A Picture is Worth 10,000 LRs

The Evaluation and Implementation of Tools to Improve Interpretation and Reporting of Mixtures Using Probabilistic Genotyping

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Why do Inter/Intra Lab Studies for PG?

- Ultimate Goal of Forensic Science(?)
  - Equal outcome for a given sample/case/Victim/Suspect
  - No matter who does the work
- Are we there yet?

Expect “different” results

- Different models
  - STRmix™
  - EuroForMix
  - TrueAllele
- Different parameters
  - Stutter variance
  - Analytical Thresholds
  - PCR cycles
  - F_{ST} values
- Different LRs
  - Conditioned vs Unconditioned
  - Point Source
  - Lowest Population
  - Normalized Population (Stratified)
  - Relatives (Unified)
  - An “interval” LR – (HPD)
- Reporting
  - The number no matter what
  - Categorized number
  - Verbal scale

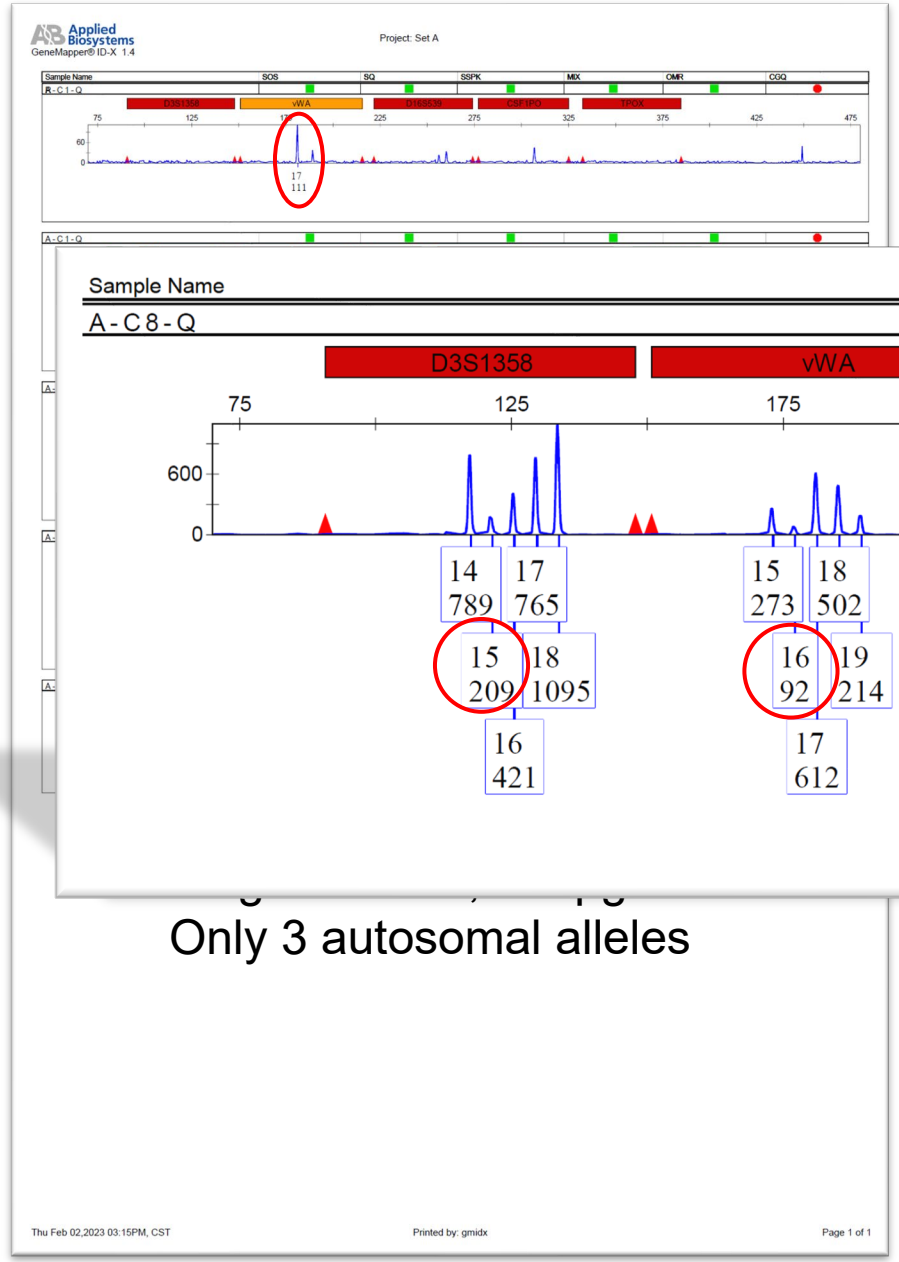
This study

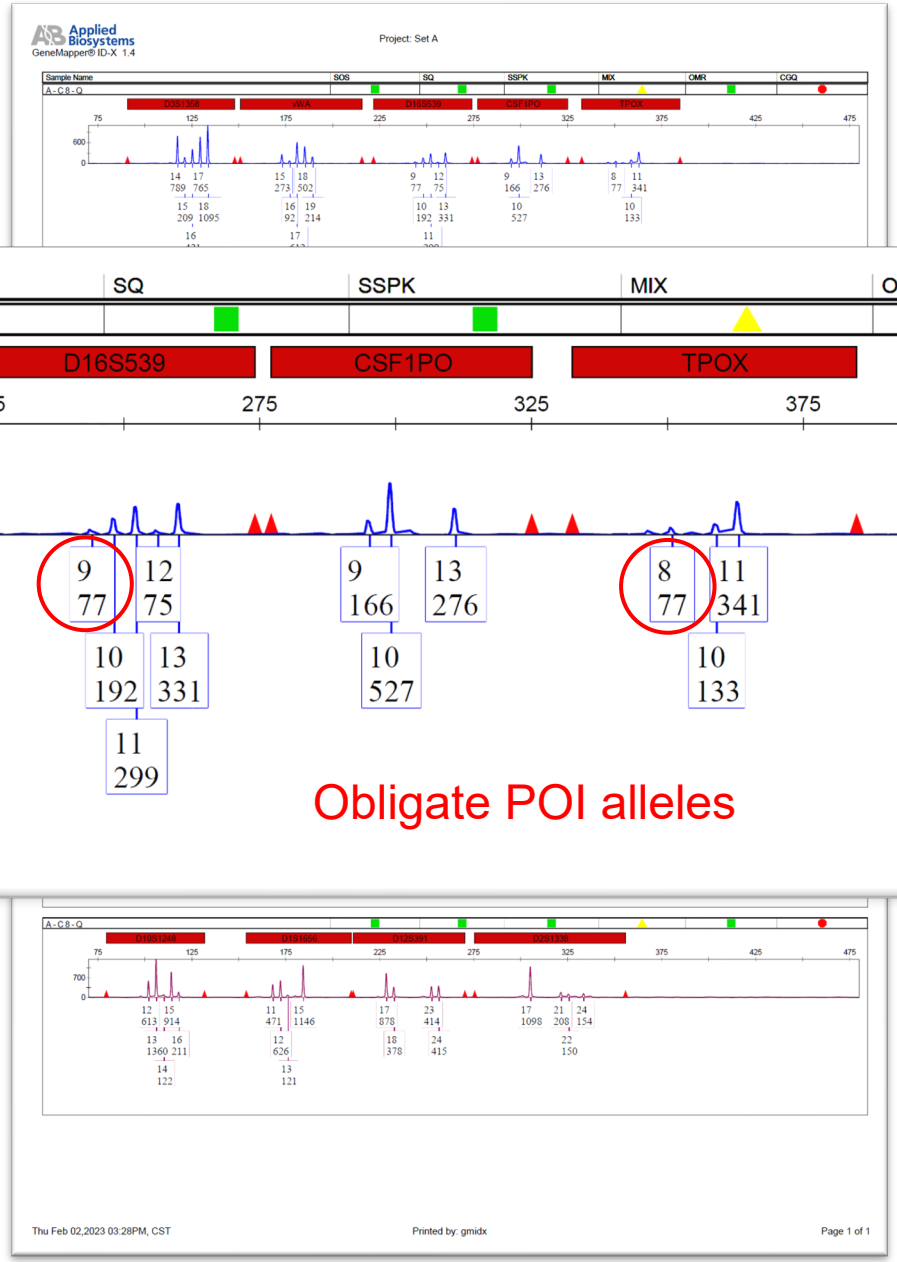
- An attempt to look at “lousy” samples
- Very low-level donors; volunteers were told 1 – 4 persons
- Expect low LRs
- Compared ground truth donors to the mixtures

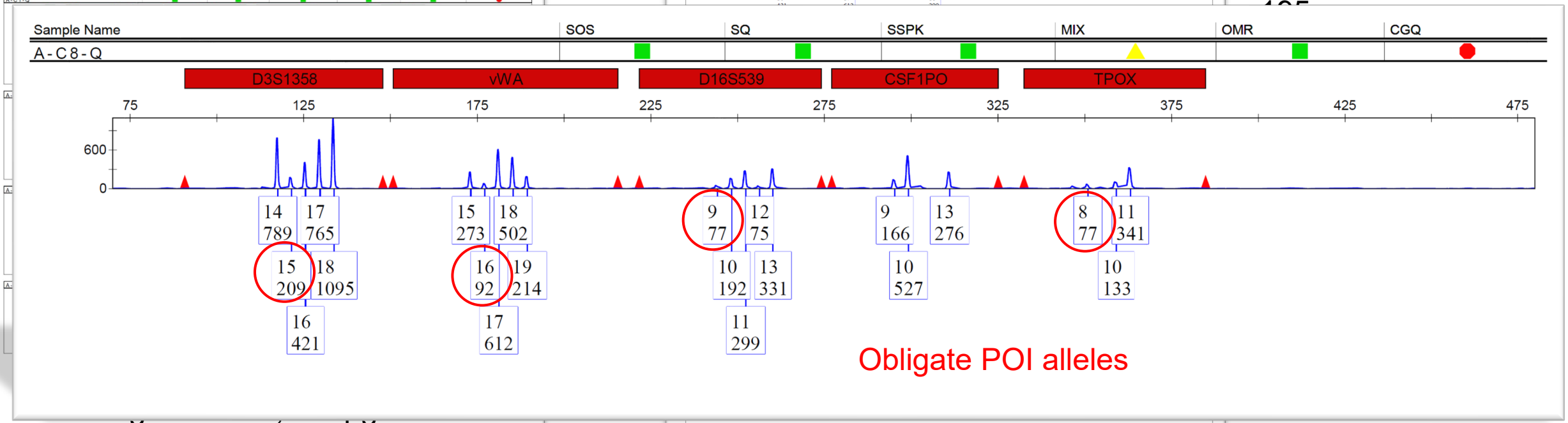
- PROVEDIt mixtures used; validated parameters (Kelly, et al)
- “Same” GMID analysis parameters
- Same STRmix™ parameters
- 10 laboratories, 25 volunteers

- Goal was to look at the “bottom line” – including report wording

How lousy is a “lousy” profile?







Obligate POI alleles

4 person; 1:4:4:4

Only 3 autosomal alleles

Most likely by realizing the other samples were 1, 2, or 3 persons

Samples examined twice

- Set A and Set B
  - 8 mixtures each
  - Assigned to different people
  - We asked for no peaking at the other set

- Round 1
  - Interpret exactly as your protocol says (Caveat: AT's provided by us)
  - Reporting statement, including LR (if any), verbal scale (if used), etc.

- Round 2 and 3
  - Swap sample sets (still no peaking/"TR")
  - Use two different tools to evaluate the LR
  - Hybrid reporting statement (normal language, but based on tools)

Round 1 Results (Lab Protocols)

- We recorded the reporting category (bottom line)

- Lots of different terms used
- Verbal scale used by some but not all
  - Verbal scale with “context”
  - Verbal scale wording – “This _____ provides…”
    - “analysis”
    - “LR”
    - Just “this”
    - “evidence” is best term?
- Suitability threshold (no interpretation/STRmix™)
- Report the number no matter how big/small
- Some have inconclusive zones

Round 1 converted to common terms

- Inclusionary = "included as a possible contributor"
- Exclusionary = LR<1
- Inc = inconclusive
- Uninformative = LR rounds to 1
- Unsuitable = Not used
- We counted them up

Sample Set	Case #	Internal Protocol				
		Unsuitable	Exclusionary	Inc	Uninformative	Inclusionary
A	C1	8		1		3
A	C2	6				6
A	C3	1		1		10
A	C4		1		1	10
A	C5	1		1		10
A	C6	2	1	1		8
A	C7	4	1	1		6
A	C8	3	2	1		6
B	C1	8				5
B	C2	8				5
B	C3	5				8
B	C4	1	1			11
B	C5	1		2		10
B	C6	2	2	1	1	7
B	C7	5	1	1		6
B	C8	4	1	1	2	5

Use of Advent*LR* and DBLR™

- Two tools that allow for non-contributor testing
- “Calibration” of the LR for POI compared to non-donors

- Advent*LR* (Schuerman *et. al.* 2020, *JFS*)
  - Graphical presentation of a STRmix™ database search
  - 10,000 random profiles based on NIST 1036 allele frequencies (static)
  - H_d or H_2 or H_A only

- DBLR™ (Kelly *et. al.* 2021, *FSI: Reports*)
  - STRmix™ companion tool
  - 10,000 to 10 million non-donors base on allele freqs (dynamic)
  - Both H_p/H_1 and $H_d/H_2/H_A$

Proposed use of non-contributor/calibration

- Schuerman et al (based on Gill, etc) - 99.9%ile LR
  - 99.9%ile LR as a “utility threshold” to aid the expert and the jury

  - LR_{POI} > 99.9%ile LR; Inclusionary support
  - LR_{POI} < 99.9%ile LR; Inconclusive due to non-contributor testing

- Initial testing of Advent*LR* and DBLR™ were both suitable
- High level of agreement (10,000 static profiles vs 100K)

- ***Volunteers were trained in use of both tools and theory***

Non-contributor Testing

- Used the full NIST 1036 combined allele frequencies for “test LR”
  - For POI in the case
  - For all profiles in the database

- Compared point source (Factor of N! or Sub-source) LR for POI
- To LRs generated from profile database (10K or 100K)

- If LR_{POI} “passes” then asked labs to report their “normal” LR
  - HPD from smallest of 3 or 4 populations
  - Unified LR was used also (4 populations normalized per census)

A picture is worth 1000 words - Advent*LR*

- Picture that shows how LR_{POI} compares to population

Set-A-Q1_K

CASE NUMBER	Set-A-Q1
SAMPLE ID	K1
NOC	1
H_p Profiles (K)	
H_d Profiles (K)	
AdventLR: V	2.9.0
STRmix™: V	2.5.11

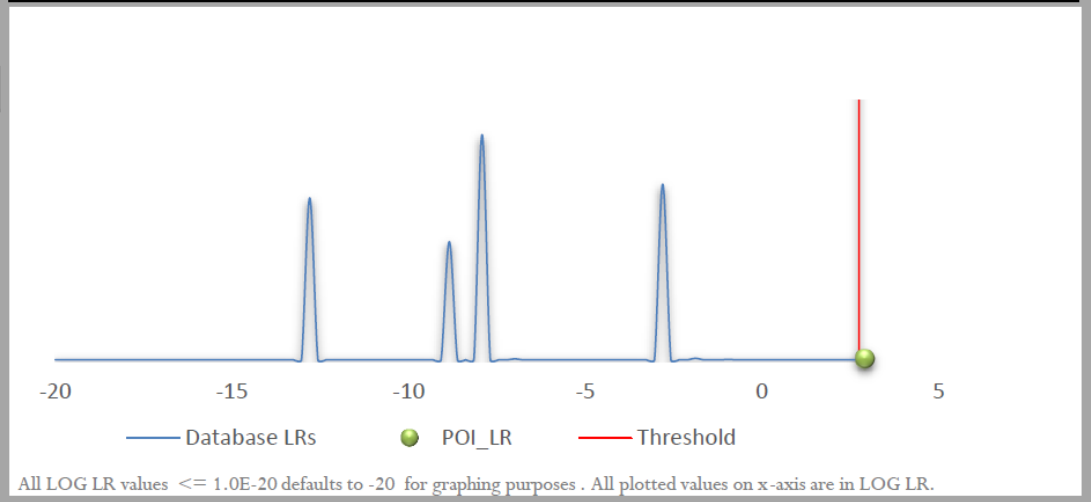
CASE NOTES	

SUMMARY OF PROFILES IN DATABASE

	Total Profiles	Average LR
Entire DB	10000	2
LR = 0	0	
LR > 0	10000	2
LR >= 1	21	714

Largest LR from search	1547
Known non-contributors > POI	6
*Point source LR for POI	876
Uninformative threshold	560

* Point source LR calculated using the NIST1036_Combined allele frequencies



REPORTED LR=93

Point source LR = 876

Green Dot

99.9%ile LR = 560

Red Line

Single Source sample (previous epg)

A picture is worth 1000 words - Advent*LR*

- Picture that shows how LR_{POI} compares to population

A-Q8_K

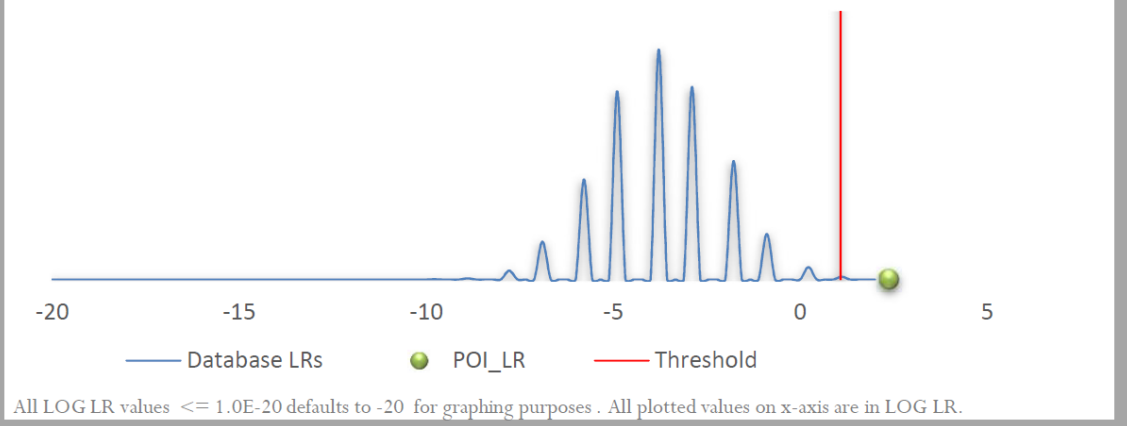
CASE NUMBER	
SAMPLE ID	
NOC	
H_p Profiles (K)	
H_d Profiles (K)	
AdventLR: V	2.9.0
STRmix™: V	2.5.11

CASE NOTES	

SUMMARY OF PROFILES IN DATABASE

	Total Profiles	Average LR
Entire DB	10000	1.02E-01
LR = 0	0	
LR > 0	10000	1.02E-01
LR >= 1	80	11

Largest LR from search	246
Known non-contributors > POI	0
*Point source LR for POI	247
Uninformative threshold	12



* Point source LR calculated using the NIST1036_Combined allele frequencies

REPORTED LR=10

Point source LR = 247

99.9%ile LR = 12

4p sample (previous epg)

A picture is worth 1000 words – DBLR™

SIMULATION RESULTS TABLE

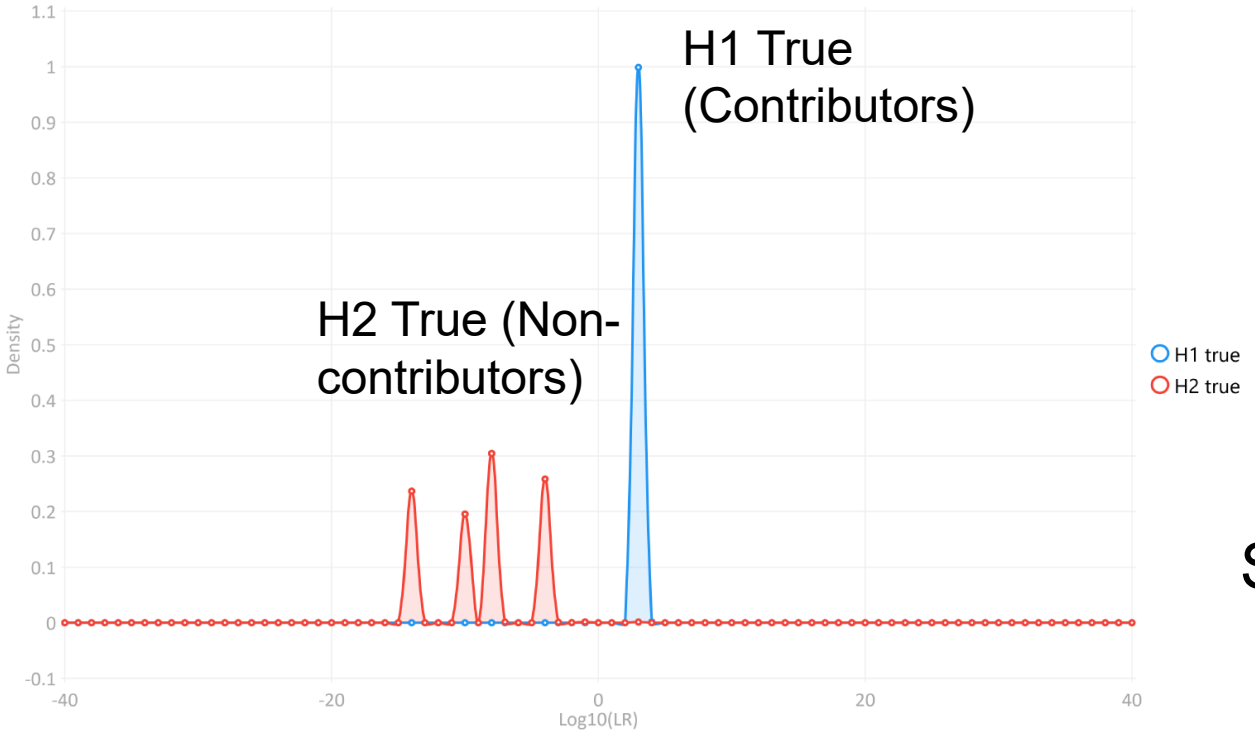
	ESTIMATE	
P(Log10LR >= 2.93752 | H1)	0.50831	
P(Log10LR >= 2.93752 | H2)	0.00048	
	Importance sampling from H1:	0.00040791

P(Log10LR>=2.93752|H2) = 0.00040791

Probability of LR > POI Given H2 is 0.040791%

(logLR used instead of LR)

SIMULATION RESULTS PLOT



1.0 – 0.00040791 = 0.99959

LR of POI is greater than 99.9%ile LR

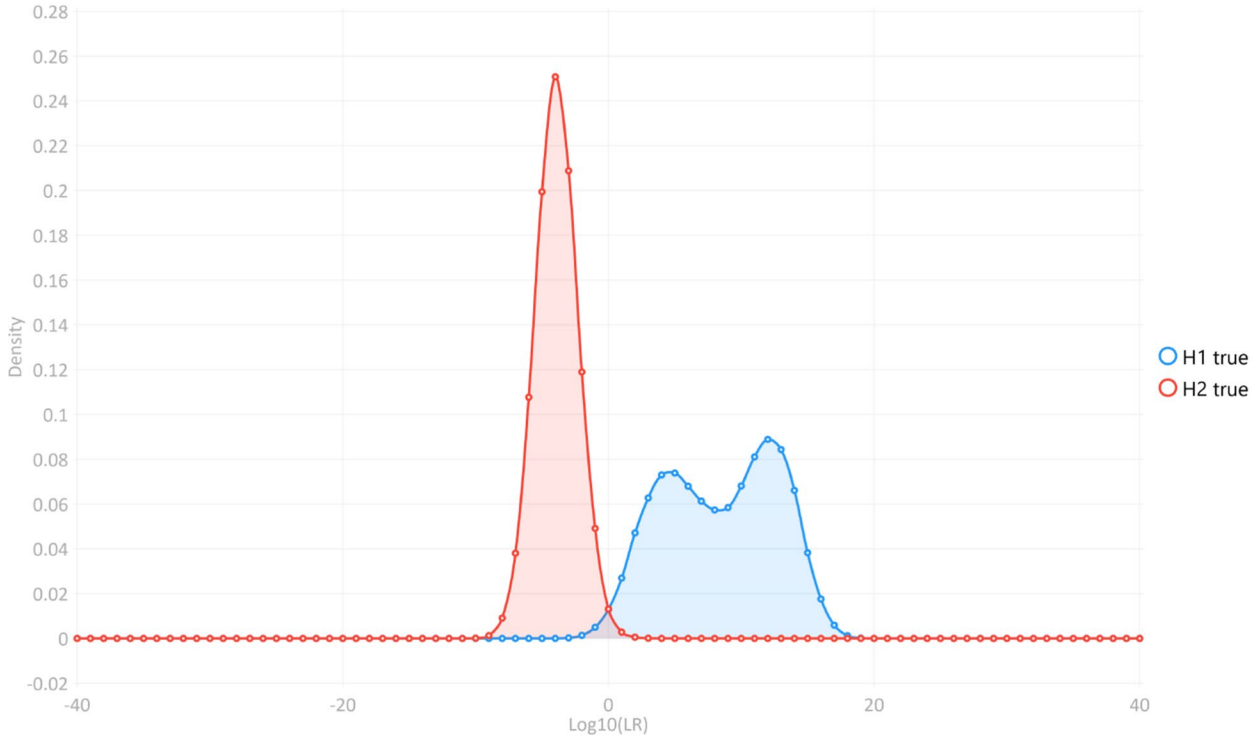
Single Source sample (previous epg)

A picture is worth 1000 words – DBLR™

SIMULATION RESULTS TABLE

	ESTIMATE	
P(Log10LR >= 1.79239 | H1)	0.94167	
P(Log10LR >= 1.79239 | H2)	0.00041	
	Importance sampling from H1:	0.00035068

SIMULATION RESULTS PLOT



1 – 0.00035068 = 0.99964

LR of POI is greater than 99.9%ile LR

4p sample (previous epg)

Round 2/3 reporting in common terms

- Dropped unsuitable
- Added “Inc – NCT”

Sample Set	Case #	AdventLR Non-contributor testing (10,000)				DBLR Non-contributor testing (100,000)			
		Exclusionary	Inc-NCT	Uninformative	Inclusionary	Exclusionary	Inc-NCT	Uninformative	Inclusionary
A	C1		1		11		9		4
A	C2				12				13
A	C3				12				13
A	C4				11			1	12
A	C5				12				13
A	C6				12				13
A	C7	1			11				13
A	C8	1			11	1			12
B	C1		1		11		8		4
B	C2				12				12
B	C3				12				12
B	C4				12				12
B	C5				12				12
B	C6	1		1	10	1		1	10
B	C7				12				12
B	C8	1		3	8	1		3	8

Overall comparison

- Much higher agreement between laboratories
- “Classification” based on empirical data

Sample Set	Case #	Internal Protocol					AdventLR testing (10,000)				DBLR (100,000)			
		Unsuitable	Exclusion	Inc	Uninformative	Inclusion	Exclusion	Inc-NCT	Uninformative	Inclusion	Exclusion	Inc-NCT	Uninformative	Inclusion
A	C1	8		1		3		1		11		9		4
A	C2	6				6				12				13
A	C3	1		1		10				12				13
A	C4		1		1	10				11			1	12
A	C5	1		1		10				12				13
A	C6	2	1	1		8				12				13
A	C7	4	1	1		6	1			11				13
A	C8	3	2	1		6	1			11	1			12
B	C1	8				5		1		11		8		4
B	C2	8				5				12				12
B	C3	5				8				12				12
B	C4	1	1			11				12				12
B	C5	1		2		10				12				12
B	C6	2	2	1	1	7	1		1	10	1		1	10
B	C7	5	1	1		6				12				12
B	C8	4	1	1	2	5	1		3	8	1		3	8

Bonus information

- Controlling variables in comparison studies is hard!
- GMID settings affected number of called peaks
  - No trend for difference in 99.9%ile
  - Reported LR in billions compared to 10,000s
- STRmix version less influence than GMID settings
- (Just initial observations; digging into this is taking time)

Looking forward

- If the goal is to get same “inclusion/exclusion” – no matter who does the work
- Non-contributor testing may be useful
  - Empirical data approach
  - Considers the quirks of the actual mixture
- But there are still unexpected things that play a role

Acknowledgments

- Participating laboratories (esp. Management)
- The volunteers
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