Rapid DNA Testing at NIST

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CODIS Conference

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National Institute of Standards and Technology

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Outline

- Rapid DNA Platforms
- 2013 Rapid DNA Interlaboratory study
- Rapid DNA work performed at NIST
- 2014 Rapid DNA Maturity Assessment

Rapid DNA platforms

- Testing on behalf of Chris Miles DHS S&T

- ANDE (NetBio)
  - PowerPlex 16 STR chemistry

- RapidHIT 200 (IntegenX)
  - PowerPlex 16 STR and GlobalFiler Express chemistry

Rapid DNA Instruments

ANDE (NetBio)

- Electrophoresis takes place on chip
- One biochipset
  - Stored at RT
  - Shelf life ≈ 6 months
- RFID tagged swabs

PowerPlex 16 loci
≈86 min runtime
(5 samples)

Rapid DNA Analysis
Automated profile interpretation

RapidHIT 200 (IntegenX)

- Electrophoresis takes place on an 8 capillary array
- Kit = 4 components
  - Stored between RT-4ºC
  - Shelf life ≈ 5 months @ 4ºC
- Cotton swabs

PowerPlex 16 loci
≈90 min runtime
(5 samples)
GlobalFiler Express loci
≈116 min runtime
(1-7 samples)

Modified Rapid DNA Analysis
Manual profile interpretation

Analysis: FBI Definitions

- **Rapid DNA Analysis**: describes the fully automated (hands free) process of developing a CODIS Core STR profile from a reference sample buccal swab. The "swab in – profile out" process consists of automated extraction, amplification, separation, detection and allele calling without human intervention.

- **Modified Rapid DNA Analysis**: describes the automated process of developing a CODIS Core STR profile from a reference sample buccal swab. This process consists of integrated extraction, amplification, separation, detection without human intervention, but requires human interpretation and technical review.

http://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-analysis

NIST R-DNA Interlaboratory Study **Fall 2013**

- Presented last September at BCC
- Two R-DNA developers
- Three testing sites
- A total of 350 reference buccal swabs run
- Success defined as the automated calling of the 13 core STR loci
- **Overall success = 87.4%**

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Vallone_BCC_Talk_Sept2013.pdf

Update since last year

September 2013-2014

- Run a total of 452 single source samples between both R-DNA platforms
  - 727 total (Not including negative controls, tests with non-buccal swabs)
- Success measured by concordant CODIS 13 loci called **Overall success = 84.8%**
- Two instrument upgrades for each platform
- Two software upgrades for each platform

Participation in developmental validation studies

- IntegenX RH200 (PowerPlex 16 chemistry)
  - 100 samples (NIST provided buccal swabs)
  - Age range (~1.5 years old)
  - 10 unique individuals
  - Results contributed to concordance and aged swab study
- NetBio ANDE (PowerPlex 16 chemistry)
  - 150 samples (reference swabs) provided by NetBio
  - Samples run over 3 weeks
  - Results provided back to NetBio/GEHC electronically

DV data is in the hands of the developers in the support of peer-reviewed studies

Positive and negative control experiments to support SWGDAM

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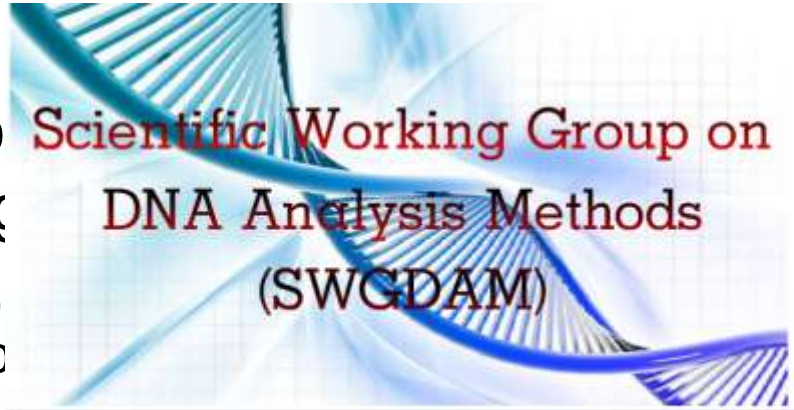
  - Swab n…



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FBI Quality Assurance Standard (QAS) Documents

- The FBI Director's Databasing Quality Assurance Standards for DNA Datasing Laboratories - Effective 09/01/2011
- The FBI Quality Assurance Standards Audit for DNA Databasing Laboratories - Effective 09/01/2011
- The FBI Director's Forensic Quality Assurance Standards for DNA Testing Laboratories - Effective 09/01/2011
- The FBI's Forensic Quality Assurance Standards Audit for Forensic DNA Testing Laboratories - Effective 09/01/2011
- The FBI Director's Addendum to the Quality Assurance Standards for DNA Databasing Laboratories performing Rapid DNA Analysis and Modified Rapid DNA Analysis Using a Rapid DNA Instrument – Effective 12/01/2014
- The FBI Director's Addendum to the Quality Assurance Standards Audit for DNA Databasing Laboratories performing Rapid DNA Analysis and Modified Rapid DNA Analysis Using a Rapid DNA Instrument – Effective 12/01/2014

O = Known Sample
X = Blank

Control Data Experiments

- Checkerboard and Zebra Stripe patterns to assess contamination

Checkerboard Pattern

Lane	1	2	3	4	5
Chip 1	X	O	X	O	X
Chip 2	O	X	O	X	O

3 replicates of pattern

Zebra Stripe Pattern

Lane	1	2	3	4	5
Chip 1	X	X	X	X	X
Chip 2	O	O	O	O	O

2 replicates of pattern

- No contamination or sample carryover observed
- Low-level artifacts which were called were properly flagged and not transferred into CMF file

Positive and negative controls

- Presence or absence of signal from a positive or negative control is not a good indicator of the success of other lanes
- This led the recommendation that positive and negative controls are not required for every run
- However, controls will be required for
  - **Cartridge/reagents check (lot check): run a positive and negative control (before or in parallel with reference samples)** *Standard 9 Analytical Procedures*

Positive	Negative	Sample 1	Sample 2	Sample 3

  - **Performance check: run positives on all lanes** *Standard 10 Equipment Calibration and Maintenance*

Positive	Positive	Positive	Positive	Positive

Making materials traceable to NIST SRM 2391c

- SRM = standard reference material ***Reference material*** is a material for which values are certified by a technically valid procedure and is accompanied by, or traceable to, a certificate or other documentation, which is issued by a certifying body.
- QAS 9.5.5 The laboratory shall check its DNA procedures ***annually*** or ***whenever substantial changes*** are made to a procedure against an appropriate and **available NIST standard reference material** *or* ***standard traceable to a NIST standard***.

http://www.nist.gov/traceability/
From the QAS
http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas_testlabs

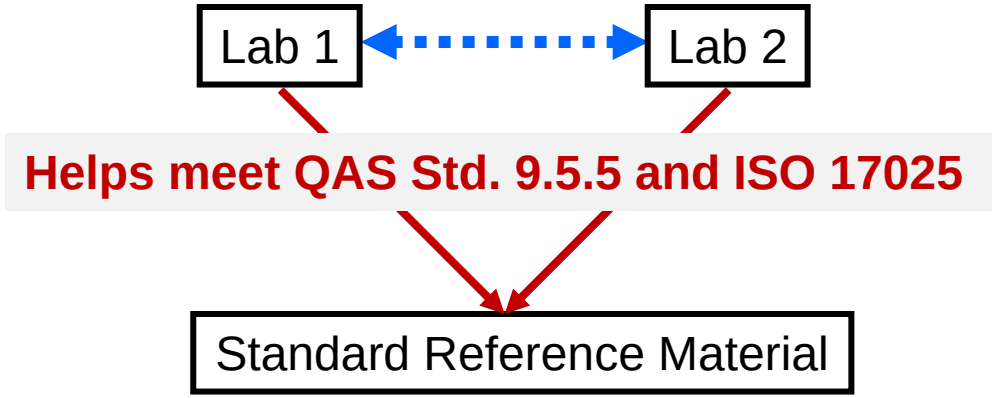
Standard **R**eference **M**aterial 2391c : PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are cells spotted on 903 paper or FTA paper

No buccal swabs in SRM 2391c

The paper components may not contain enough cells for R-DNA analysis

Genomic DNAs characterized for the expanded CODIS core loci and Y-STRs



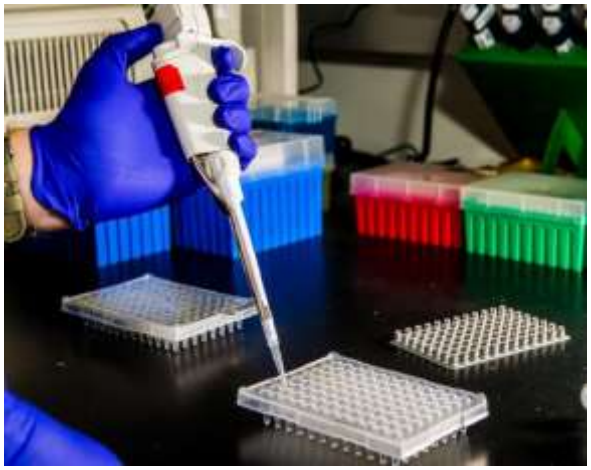
Calibration with SRMs enables confidence in comparisons of results between laboratories

How to make a NIST traceable swabs (SRM 2391c) - example

Collect a lot of 10 Buccal swabs from single individual

You are making this lot of swabs traceable to the SRM

Extract the DNA from two swabs from the lot (traditional lab methods)



Amplify extracted swabs along with components from SRM 2391c

Verify SRM 2391c allele calls are accurate against the certificate and make allele calls for the (now) traceable swab lot

How to make a NIST traceable swabs (SRM 2391c)

- These swabs can be used on R-DNA instruments now as a NIST traceable material
  - Must confirm typing results after running on a R-DNA platform
  - The process must be repeated to make another traceable lot of materials

- Use of traceable swabs:
  - **Annually or when upgrades are made (9.5.5 of QAS)**

  also if desired
  - During a critical reagents and R-DNA cartridge check (Standard 9)
  - R-DNA performance check (Standard 10)

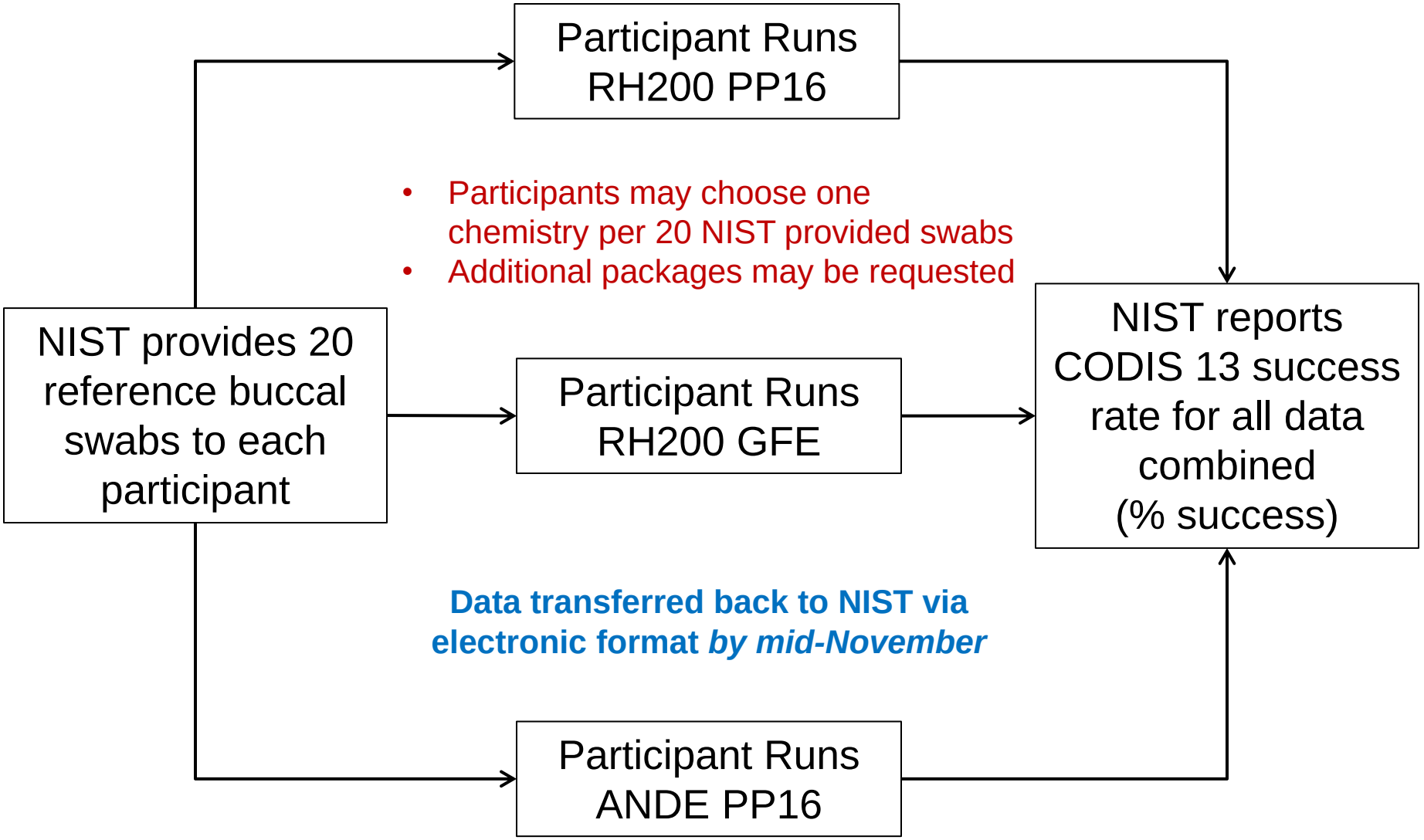
Rapid DNA Maturity Assessment

Preliminary Results

Rapid DNA Maturity Assessment

- Fall of 2014 assessment of the current status of rapid DNA typing technology for the CODIS Core Loci
  - In support of lab and future external (non-lab-based) Rapid DNA implementation
- Each laboratory runs 20 reference buccal swabs
  - 10 individuals in duplicate (provided by NIST)
- Data returned to NIST for analysis

R-DNA Maturity Assessment



Preliminary Maturity Assessment Results

- 5 laboratories have submitted results
  - 7 sets of PP16 data = 140 profiles
  - 5 sets of GFE data = 100 profiles

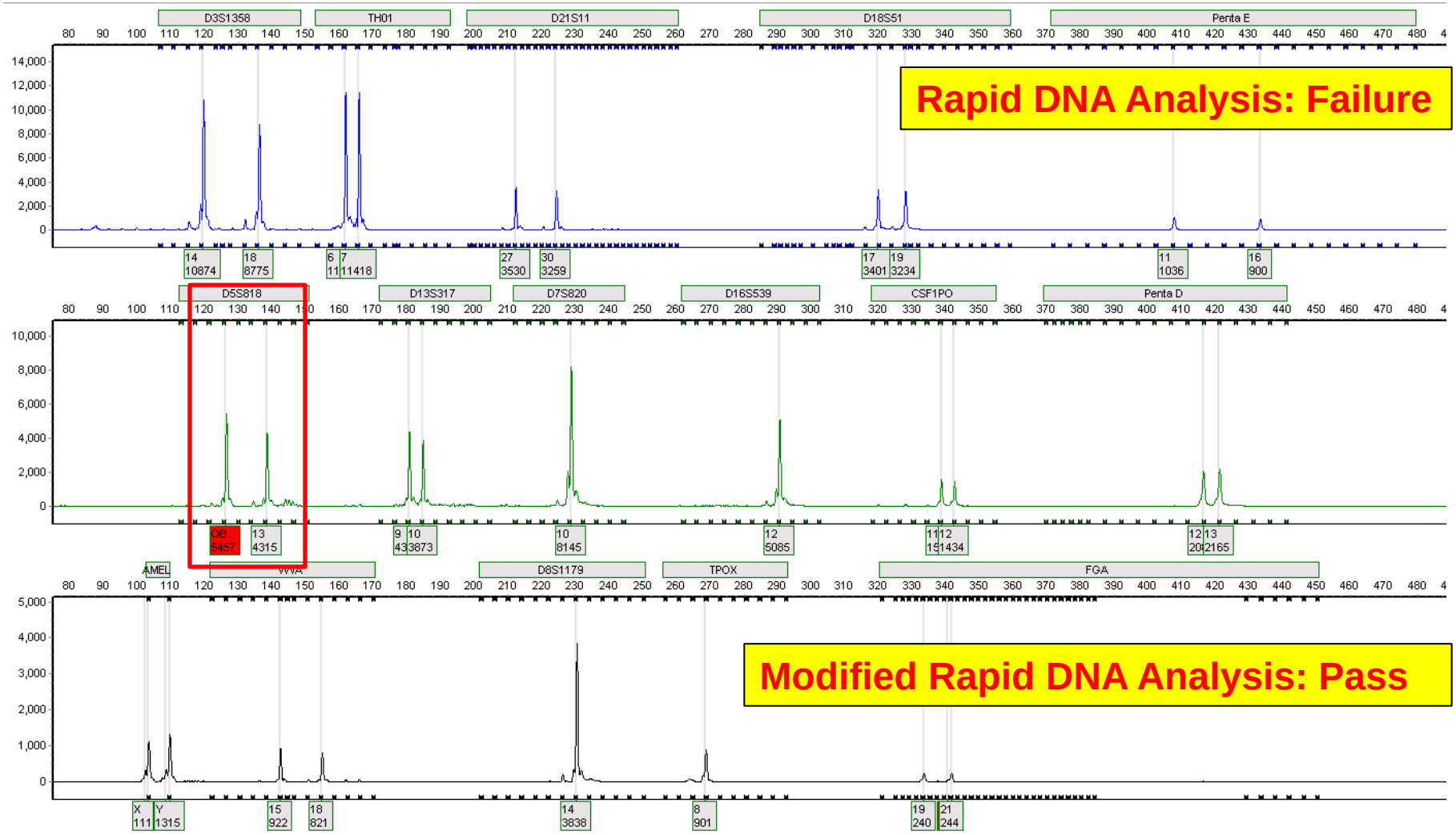
- A total of 240 samples have been analyzed to date by NIST
  - Employed both *Rapid DNA Analysis* and *Modified Rapid DNA Analysis* for all data

NIST Analysis Parameters

- **Rapid DNA Analysis**: Without human intervention, CMF file and electropherogram were evaluated for correct concordance. Any flagged alleles not reported in the CMF file and incorrect allele calls were considered failures. Success was measured for all CODIS Core STR loci.

- **Modified Rapid DNA Analysis**: Expert interpretation and analysis of electropherogram with annotated corrections, to include confirmation of flagged alleles, removal of pullup, removal of spikes, etc. Success was measured for all CODIS Core STR loci

D3S1358	TH01	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	AMEL	vWA	D8S1179	TPOX	FGA



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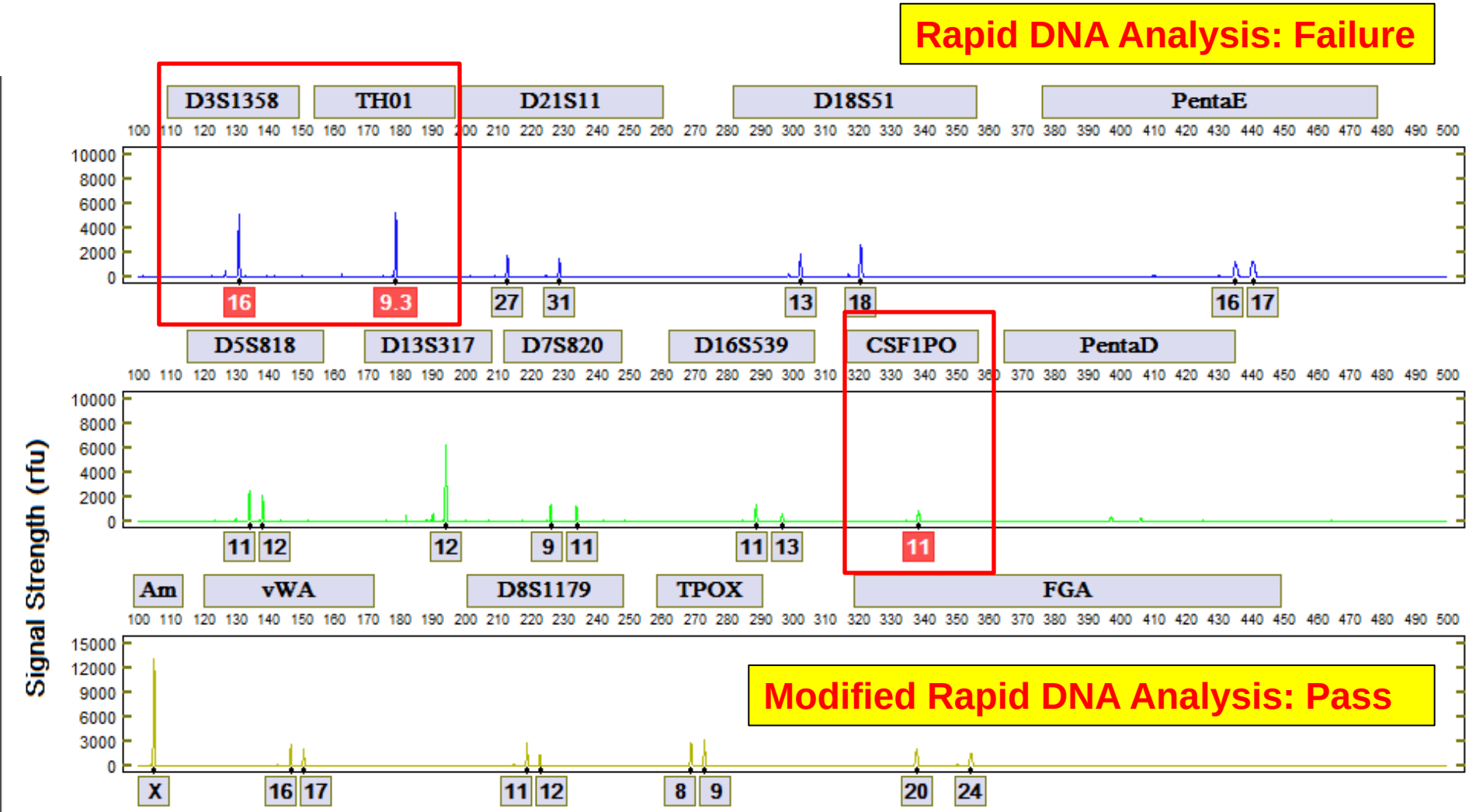

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Preliminary Results

Rapid DNA Instrument Platforms	Number of Participating labs	Total instruments	Samples attempted	Core CODIS Success (Rapid DNA Analysis)	Core CODIS Success (Modified Rapid DNA Analysis)
2	5	9	240	79%	82%

Once all results have been analyzed the overall success for the R-DNA maturity assessment will be reported:

http://www.nist.gov/mml/bmd/genetics/dna_biometrics.cfm

Summary

- Continuing to run R-DNA platforms with newer kits/chemistries
- Continuing to provide data in support of discussion within the SWGDAM R-DNA committee
- Example of material traceability to SRM 2391c for R-DNA platforms
- R-DNA maturity assessment: finalizing study (pending data from 3 more participants)

Thank you for your attention!

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Funding

DHS – Rapid DNA Prototype and Kinship Performance Evaluation

FBI - the Evaluation of Forensic DNA Typing as a Biometric Tool