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2018 Rapid DNA Maturity Assessment
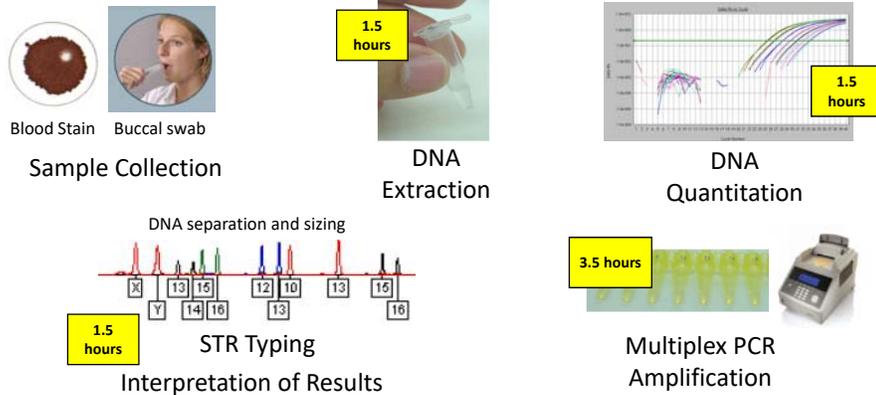
Erica Romsos & Peter Vallone

Applied Genetics Group, NIST

November 7, 2018

APPLIED GENETICS

Steps in Forensic DNA Analysis

Steps Involved

- Collection
- **Extraction**
- **Quantitation**
- **Multiplex PCR**
- **STR Typing**
- **Interpretation of Results**

Blood Stain   Buccal swab

Sample Collection

1.5 hours

DNA Extraction

1.5 hours

DNA Quantitation

DNA separation and sizing

| X | 13 | 15 | | 12 | 10 | | 13 | | 15 |
| Y | | 14 | 16 | | | 13 | | | 16 |

1.5 hours

STR Typing

Interpretation of Results

3.5 hours

Multiplex PCR Amplification

1-2 day process (a minimum of ~8 hours) with current laboratory procedures and technology

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Commercial RDNA Instruments

IntegenX

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

3-7 Samples
2 hour run time

1 Sample
90 minute run time

ANDE

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

5 Samples
86 minute run time

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Rapid DNA Instrument Testing

2012-2015: Ongoing communication with Developers

08/2017: Rapid DNA Act Passes

2008   2010   2012   2014   2016   2018

2010: Apollo 200 Testing (**136 samples**)

09/2012: First prototypes delivered to NIST (DHS Instruments)

2015: NIST Participates in IXI PP16 Dev. Validation (**100 samples**)

2016: NIST Participates in ANDE PP16 Dev. Validation (**150 samples**)

2016: NIST Participates in testing ParaDNA

2013: NIST Interlab Study (**350 samples**)

2014: NIST Maturity Assessment (**280 samples**)

2017: NIST Participates in ANDE FlexPlex Dev Validation (**250 samples**)

2018: NIST Rapid DNA Maturity Assessment (**240 samples**)

Forensic Science International: Genetics Supplement Series

Rapid DNA maturity assessment

Erica L. Romsos, Sanae Lembirick, Peter M. Vallone

NIST: Rapid DNA Maturity Assessments

- Collection and distribution of samples to all participating laboratories
  - 2013: 3 labs, 150 samples
  - 2014: 7 labs, 280 samples
  - 2018: 9 participants, 240 samples
- Coordination of all testing sites to include return of all data to NIST for analysis and review
  - Includes IRB approval for swab collection and MTA for distribution
- Analysis and compilation of all data
- Summary of results presented across multiple meetings within the forensic and biometric communities

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2013 Rapid DNA Maturity Assessment

- Summer 2013 we felt confident that we could carry out an interlab assessment of the R-DNA prototypes
- Data was collected and analyzed in August 2013
  - All instruments had the same version of software and scripts for testing
- Three federal testing sites (7 individual instruments)
  - 50 single source reference buccal swabs were provided by NIST for each instrument tested
- 350 single source reference buccal swabs tested
  - Success defined as the automated calling of the 13 CODIS core STR loci
- **Overall success = 88.3%**

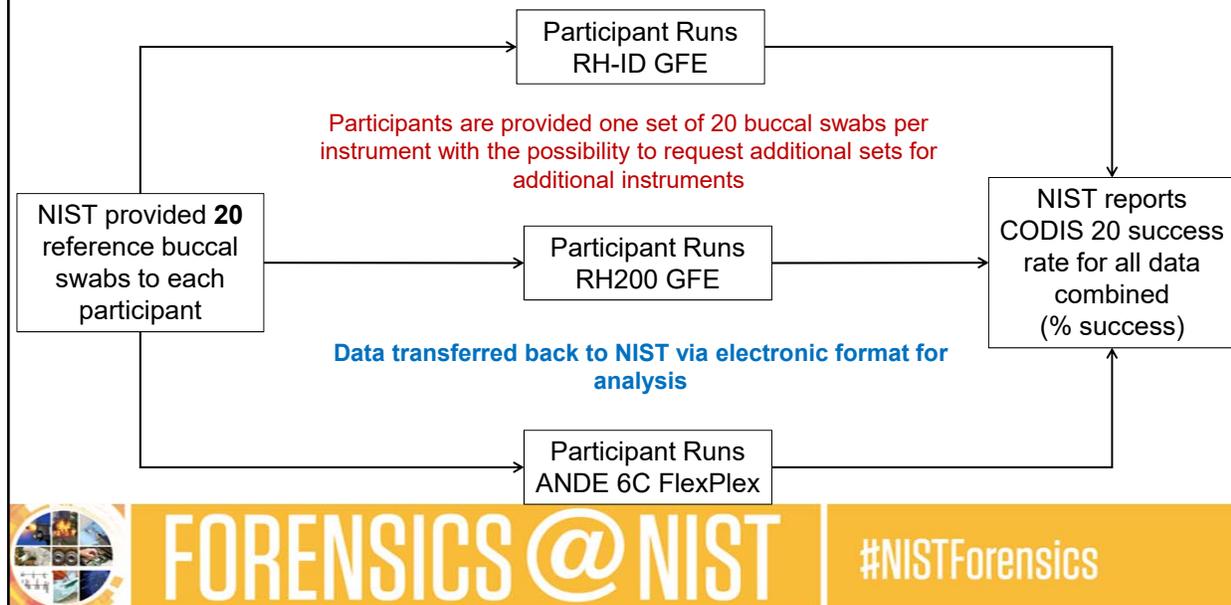**FORENSICS @ NIST** | #NISTForensics

2014 Rapid DNA Maturity Assessment

- Fall 2014 assessment of the current status of rapid DNA typing technology for the CODIS Core Loci
    - In support of lab and future booking station Rapid DNA implementation
- Many modifications to both hardware and software were made between 2013 and 2014
- 7 participating laboratories (11 independent instruments)
    - 20 single source reference buccal swabs tested
- 280 single source reference buccal swabs tested
    - Success defined as automated (lights out) calling of the 13 CODIS core loci
- **Overall Success = 76.1%**

Forensic Science International: Genetics Supplement Series

Rapid DNA maturity assessment
Erica L. Romsos, Sanae Lembirick, Peter M. Vallone
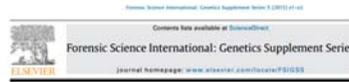
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2018 Rapid DNA Maturity Assessment

- **Goal**: To measure the status of rapid DNA typing technology for the 20 CODIS core loci in support of booking station Rapid DNA implementation

- Rapid DNA instruments capable of genotyping the 20 CODIS core loci were eligible for participation

- 20 single source reference buccal swabs were distributed to participating laboratories

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2018 Rapid DNA Maturity Assessment Study Outline

Participant Runs
RH-ID GFE

Participants are provided one set of 20 buccal swabs per
instrument with the possibility to request additional sets for
additional instruments

NIST provided **20**
reference buccal
swabs to each
participant

Participant Runs
RH200 GFE

NIST reports
CODIS 20 success
rate for all data
combined
(% success)

Data transferred back to NIST via electronic format for
analysis

Participant Runs
ANDE 6C FlexPlex

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Timeline of 2018 Maturity Assessment

February 2018-Present: Buccal samples collected at NIST and stored
at RT (>900 swabs have been collected from >50 unique individuals)

May 2018: Official call for Participants in the 2018 Rapid DNA
Maturity Assessment for the CODIS core 20 loci

July 2018: Samples shipped to participants

August 2018: Data returned to NIST for analysis

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Participants	Instrument Platforms	Chemistry	Independent Instruments	Total Samples Tested	Analysis Method
Federal	ANDE 6C	FlexPlex	5	100	Rapid DNA Analysis
State Police	IntegenX RapidHIT 200	GlobalFiler Express	3	60	Modified Rapid DNA Analysis
Vendor	IntegenX RapidHIT ID	GlobalFiler Express	4	80	Modified Rapid DNA Analysis
9 Participants	3 Platforms		12 Instruments	240 Samples	

20 Swabs provided

Success Metrics

- Success was measured by **complete and concordant genotypes** produced for the 20 CODIS core loci

- Allele calls by the integrated rapid DNA devices were compared to lab generated profiles for concordance
  - Fusion 6C, PP21, GFE on a 3500xL

- Two interpretation parameters were implemented
  - Rapid DNA Analysis: Without human intervention
  - Modified Rapid DNA Analysis: Expert interpretation and analysis of electropherogram https://www.fbi.gov/file-repository/addendum-to-qas-for-rapid-dna.pdf/view

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Rapid DNA Analysis-ANDE

Automated (lights-out) analysis without human intervention

Effective June 1, 2018, the following Rapid DNA system is approved for use at NDIS by an accredited forensic DNA laboratory:

Rapid DNA Analysis System for Accredited Laboratory Use

To date, ANDE 6C is the only rapid DNA system to be NDIS approved for automated rapid DNA analysis

Component	Name	Part/Version Number
Rapid DNA Instrument	ANDE 6C Instrument	A0120001003
Typing Kit	FlexPlex27	FlexPlex27
Cartridge	ANDE A-Chip (FlexPlex)	A0210001057
System Software	ANDE System Software	2.0.6
Expert System Software	ANDE Expert System	2.0.5

https://www.fbi.gov/services/laboratory/biometric-analysis/codis/rapid-dna

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CODIS 20 Success: Automated Analysis

n=85
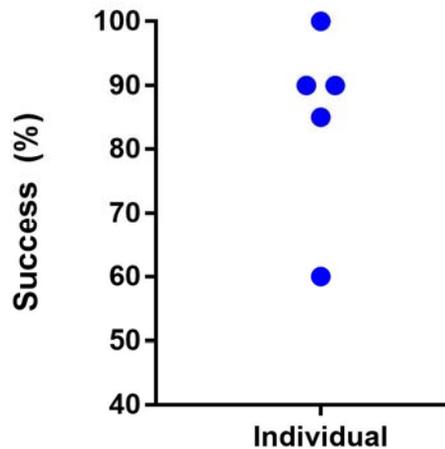
Average Success: 85%

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Modified Analysis Parameters

- Samples were manually reviewed (n=240)
  - ANDE profiles were analyzed in GeneMapper IDX v1.5
  - RapidHIT profiles were reviewed in GeneMarker HID v2.8.2
  - Heterozygote balance filter set at 0.25

- After manually interpreting a profile (PHR >0.25, low AT, recovery of "lost" data), concordance was checked against the laboratory generated reference profile

- Success was determined by **complete and concordant** profiles for the 20 CODIS core loci

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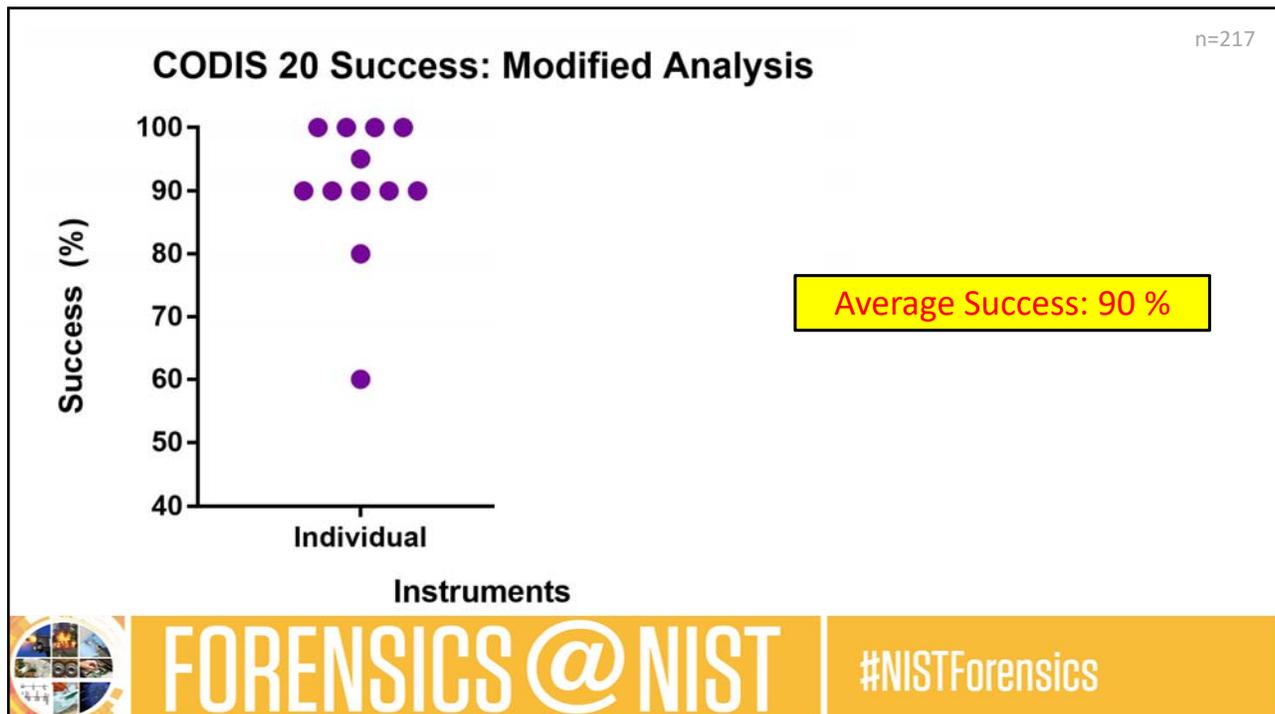
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n=217

CODIS 20 Success: Modified Analysis

Average Success: 90 %

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Unrecovered Samples

n=23

Unrecovered Samples

n=23
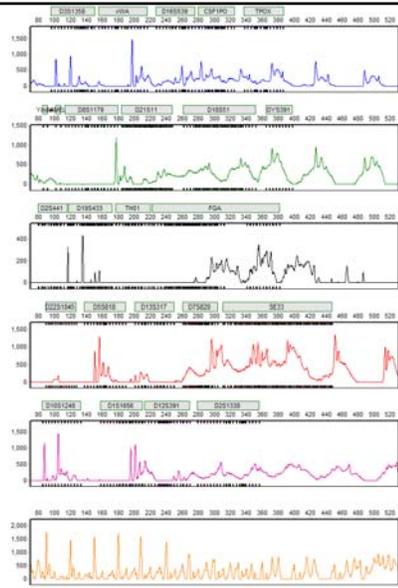
Instrument Related

| Unknown n=9 | Data Transfer Failure n=2 |

No data was transferred from the instrument to analyze

Unrecovered Samples
n=23

Instrument Related

Partial Profile
n=10

Unknown
n=9

Data Transfer Failure
n=2

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Unrecovered Samples
n=23

Instrument Related

Partial Profile
n=10

Unknown
n=9

Data Transfer Failure
n=2

Single Locus Dropout
n=2

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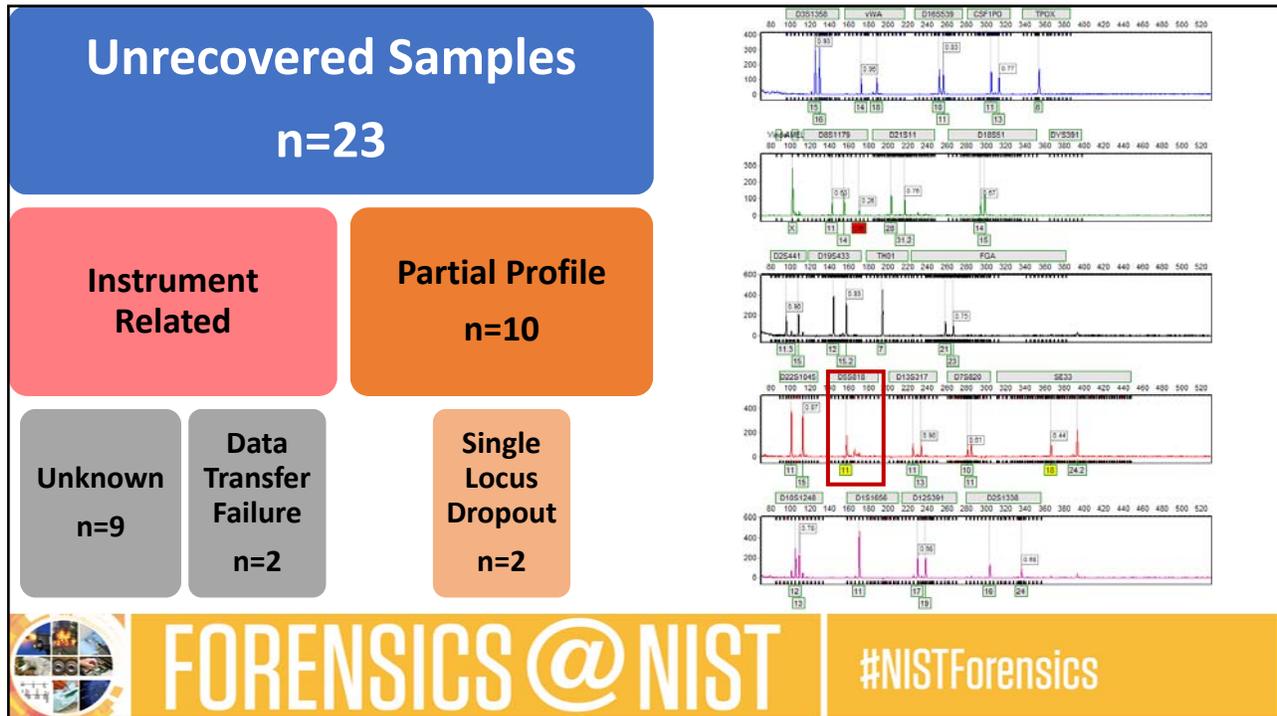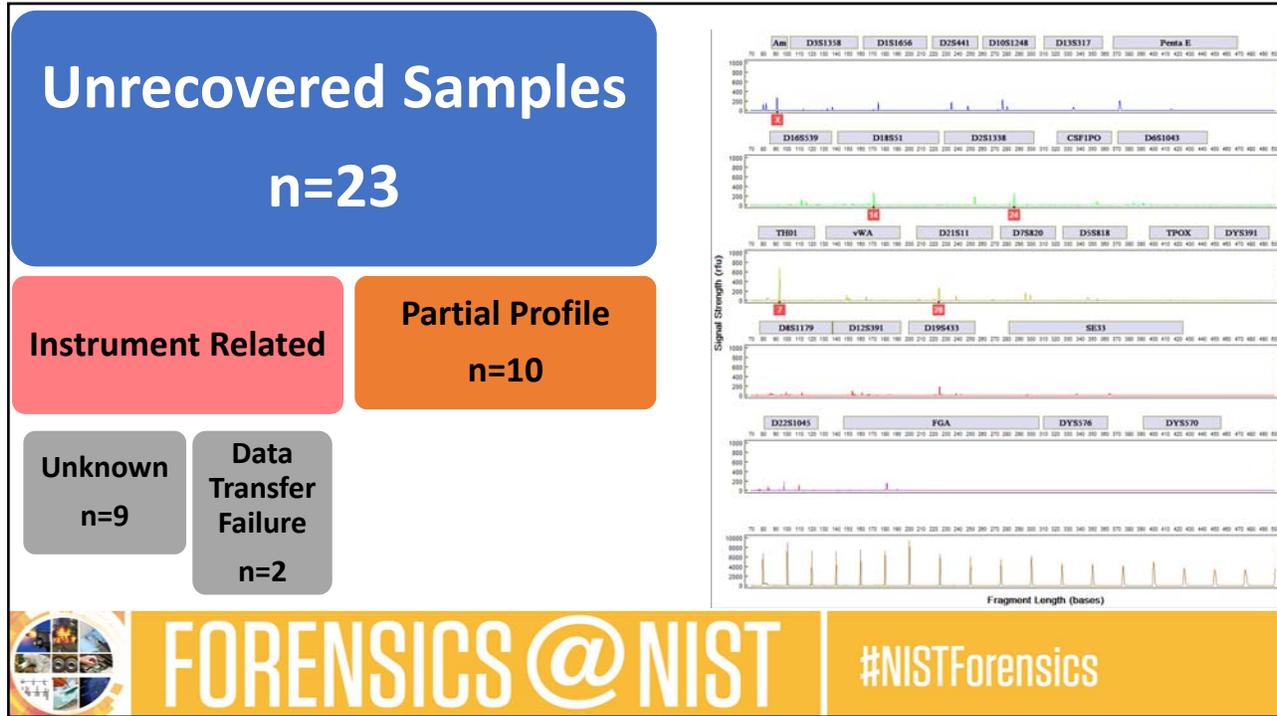
Summary of Success
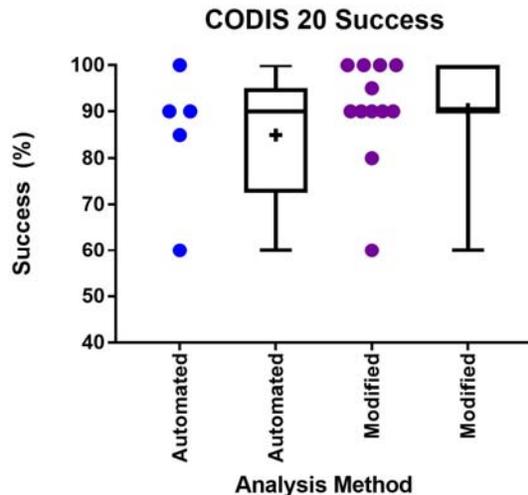
- **Automated success rate of 85% (n=100)**
  - ANDE 6C is the only NDIS approved instrument for automated analysis
  - xml files for the unsuccessful profiles were not generated by the instrument (15%)

- **Modified analysis success rate of 90%**
  - Manual interpretation of all samples (n=240 samples)
  - Increase of success for manually reviewed profiles

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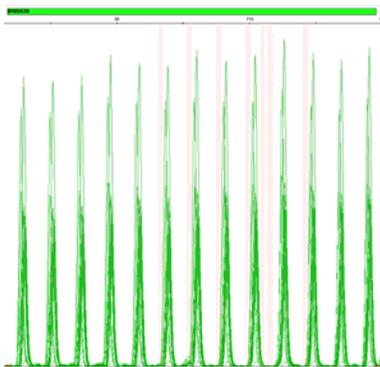
n=217 successful profiles
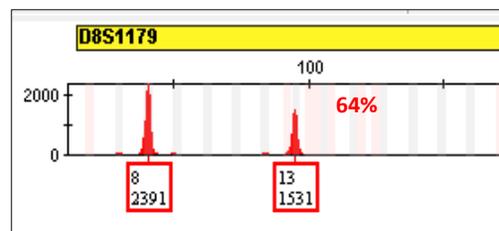
Additional Metrics Analyzed

Base pair sizing precision

Heterozygote Balance

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Precision-Base Pair Sizing

n=19 ladders for ANDE
n=8 ladders for RH 200

ANDE Size Pooled Standard Deviation
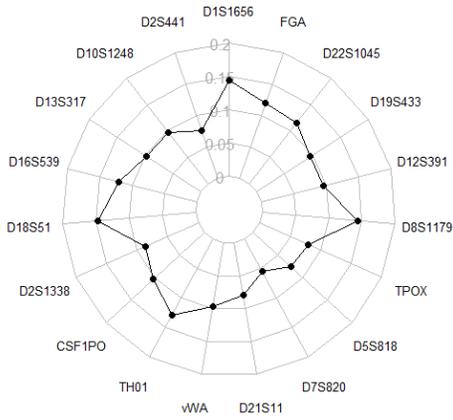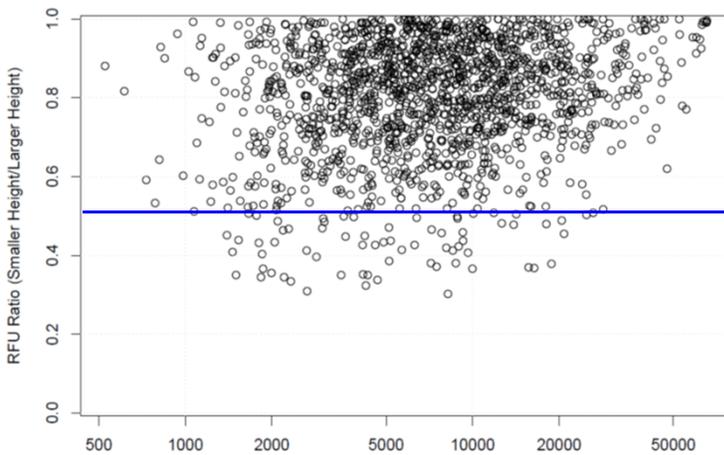
RapidHIT 200 Size Pooled Standard Deviation
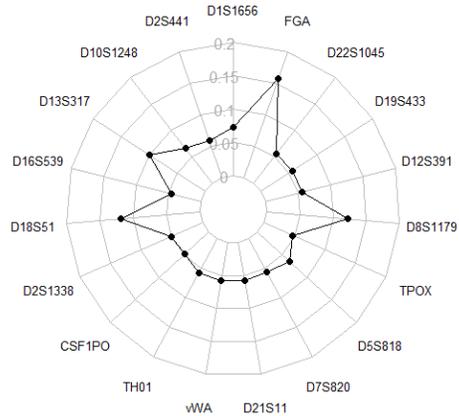
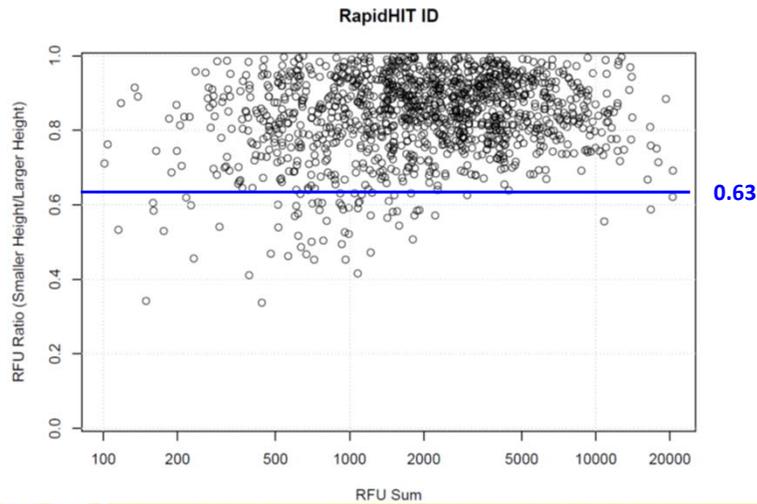**FORENSICS @ NIST** #NISTForensics

Heterozygote Balance

ANDE

95% of the samples were greater than 52%

0.52

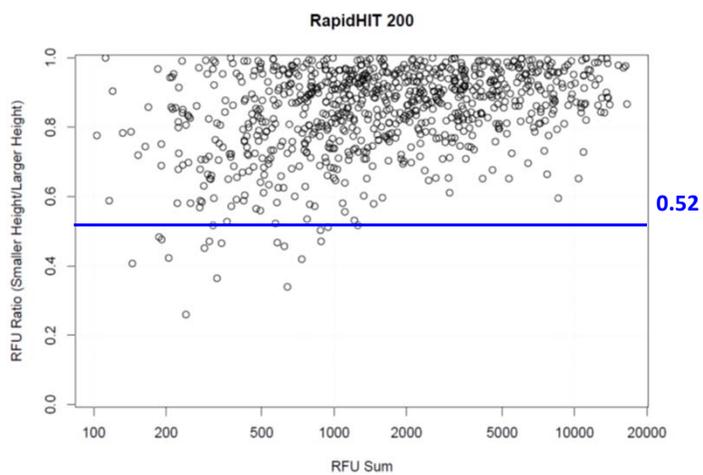**FORENSICS @ NIST** #NISTForensics

Heterozygote Balance

RapidHIT ID

95% of the samples were greater than 63%

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Heterozygote Balance

RapidHIT 200

95% of the samples were greater than 52%

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Heterozygote Balance

n=217 successful profiles
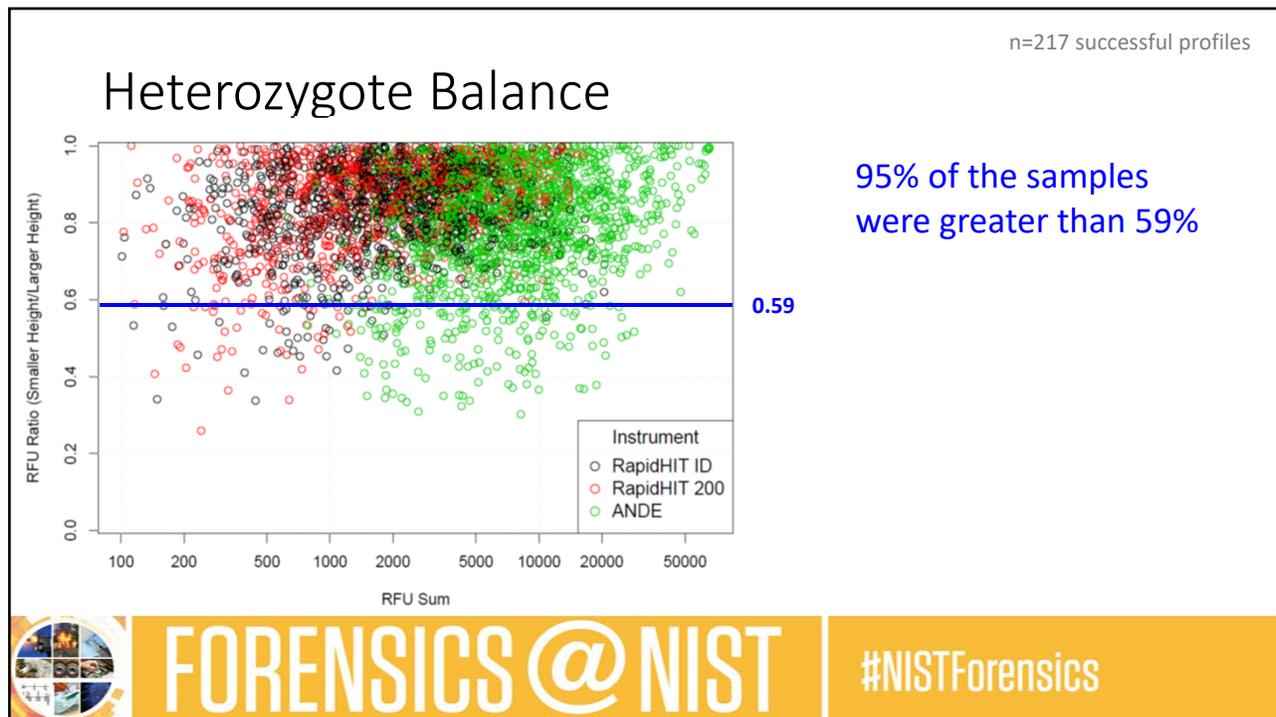
95% of the samples were greater than 59%

Maturity Assessment Summary

- 12 instruments tested across 9 laboratories
- Total of 240 samples examined
  - 85% success rate for the CODIS 20 using **Rapid DNA Analysis**
  - 90% success rate for the CODIS 20 using **Modified Rapid DNA Analysis**
  - Success ranged from 60% to 100%
  - Precision was below 0.17 bp on for both ANDE 6C and RapidHIT 200
  - Combined heterozygote balance (all three instruments) was above 59%

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Acknowledgements

Thank you to our participants

- ANDE
- Arizona Department of Public Safety
- Bensalem Police Department
- Federal Bureau of Investigation Laboratory
- Louisiana State Police Crime Laboratory
- Miami Beach Police Department
- Miami Dade Police Department
- NIST (DHS instruments, run at SNA Intl.)
- U.S. Army Criminal Investigation Laboratory

Contact Information
erica.romsos@nist.gov

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