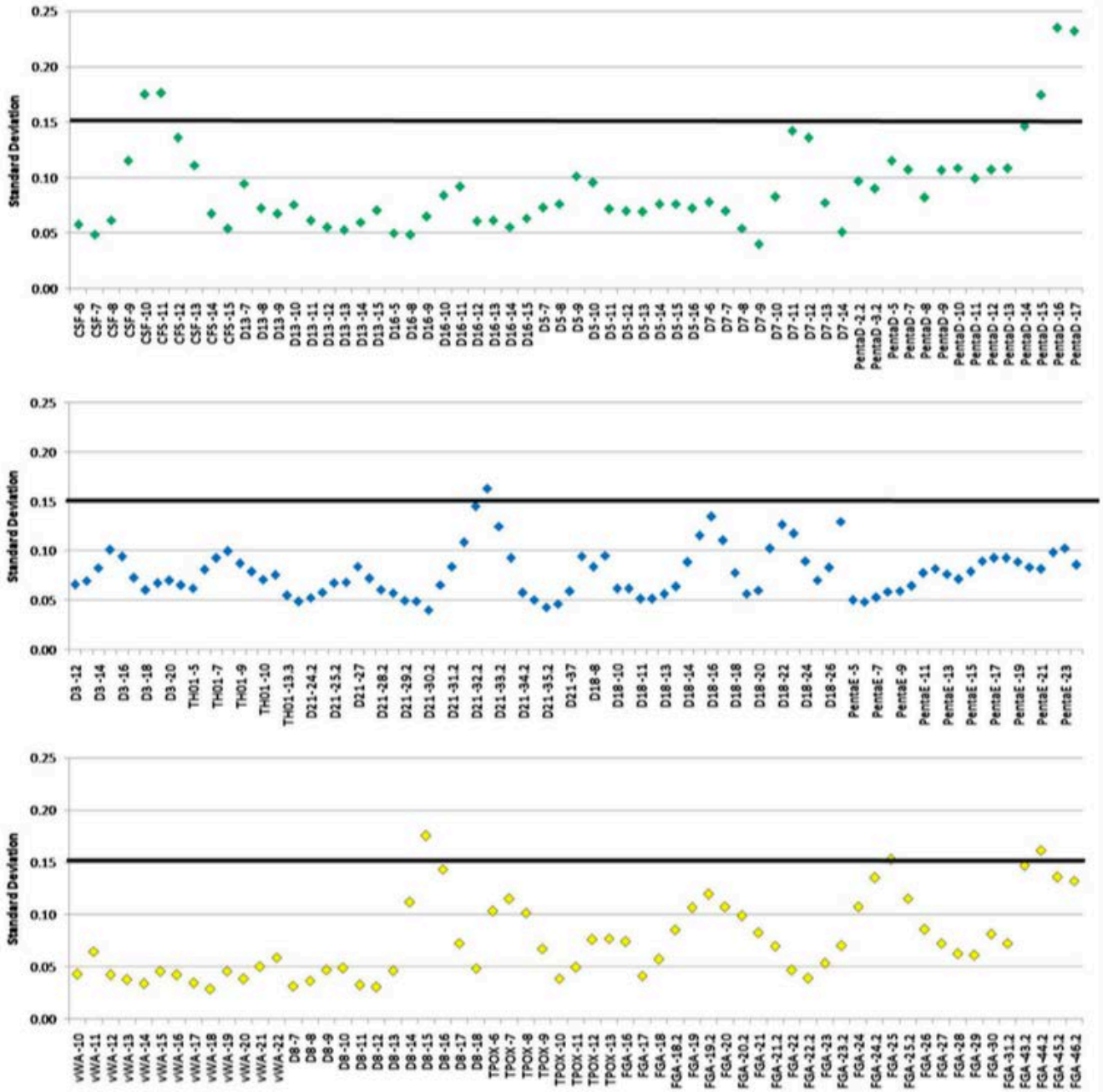
Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory (QAS)

Moreno et al. FSI:G (2017)



IV - Sensitivity

2.7. Sensitivity

Samples previously ex
methods were quantified
µl and spotted onto sw
allowed to dry before lo
Data produced from the
into GeneMapper ID-X v.
instrument, as well as t
required for the system t
of observing drop-out of

3.7. Sensitivity and interpretation threshold calculations

The results of the sensitivity study suggest the expected response to decreasing amounts of DNA. Samples with 50 ng of total input DNA or less were found to consistently yield partial or no results after processing in the DNAscan/ANDE™. When a 100 ng input amount was used, some amplification artifacts and sporadic loss of alleles were noted. Input amounts of 250 ng and higher yielded full profiles that were concordant with previous results developed by conventional analysis methods.

Interpretation thresholds are used as a benchmark for complete allele recovery; i.e. the RFU value at which it is reasonable to expect that the companion allele in a heterozygous locus has not dropped-out [8]. In the data set used for the sensitivity study, a total of 334 heterozygous occurrences were expected. Of these, there were 289

- 250 ng – full profiles
- 100 ng – some drop out
- Estimates for allele drop out thresholds

this number could be much higher than what was observed with the samples in this study, it could be used as a starting point in the event that a sample needs to be reviewed by an analyst using standard laboratory DNA analysis software such as GeneMapper IDx or equivalent.

Moreno et al. FSI:G (2017)

IV – Reagent Lots

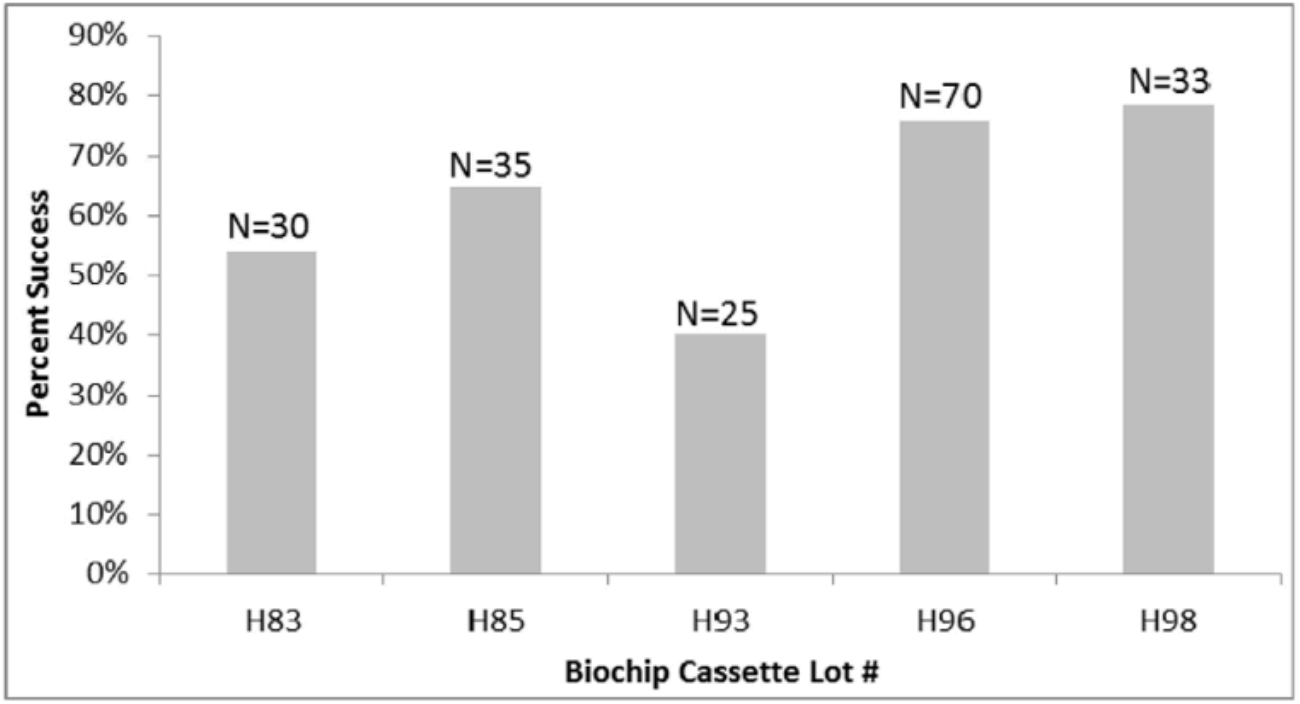


Fig. 4. Biochip cassette lot-to-lot comparison. The number of known samples run with each of the lots is included for reference. Four of the five lots used exhibited >50% success, but one of the lots (H93) exhibited a decreased level of success. Samples run with these lots and used in the sensitivity study were not counted as part of this evaluation.

Moreno et al. FSI:G (2017)

IV – Checking swab type

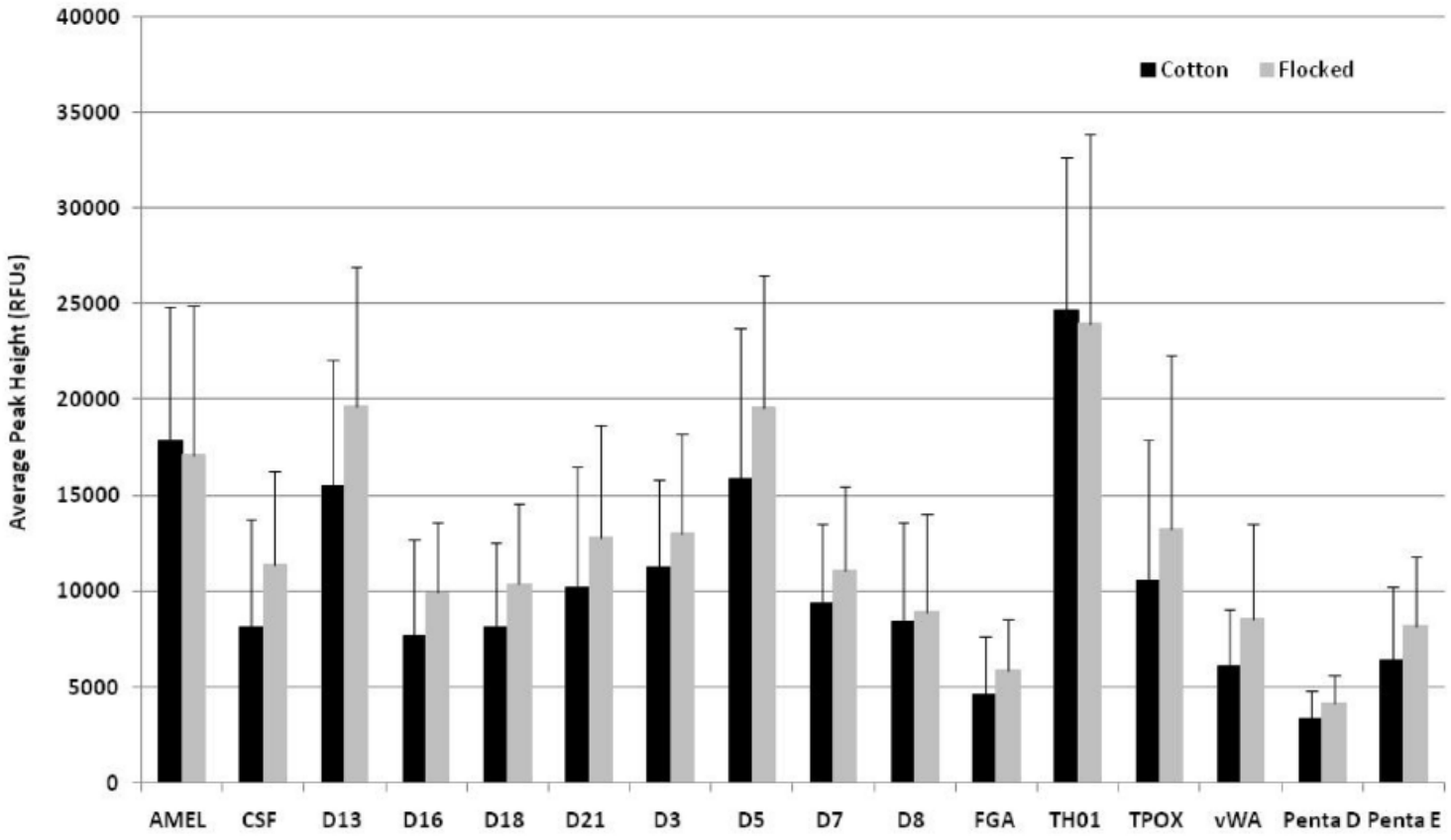


Fig. 1. Average peak heights observed for flocked and cotton swabs from all typed samples. Data suggests that both swab materials perform equally when processed using the DNAScan/ANDE™ instrument.

Moreno et al. FSI:G (2017)

Thoughts

- First pass testing
- Anything is game
- Instrument is still undergoing optimization
- Not a validation (waiting for DevVal to be made public)

Thoughts

Evaluations → **Developmental validation** → Internal validation

- Following Standards for experiments
- Define validation performance “space”
- Minimal requirements – more can (and is) done
- More robust – more samples, replicates, operators, experiments
- Instrument is optimized – final version
- **Performed one time**

Thoughts

Evaluations → Developmental validation → **Internal validation**

- Following Standards for experiments
- Verification of the developmental validation
- Define validation performance “space” ***for a specific lab***
- Additional experiments can cover ranges or experiments outside of the published developmental validation
- Performed on commercial instrument
- **Performed by a laboratory (one per lab)**

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