Inter and intra-variation observed from a NIST interlaboratory study on DNA mixture interpretation in the U.S. (MIX13)

Michael Coble[1], PhD, Jo Anne Bright[2], PhD, James Curran[3], PhD, and John Butler[1], PhD

[1]National Institute of Standards and Technology, Gaithersburg, USA
[2]The Institute of Environmental Science and Research, Auckland, NZ
[3]The University of Auckland, Auckland, NZ

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NIST and NIJ Disclaimer

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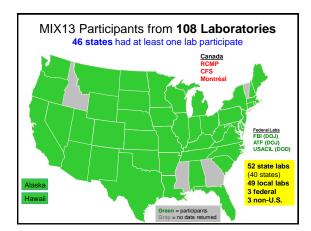
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NIST Interlaboratory Studies
on DNA Mixture Interpretation

Summary of DNA Mixture Interlaboratory Studies

Study	Year	# Labs	# Samples	Mixture Types
MSS 1	1997	22	11 stains	ss, 2p, 3p
MSS 2	1999	45	11 stains	ss, 2p, 3p
MSS 3	2000-01	74	7 extracts	ss, 2p, 3p
MIX05	2005	69	4 cases (.fsa)	only 2p
MIX13	2013	108	5 cases (.fsa)	2p, 3p, 4p

- Other recent studies
  - UK Regulator
  - DFSC (US Department of Defense Lab)

MIX13 Participants from **108 Laboratories**
46 states had at least one lab participate

Canada
RCMP
CFS
Montréal

Federal Labs
FBI (DOJ)
ATF (DOJ)
USACIL (DOD)

52 state labs
(40 states)
49 local labs
3 federal
3 non-U.S.

Alaska
Hawaii

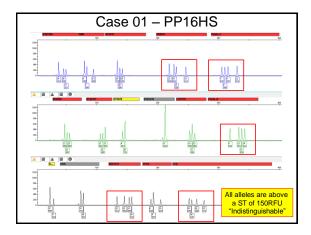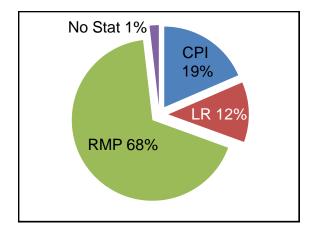**Green** = participants
Gray = no data returned

Due to the number of laboratories responding and the federal, state, and local coverage obtained, this MIX13 interlaboratory study can be assumed to provide **a reasonable representation of current U.S. forensic DNA lab procedures across the community**
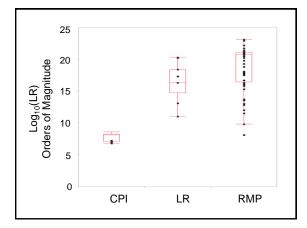
Purpose of MIX13 Cases

	Challenge provided to study responses
Case 1	~1:1 mixture (2-person)
Case 2	**Low template** profile with potential dropout (3-person)
Case 3	Potential **relative** involved (3-person)
Case 4	Minor component (2-person)
Case 5	Complex mixture (>3-person) with **# of contributors**; inclusion/exclusion issues

According to German Stain Commission (2009) mixture types: 1 = A, 2 = C, 3 = ?, 4 = B, 5 = ?
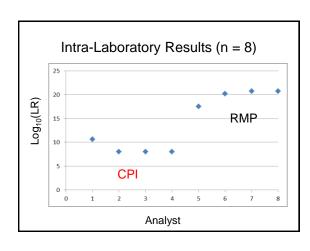
MIX13 Study (Case 01)

- Summary – Mock sexual assault, 2 person 50:50 mixture, all alleles above a ST of 150 RFU.

- Purpose – How many labs would consider the victim's profile and determine genotypes (deconvolution) for a mRMP statistic?
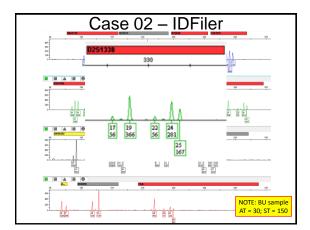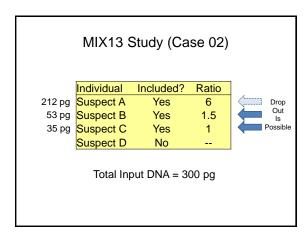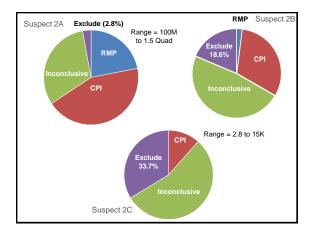
Case 01 – PP16HS

All alleles are above a ST of 150RFU "Indistinguishable"

Conclusions

- No false exclusions

- Wide range of variation in stats reported for labs that inferred genotypes (RMP or LR).

Case 02 – IDFiler

NOTE: BU sample AT = 30; ST = 150

MIX13 Study (Case 02)

Individual	Included?	Ratio
Suspect A	Yes	6
Suspect B	Yes	1.5
Suspect C	Yes	1
Suspect D	No	--

212 pg — Suspect A
53 pg — Suspect B
35 pg — Suspect C

Drop Out Is Possible

Total Input DNA = 300 pg

Suspect 2A — Exclude (2.8%), RMP, Inconclusive, CPI
Range = 100M to 1.5 Quad

RMP — Suspect 2B — Exclude 18.6%, CPI, Inconclusive

CPI, Exclude 33.7%, Inconclusive — Suspect 2C
Range = 2.8 to 15K

Intra-Laboratory Results (n = 8)

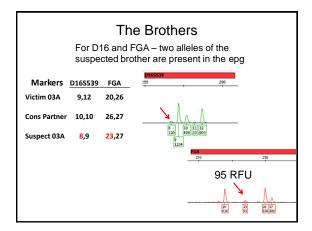| Analyst	Suspect A	Suspect B	Suspect C	Suspect D
1	Inconclusive - A, B, C			Excluded
2	6.74 Quad	23.6	Excluded	Excluded
3	Inconclusive - A, B, C			Excluded
4	9.4 for A, B, C			Excluded
5	4.1 Quint	37	Excluded	Excluded
6	230 for A, B		Inconclusive	Excluded
7	9.4 for A, B		Excluded	Excluded
8	37.3 for A, B		Excluded	Excluded

Concerns with Case 02

• One lab included Suspect D (False Inclusion).

• A substantial number of labs falsely excluded the two low-level contributors.
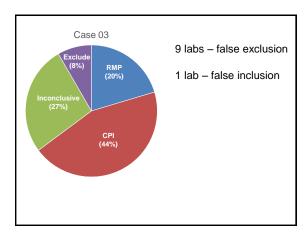
• Major concern with labs using CPI for contributor #3…

CPI with Suspect C

Contributors
A = 15, 15
B = 14, 15
C = **12**, 14

D19S433

15 of 108 labs used CPI to include Suspect C (13.8%)

4 of these 15 (26.6%) used D19 as a locus for CPI

Case 03 – IDPlus

Case 03 – Two Suspects

Individual	Inclusion?	Ratio
Victim	Included	7
Boyfriend (CP)	Included	2
Suspect 3A (Brother)	Included	1
Suspect 3B (Friend)	Excluded	--

Drop-out Possible

The Brothers

Markers	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01
Victim 03A	12,15	31.2,31.2	10,10	10,11	14,14	9.3,9.3
Cons Partner	14,14	28,35	10,11	10,12	14,18	7,8
Suspect 03A	14,15	28,35	10,11	12,12	14,18	7,8

Markers	D13S317	vWA	TPOX	D18S51	D5S818
Victim 03A	11,12	15,15	9,11	12,13	11,12
Cons Partner	12,13	17,21	6,8	13,16	10,12
Suspect 03A	12,13	17,21	8,9	13,16	10,12

The Brothers

For D16 and FGA – two alleles of the suspected brother are present in the epg

Markers	D16S539	FGA
Victim 03A	9,12	20,26
Cons Partner	10,10	26,27
Suspect 03A	8,9	23,27

95 RFU

Case 03

- Exclude (8%)
- RMP (20%)
- Inconclusive (27%)
- CPI (44%)

9 labs – false exclusion

1 lab – false inclusion

Statistical Concerns

Only 4 loci have alleles obligate to the suspected brother – ALL are below ST
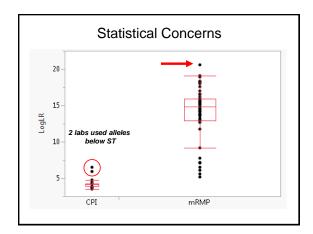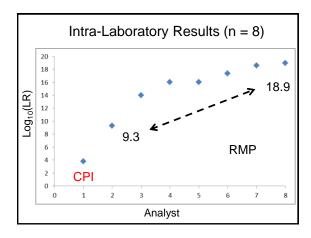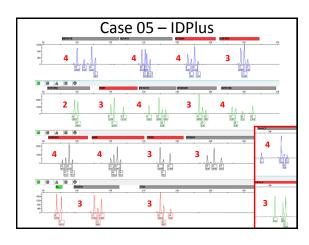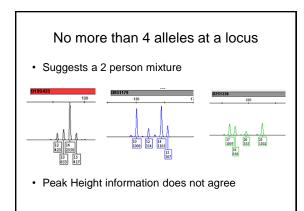
Case 04 – IDPlus

MIX13 Study (Case 04)

- Summary – Mock sexual assault, 2 person **3.5**:**1** mixture, minor component has alleles below the ST of 150 (required by all labs!)

- Purpose – How many labs would attempt to separate the two components?
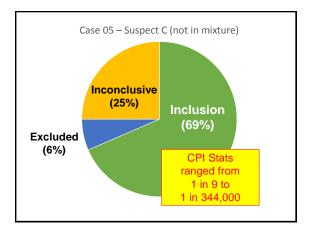
- No false exclusions.

Statistical Concerns

Intra-Laboratory Results (n = 8)

Case 05 – IDPlus

No more than 4 alleles at a locus

- Suggests a 2 person mixture

- Peak Height information does not agree

MIX13 Study (Case 05)

- Summary – Mock bank robbery with ski mask evidence (touch DNA), 4 person **1:1:1:1** mixture.

- However – this mixture had no more than 4 alleles at any locus (appears as a 2p mixture). 2 of the 4 contributors were provided along with a non-contributor.

- Purpose – How many labs would consider this mixture as too complex to interpret?

Case 05 – 3 Suspects

Individual	
Suspect 5A	Included
Suspect 5B	Included
Suspect 5C	Not in the mixture

Case 05 – Suspect C (not in mixture)

Inconclusive (25%)

Inclusion (69%)

Excluded (6%)

CPI Stats ranged from 1 in 9 to 1 in 344,000

Concluding Thoughts

- Despite the improvements in protocols and interpretation guidelines since 2010, mixture interpretation is still all over the place.
- Some of this is a consequence of using a statistical approach that is inappropriate for complex mixture interpretation – CPI is often being used as a substitute for interpretation, and has the risk of including a non-contributor.

Concluding Thoughts

- Better training and improved validations for mixture interpretation is needed!

- Software solutions may assist in mixture interpretation, statistical evaluation, and removal of bias – however, "black box" solutions are not helpful for the analyst who will need to explain the process to the trier of fact.

Thank you for your attention

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Contact Information

Michael D. Coble, Ph.D.
Research Biologist, NIST Applied Genetics Group
michael.coble@nist.gov