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Training Level Effects on RDNA Device Results and Analysis

Rapid DNA Technology Forum

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Study Objective

To provide an objective third party evaluation of Rapid DNA devices commercially available (IntegenX and NetBio). Categories of this evaluation include:

1. Training necessary for reproducible results by expert software

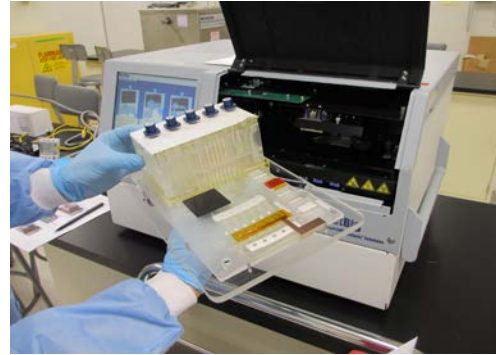2. Ease of use for running the device

3. Data interpretation pitfalls

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Devices Assessed

IntegenX RapidHIT 200

- 180 lb
- 24 loci
- 7 samples
- Run time: 90 min
- Refrigerated reagents

NetBio ANDE 4C

- 117 lb
- 16 loci
- 5 samples
- Run time <85 min
- Room temperature reagents

NetBio ANDE 6C

- 117 lb
- 27 loci
- 5 samples
- Run time <85 min
- Room temperature reagents

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Buccal Swab Sample Collection

IntegenX: wooden handled Puritan swabs

NetBio Proprietary RFID tagged swabs with desiccant

Image from: Croatian Medical Journal 55(1):3-9 2014

Experimental Design

Specialist
- 2 operators
- 48 buccal swabs
  - 2 per donors
  - 12 donors
- One blank swab per run

Non-Specialist
- 2 operators
- 24 buccal swabs
  - 1 per donor
  - 12 donors
- One blank swab per run

- 72 sample swabs per device
- 12 blanks on IntegenX RapidHIT 200
- 18 blanks on NetBio ANDE 4C or 6C

Loci Covered for Truth Data

Expanded US CODIS Core Loci	Other Loci
CSF1PO	Amelogenin
FGA	DYS391
TH01	Penta D
TPOX	Penta E
vWA	SE33*
D3S1358	DYS576*
D5S818	DYS570*
D7S820	
D8S1179	
D13S317	
D16S539	
D18S51	
D21S11	
D2S1338	
D19S433	
D10S1248	
D22S1045	
D2S441	
D1S1656	
D12S391	

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Operational Training

- For RapidHIT 200 and ANDE 4C:
  - ~½ hour training on how to safely operate the device
  - Training on how to download the data from the device

- ANDE 6C:
  - Training provided by NetBio Field Application Scientist on how to run the device.
  - Trained on downloading of data from the device.

- No training on how to interpret the data was provided to either specialist or non-specialist groups.

- NetBio provided training on interpretation of data to project team members directing the project, but not operating the devices.

IntegenX RapidHIT 200 Procedural Assessment

All operators described the sample preparation and loading of samples as very easy.

However, it was suggested that a way to individually seal the lanes after addition of a sample would improve the device.
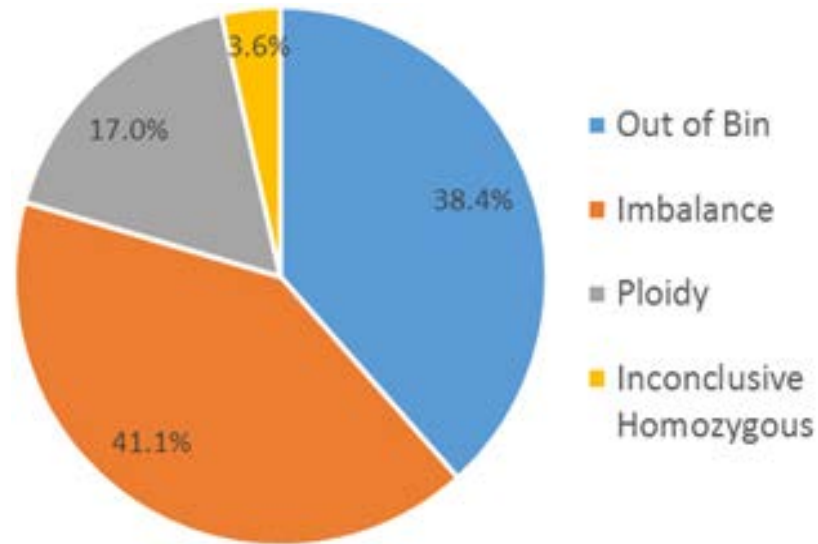
IntegenX RapidHIT 200 Allele Report

Locus	01SD4S5
D3S1358	16
vWA	18, 19
D16S539	12, 13
CSF1PO	12, 13
TPOX	8, 11
Y indel	
AMEL	X
D8S1179	11, 15
D21S11	28
D18S51	12, 15
DYS391	
D2S441	11, 14, 16 (PL, IMB)
D19S433	11, 14
TH01	7, 9.3
FGA	13 (PL, IMB), 19, 23
D22S1045	15
D5S818	11,12
D13S317	11
D7S820	12, 13
SE33	6.3, 27.2, 30.2 (PL)
D10S1248	14, 16
D1S1656	13, 15.3
D12S391	18, 22
D2S1338	20

Flags indicating loci should be reviewed

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IntegenX RapidHIT 200

Operator Type	Swabs to be Reviewed	Number of Flagged Loci	Number of Flags
Specialist	17/48 (35.4%)	43	85
Non-Specialist	3/24 (12.5%)	11	27

Pie chart legend:
- Out of Bin — 38.4%
- Imbalance — 41.1%
- Ploidy — 17.0%
- Inconclusive Homozygous — 3.6%

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IntegenX RapidHIT 200- Unmodified
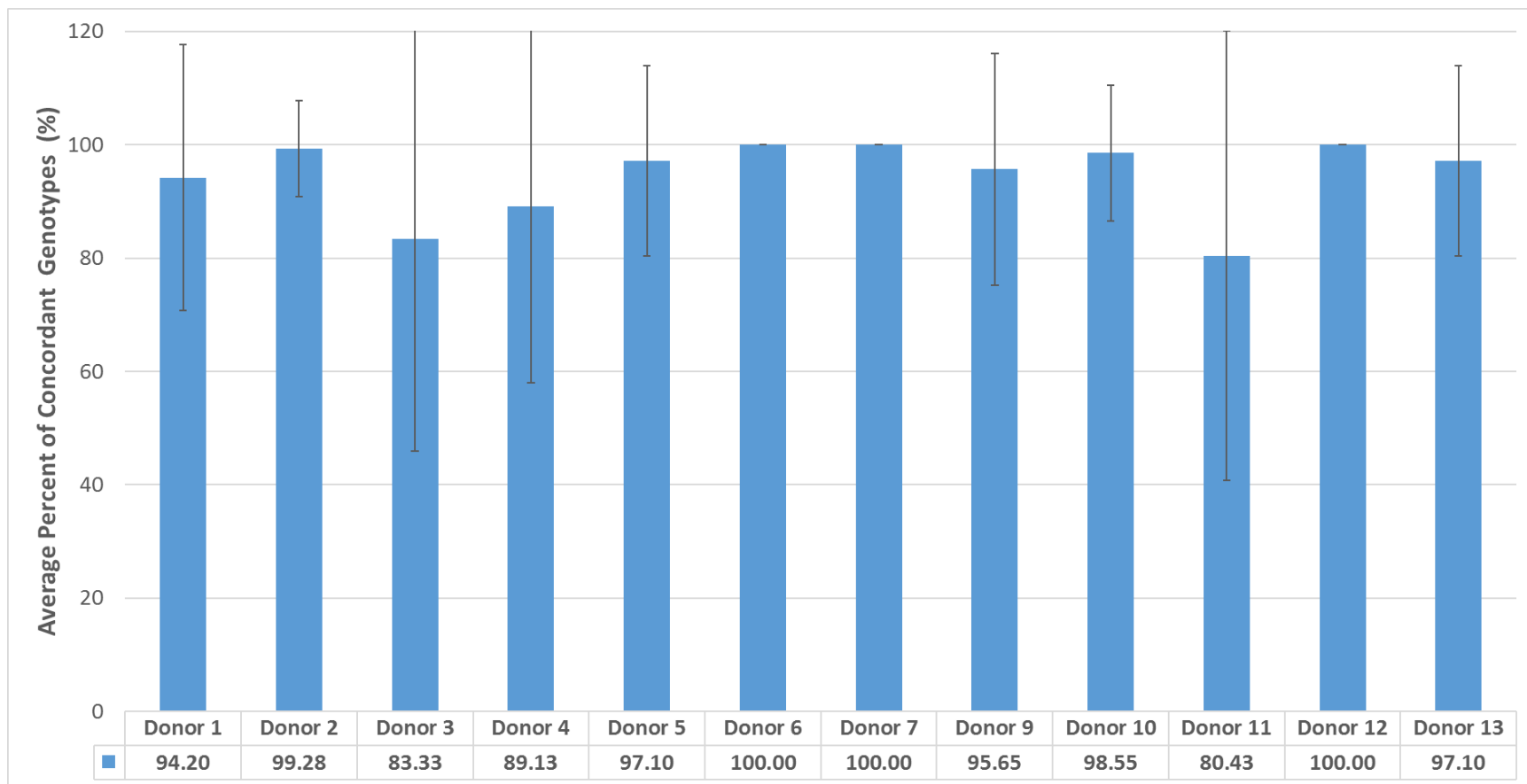
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 9	Donor 10	Donor 11	Donor 12	Donor 13
■	94.20	99.28	83.33	89.13	97.10	100.00	100.00	95.65	98.55	80.43	100.00	97.10

Y-axis: Average Percent of Concordant Genotypes (%)

100% of expert software confidently called alleles were concordant to the truth.

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IntegenX RapidHIT 200- Modified

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 9	Donor 10	Donor 11	Donor 12	Donor 13
■	99.28	99.64	83.33	99.64	99.28	100.00	100.00	100.00	100.00	82.97	100.00	100.00
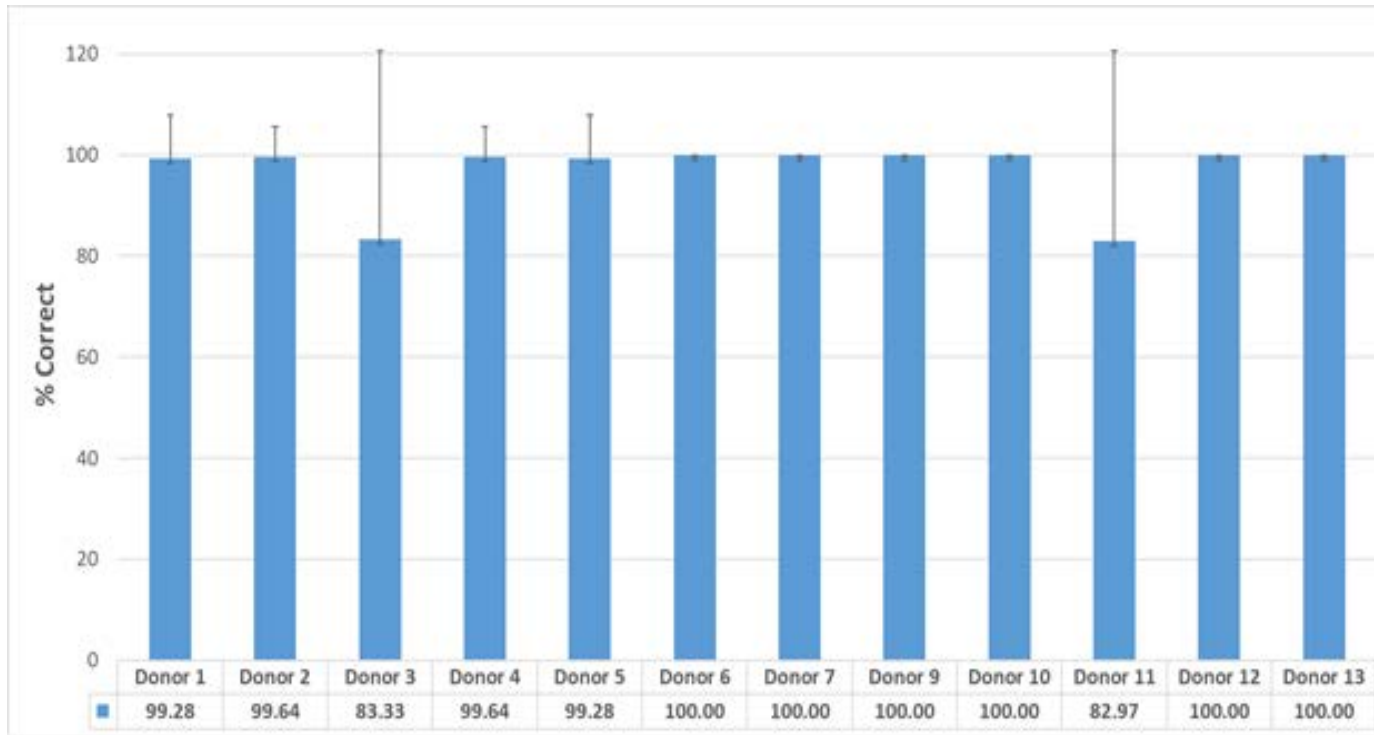
Expert software called alleles plus specialist calls after manual review of flagged loci.

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IntegenX RapidHIT 200
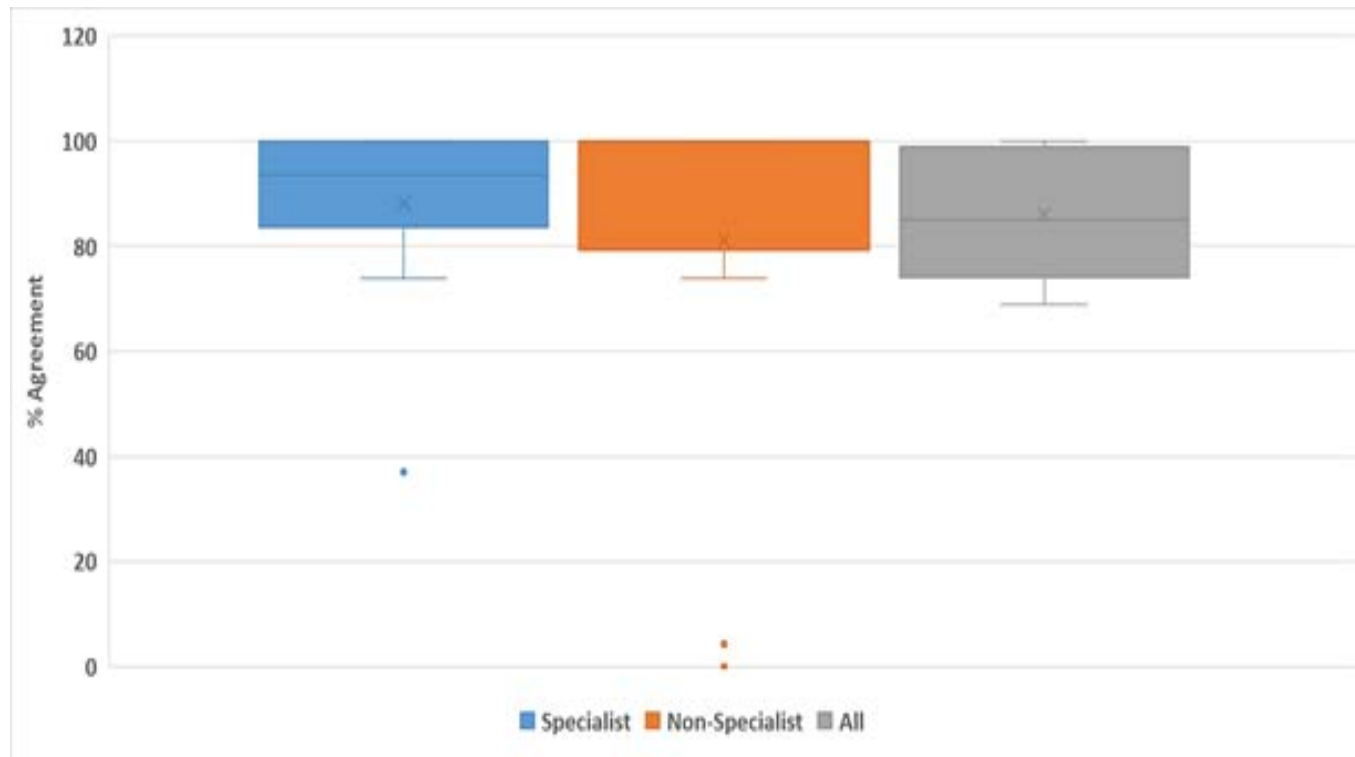
RapidHIT 200: Timing

| Process	Specialist (Hours:Minutes:Seconds)	Non-specialist (Hours:Minutes:Seconds)
Hands-On-Time	0:13:00 ± 0:04:32	0:29:00 ± 0:14:36
Run Time	1:54:47 ± 0:00:54	1:54:30 ± 0:02:23
Cleanup Time	0:01:35 ± 0:00:24	0:02:15 ± 0:00:30

NetBio ANDE 4C Procedural Assessment

All operators described the sample preparation and loading of samples as very easy.

A suggested improvement is a mobile RFID scanner.

- Once a swab is locked into the BioChipCassette, currently, the RFID tag cannot be scanned.
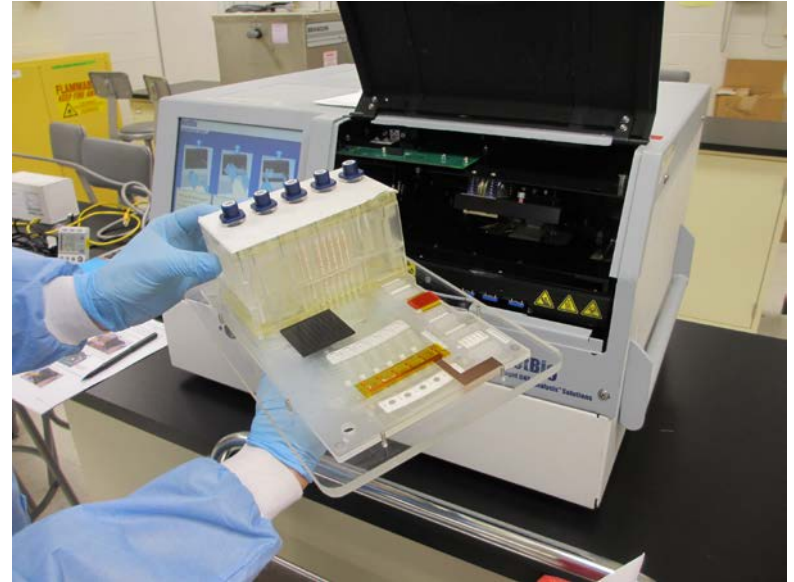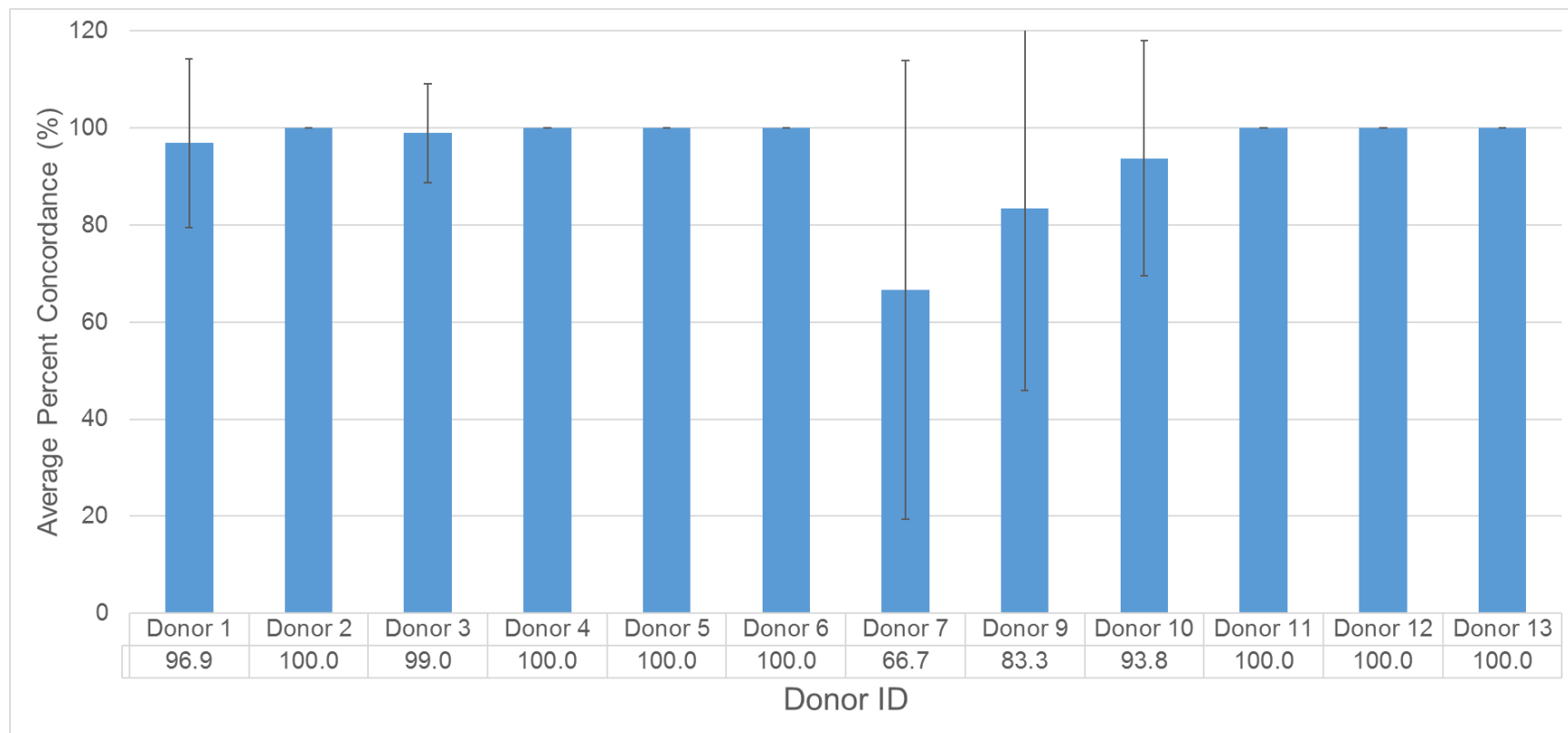
NetBio ANDE 4C
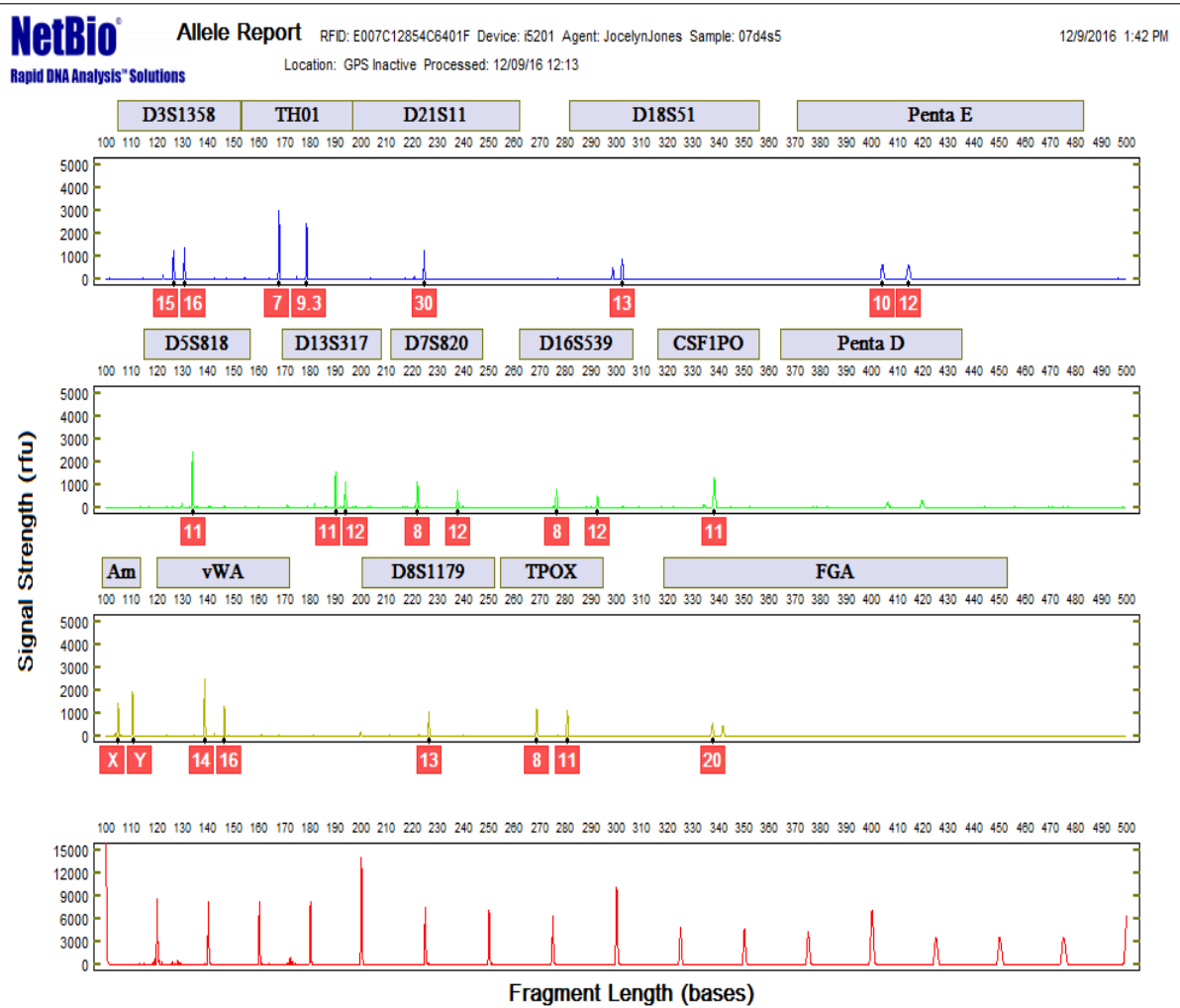
Red boxes indicate unapproved alleles that need a specialist operator to review
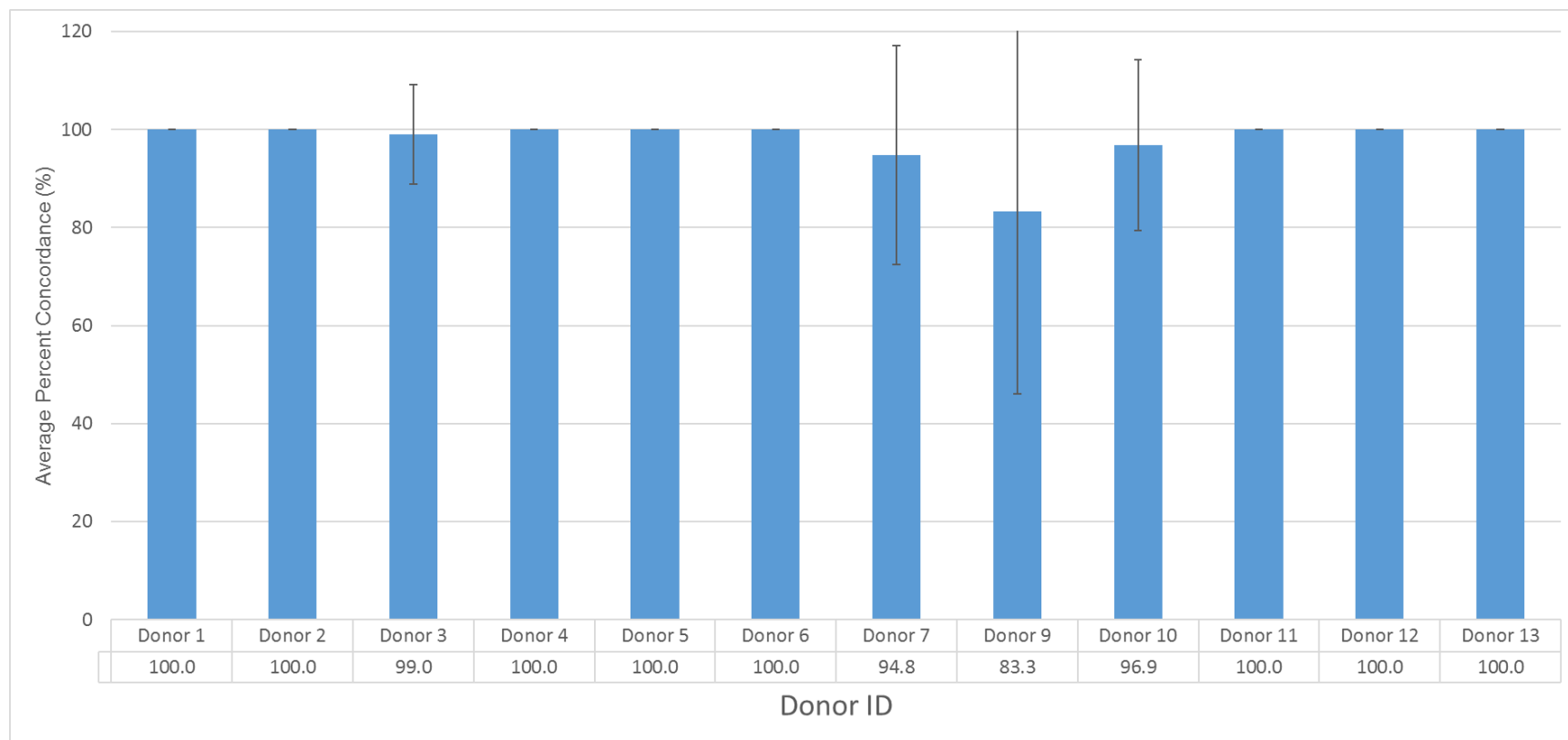
NetBio ANDE 4C

| Data Set	Full PowerPlex 16 Pass Rate	CODIS Core 13 Pass Rate
All data (n=72)	91.7% (66/72)	94.4% (68/72)
Specialist only (n=48)	89.6% (43/48)	93.8% (45/48)
Non-specialist only (n=24)	95.8% (23/24)	95.8% (23/24)

NetBio ANDE 4C- Unmodified

Donor ID	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 9	Donor 10	Donor 11	Donor 12	Donor 13
	96.9	100.0	99.0	100.0	100.0	100.0	66.7	83.3	93.8	100.0	100.0	100.0

100% of expert software confidently called alleles were concordant to the truth.

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NetBio ANDE 4C- Modified

Donor ID	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 9	Donor 10	Donor 11	Donor 12	Donor 13
	100.0	100.0	99.0	100.0	100.0	100.0	94.8	83.3	96.9	100.0	100.0	100.0

Expert software called alleles plus specialist calls after manual review of unapproved loci.
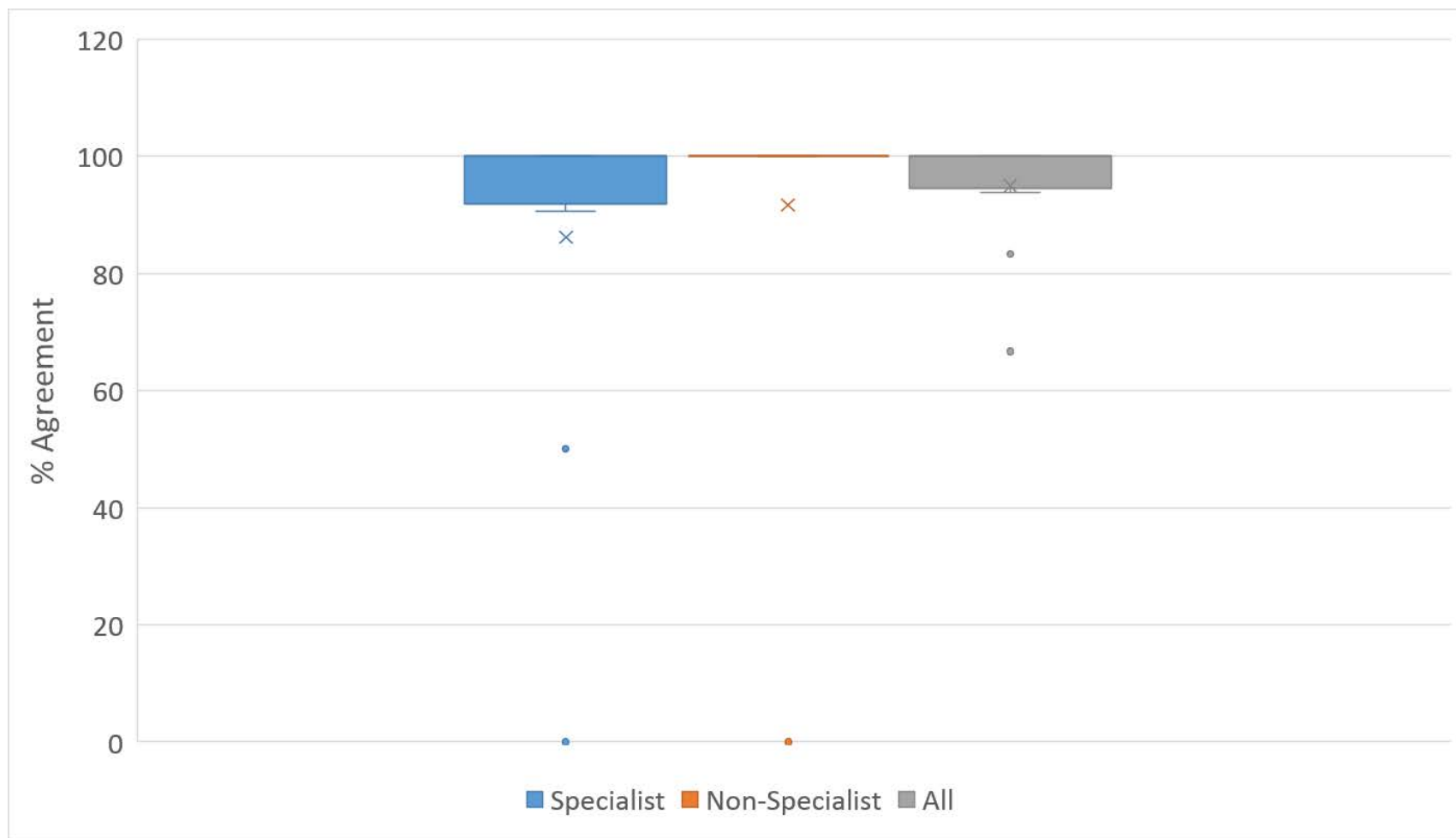
NetBio ANDE 4C

NetBio ANDE 4C: Timing

Process	Specialist (Hours:Minutes:Seconds)	Non-specialist (Hours:Minutes:Seconds)
POST Check and Software Launch	0:15:41 ± 0:00:28	0:15:30 ± 0:00:35
Hands-On Time	0:13:20 ± 0:07:42*	0:23:40 ± 0:16:14**
Run Time	1:28:44 ± 0:01:45	1:29:00 ± 0:00:42

*One outlier of 0:08:02 caused the wide standard deviation.
**One outlier of 0:19:00 caused the high average and standard deviation.

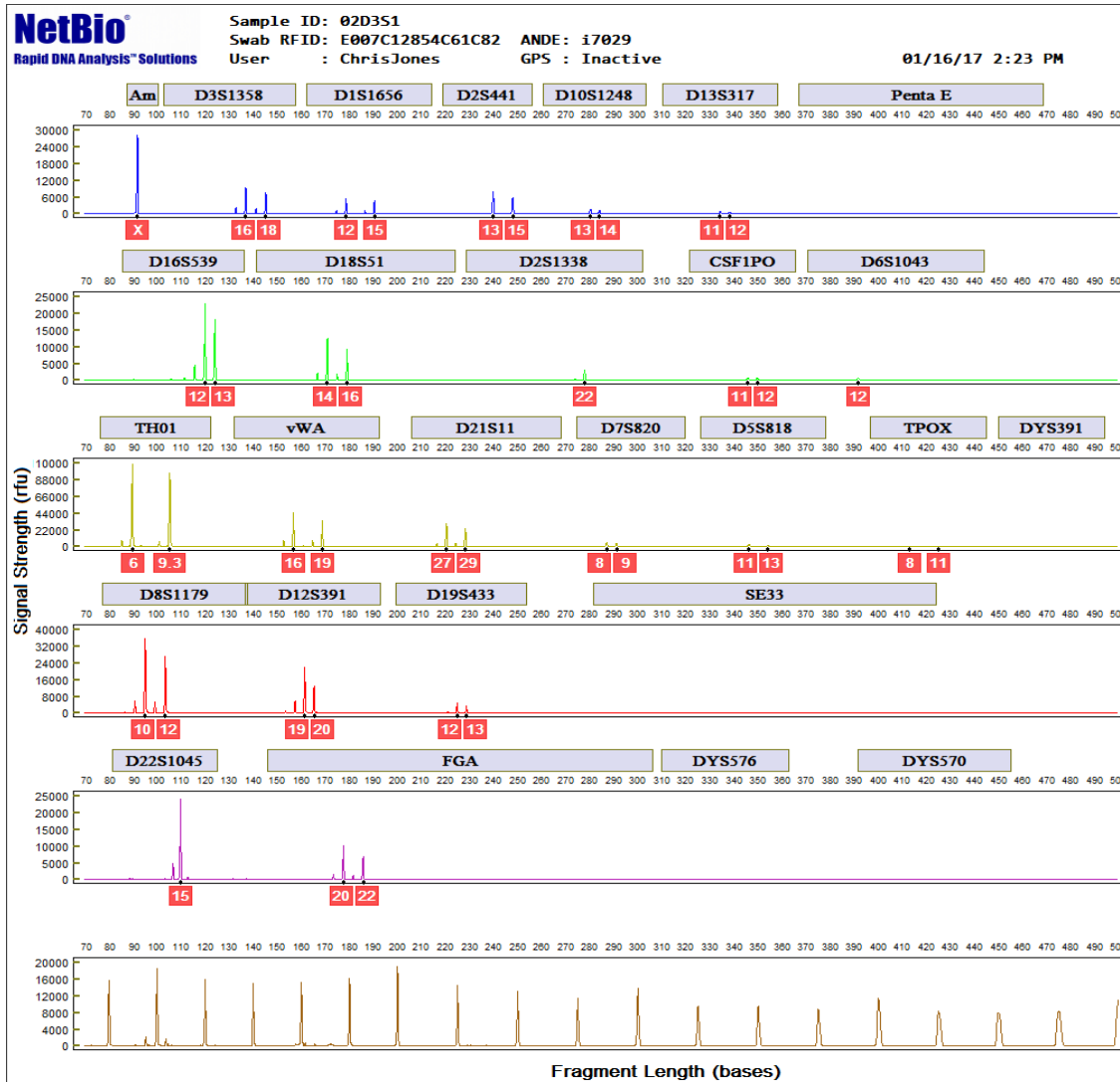**BATTELLE**

NetBio ANDE 6C Procedural Assessment

All operators described the sample preparation and loading of samples as very easy.

A suggested improvement is a mobile RFID scanner.

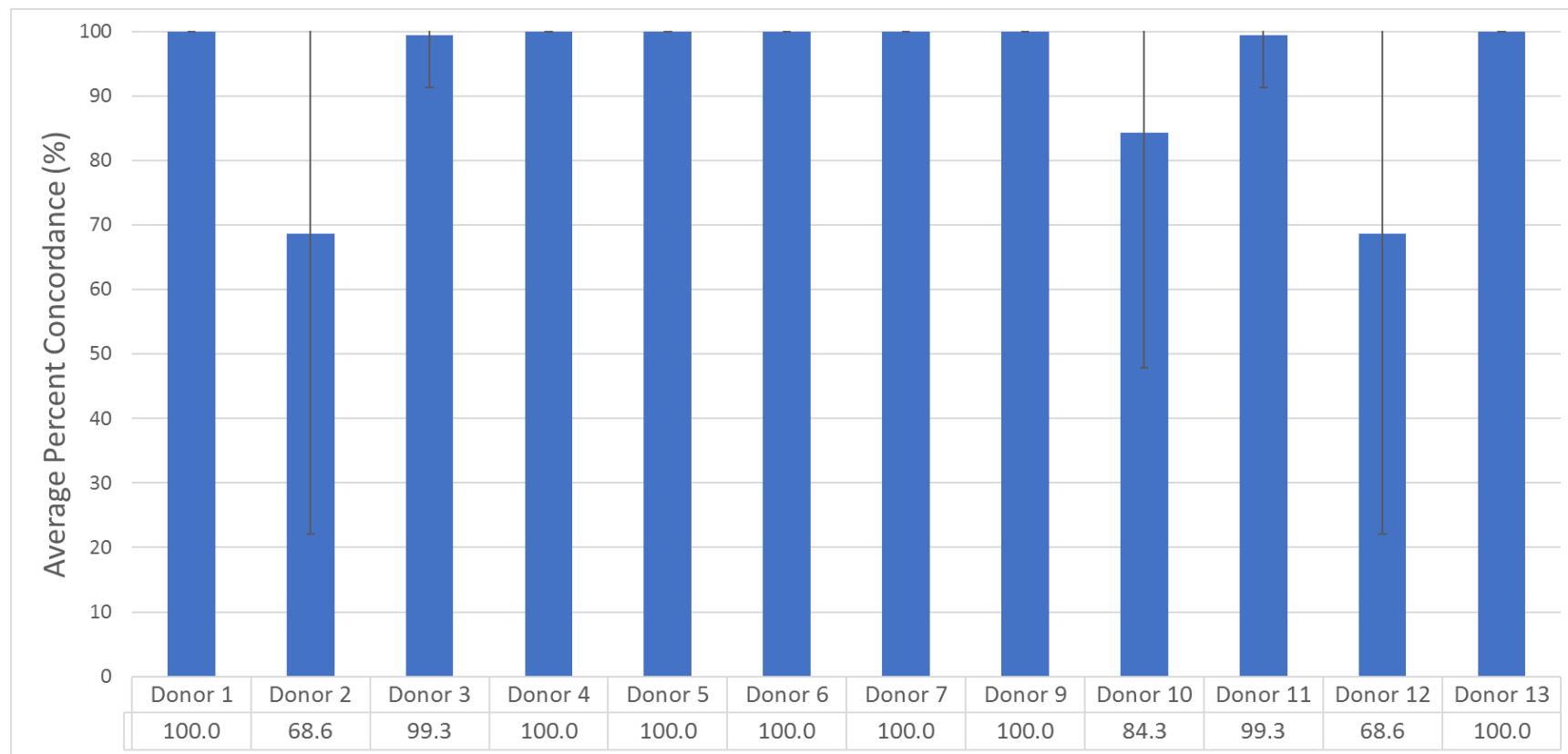- Once a swab is locked into the BioChipCassette, currently, the RFID tag cannot be scanned.

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NetBio ANDE 6C

Red boxes indicate unapproved alleles that need a specialist operator to review

NetBio ANDE 6C

Operator Type	Swab ID	Number of Unapproved Loci in Genotype	Unapproved Loci	Loci with No Allele Calls
Non-specialist	02d3s1	22	All but YSTRs, Penta E, SE33	Penta E, SE33, YSTRs*
Non-specialist	10d3s1	24	All but YSTRs	YSTRs*
Non-specialist	11d4s1	1	SE33	YSTRs*
Non-specialist	12d2s4	24	All but YSTRs	YSTRs*
Specialist	02d2s3	0	None	All
Specialist	03d2s3	0	None	Penta E
Specialist	12d3s3	24	All but YSTRs	YSTRs*

*YSTRs had no calls in these samples because the donor was female.

NetBio ANDE 6C-Unmodified

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 9	Donor 10	Donor 11	Donor 12	Donor 13
	100.0	68.6	99.3	100.0	100.0	100.0	100.0	100.0	84.3	99.3	68.6	100.0

100% of expert software confidently called alleles were concordant to the truth.

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NetBio ANDE 6C- Modified
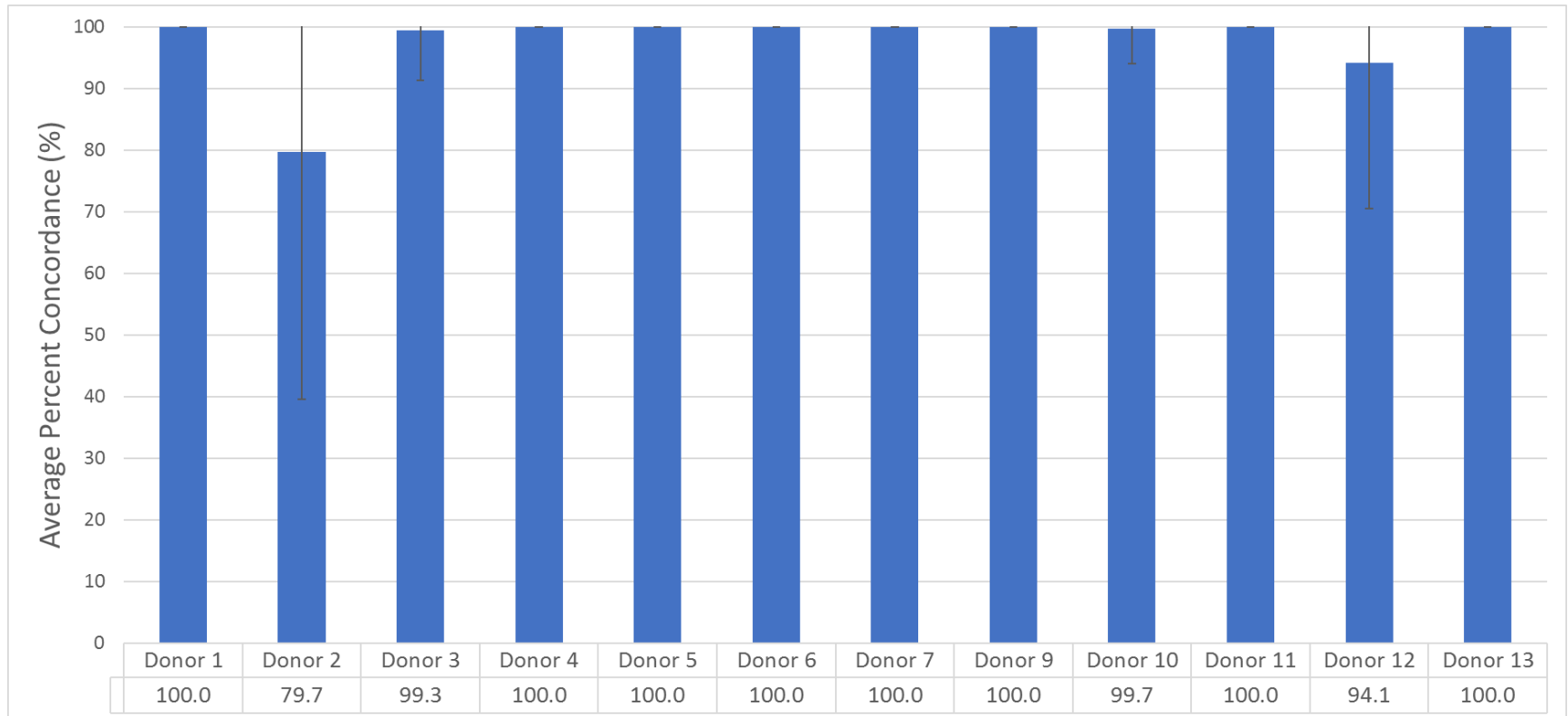
Expert software called alleles plus specialist calls after manual review of unapproved loci.

NetBio ANDE 6C
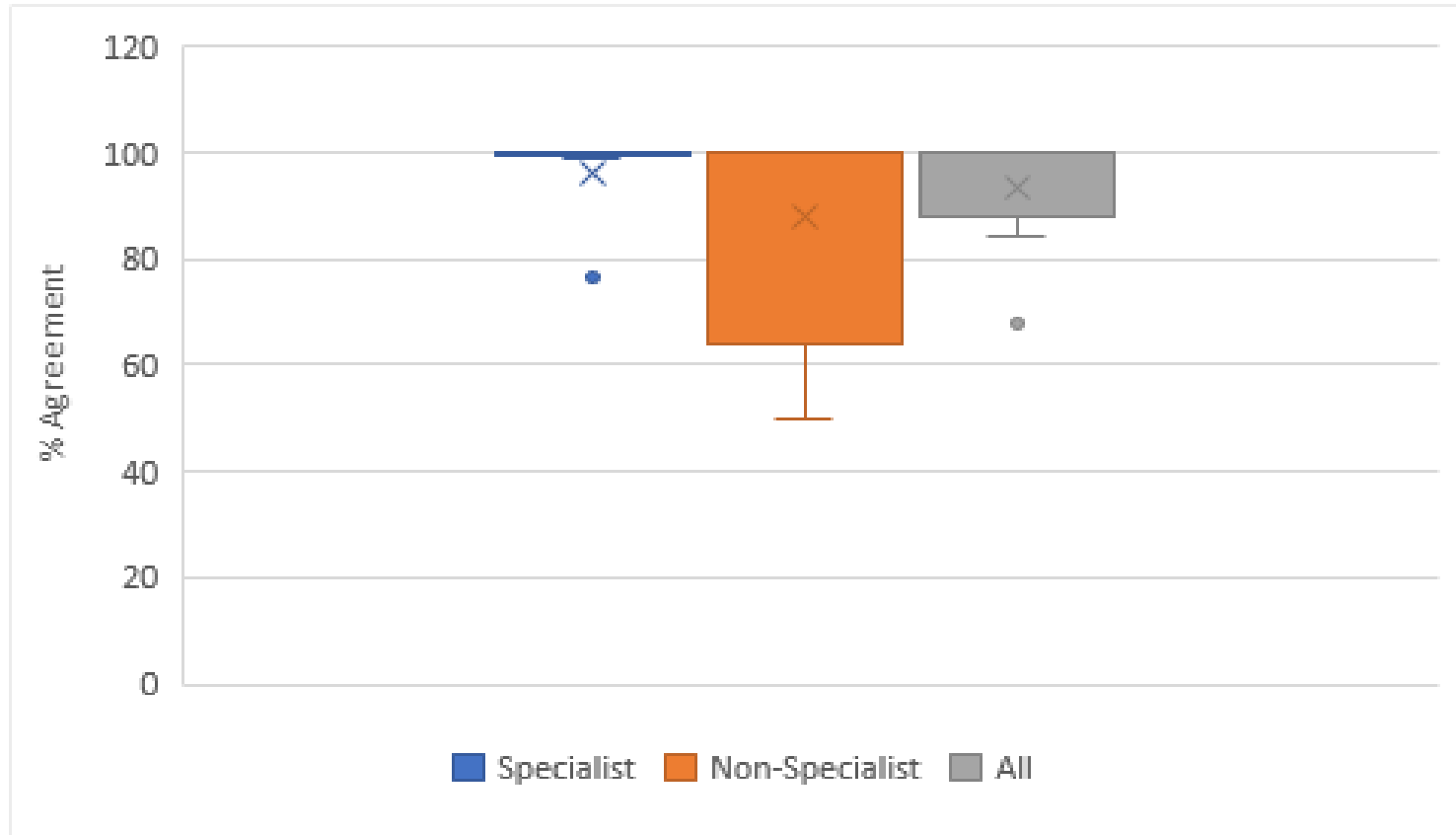
NetBio ANDE 6C: Timing

Process	Specialist (Hours:Minutes:Seconds)	Non-specialist (Hours:Minutes:Seconds)
POST Check and Software Launch	0:32:25 ± 0:24:52*	0:30:45 ± 0:22:47*
Hands-On Time	0:13:07 ± 0:09:04**	0:17:00 ± 0:08:26**
Run Time	1:36:35 ± 0:01:52	1:38:00 ± 0:00:38

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Conclusions

- Experience level of the operator did not affect data quality for either NetBio or IntegenX devices.

  - All approved (NetBio) or unflagged (IntegenX) loci were concordant with the truth data.

- RapidHIT 200 flagged 9 alleles that were true alleles instead of the sequence artifact.

  - 6 out of 9 were SE33.

- NetBio devices unapproved several usable loci.

- For all three devices, specialists were able to increase the number of usable allele calls upon manual data review.

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It can be done