Establishing High Performance Analyte Cutoffs in Metabolic Disorders Screened by Mass Spectrometry through Understanding of False Negative Risk

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Bio update and background information

- 10/14: NIH/NCI grant to investigate human plasma proteins stability when spotted on filter paper for downstream proteomic and clinical applications. Trying to expand the study to include blood spots.
- 02/14: Joined AZDHS, Newborn screening division
- First task to evaluate our MS/MS cutoffs
- In 2013 our cutoffs changed; a lot of ratios were introduced at the request of the Phoenix Children's Hospital metabolic team which had been overwhelmed by the number of false positives (>550 per year, excluding TPNs)
- Many disorders went from 10's of false positives per year to no false positives per year, raising suspicions of the cutoffs being too "strict" and increased likelihood of false negatives.

Challenges associated with establishing high-performing cut-offs

Estimating False Positives and False Negatives

- The availability of a large number of True Negatives (healthy) allow us to easily estimate our risk for false positives.
- The absence of a statistically significant number of True Positives (confirmed cases) compromises our ability to estimate our risk for false negatives.
- Without having an understanding of how you affect your risk for false negatives; establishing cut-offs might be a dangerous exercise.
- This is particularly true when more than one marker is used per disorder since each marker can bring in it's own set of false negatives.

Training set

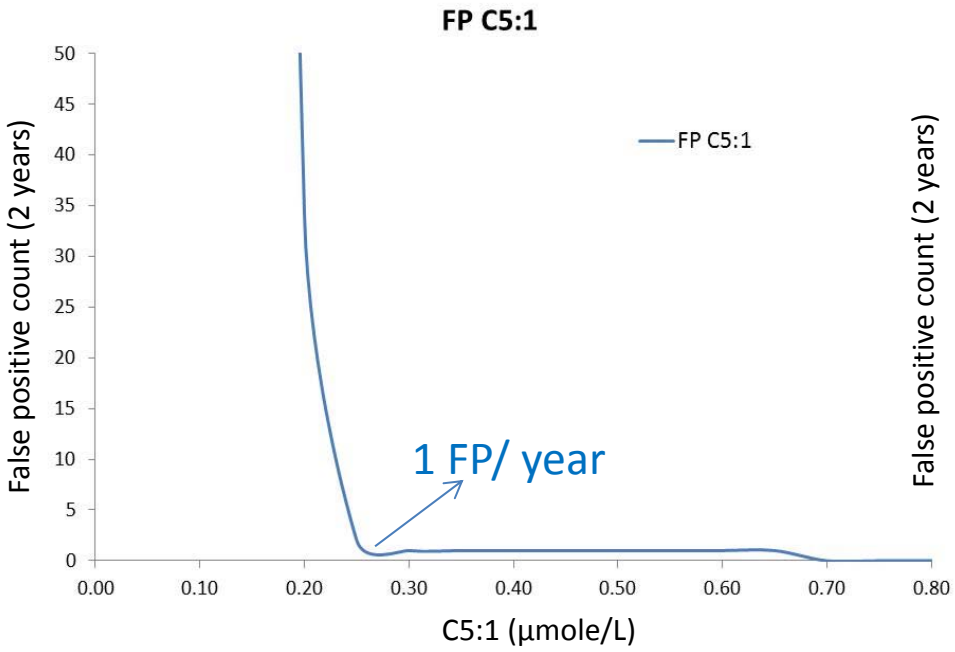
Arizona Data:

- Confirmed cases: 2006-2014 (N~280) –primary and secondary disorders-
- Healthy/Normal cases: 2012-2013 (N~270,000)
- Filtered out of Normal cases: TPN, QNS, QNS2, UIO, UMA, UNI, UNS, UTO, all cases of >1000 hours of age)
- Data from Region 4 stork (R4S) collaborative project, also hosted at the Newborn Screening Translational Research Network (NBSTRN) and freely available to labs performing newborn screening

False positive estimation is relative easy since it uses healthy data

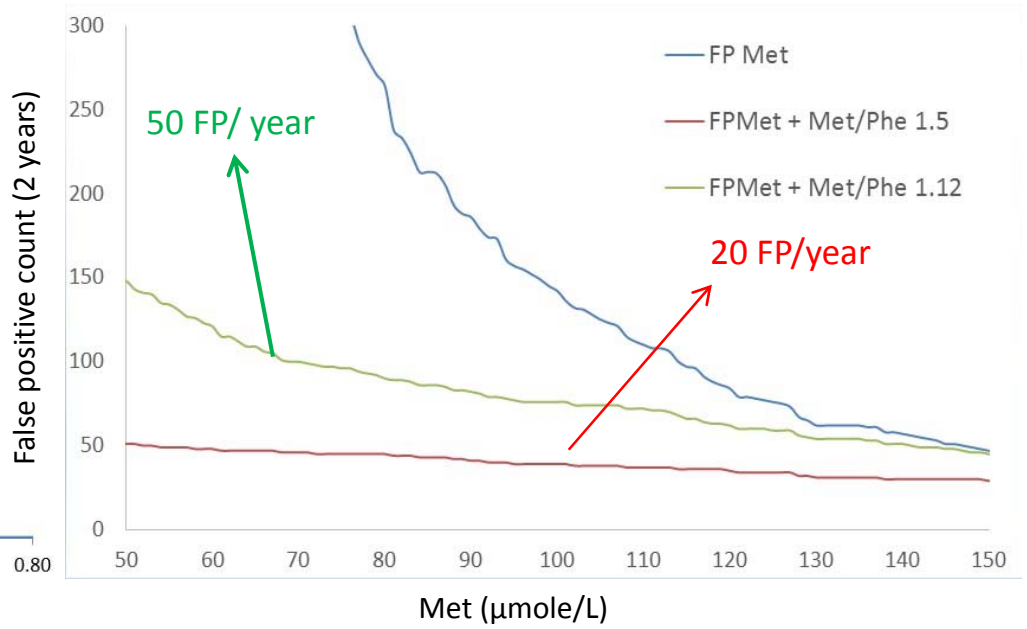
Distribution of false positive count vs. different cutoff value scenarios

BKT



Narrow optimum

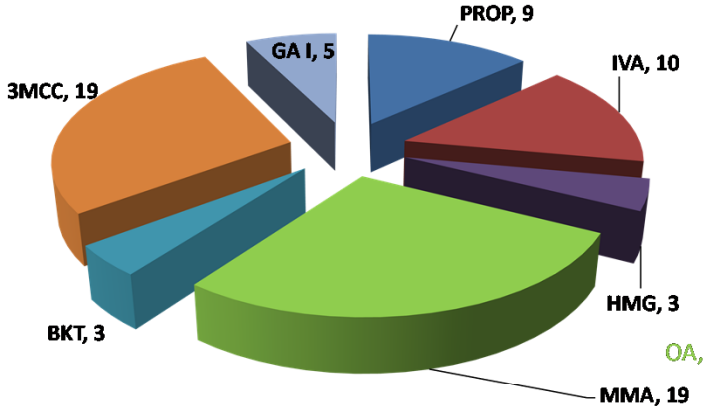
HCY

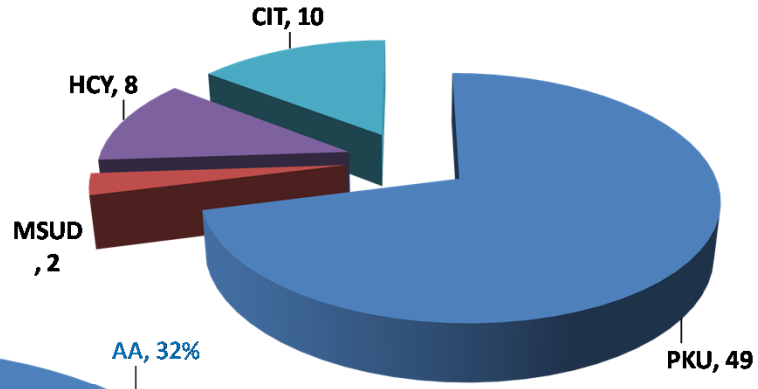


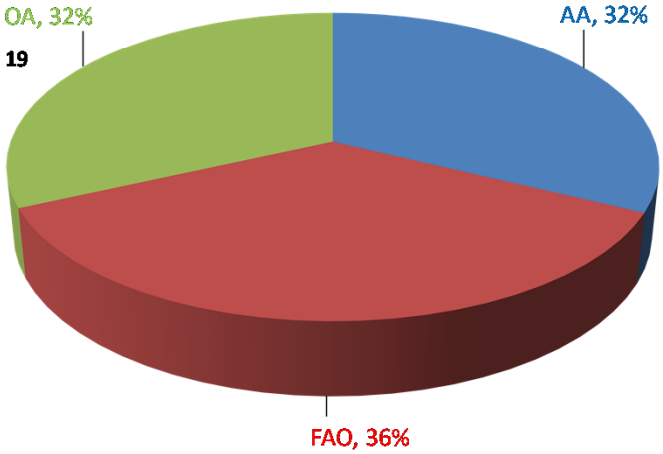
Challenging optimum

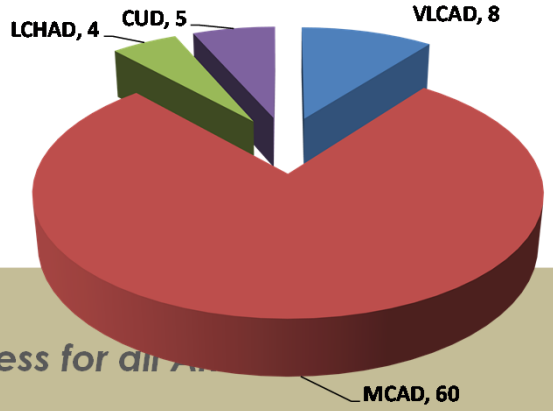
2012-2013 healthy values ≈ 270,000

Training set – Confirmed cases in our database



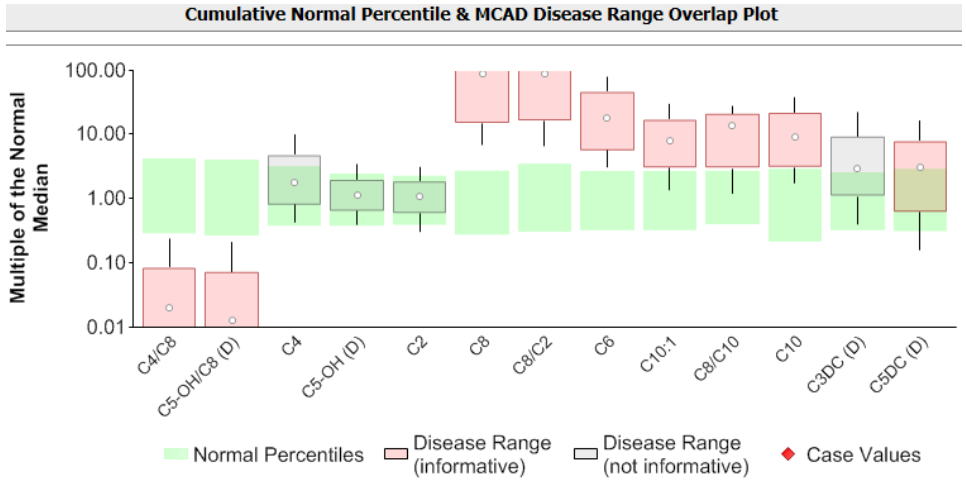






Challenges associated with establishing high-performing cut-offs

The R4S has >17,000 relevant confirmed cases in their database as well as information and tools to help make informed decisions:

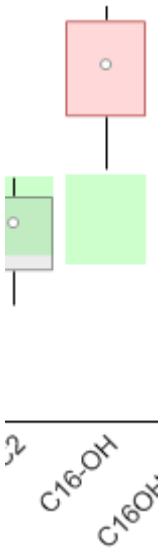


	Normal 99%ile	Overlap %ile	Disorder 1%ile
C8	0.19	0.0 %	0.49
C8/C2	0.01	0.0 %	0.02
C6	0.16	0.0 %	0.19
C10:1	0.16	6.2 %	0.08
C8/C10	2.03	7.4 %	0.91
C10	0.26	7.5 %	0.16
C3DC (D)	0.15	41.0 %	0.02
C5DC (D)	0.18	45.9 %	0.01

Aggregate data from laboratories around the world performing NBS in blood spots
Normal and disease percentiles are available as well as overlap between 99% healthy and 1% disease percentiles
Separate data for derivatized vs. non-derivatized approaches

Challenges associated with establishing high-performing cut-offs

Although there is a lot of information in the R4S database, if we try to use the R4S data with our data directly, there are significant inconsistencies since the R4S is an amalgam of data from different sites



LCHAD

R4S Disease %
R4S Normal %

95%	2.64
75%	1.44
50%	0.83
25%	0.43
5%	0.19
1%	0.08
99%	0.07
95%	
75%	
50%	

C16OH

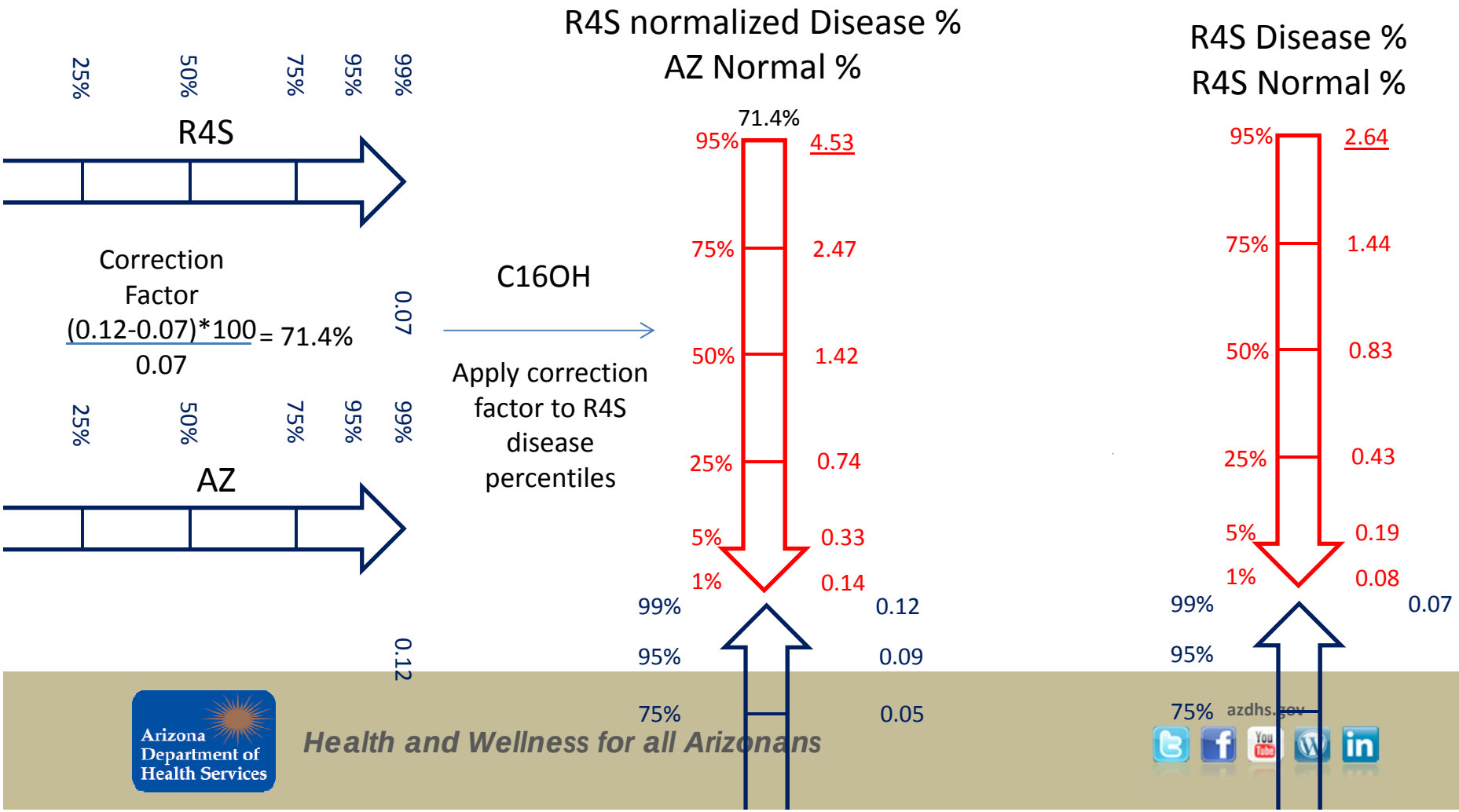
R4S Disease %
AZ Normal %

95%	2.64
75%	1.44
50%	0.83
25%	0.43
5%	0.19
1%	0.08
99%	0.12
95%	
75%	
50%	
25%	

Some kind of normalization is necessary to be able to use the R4S data

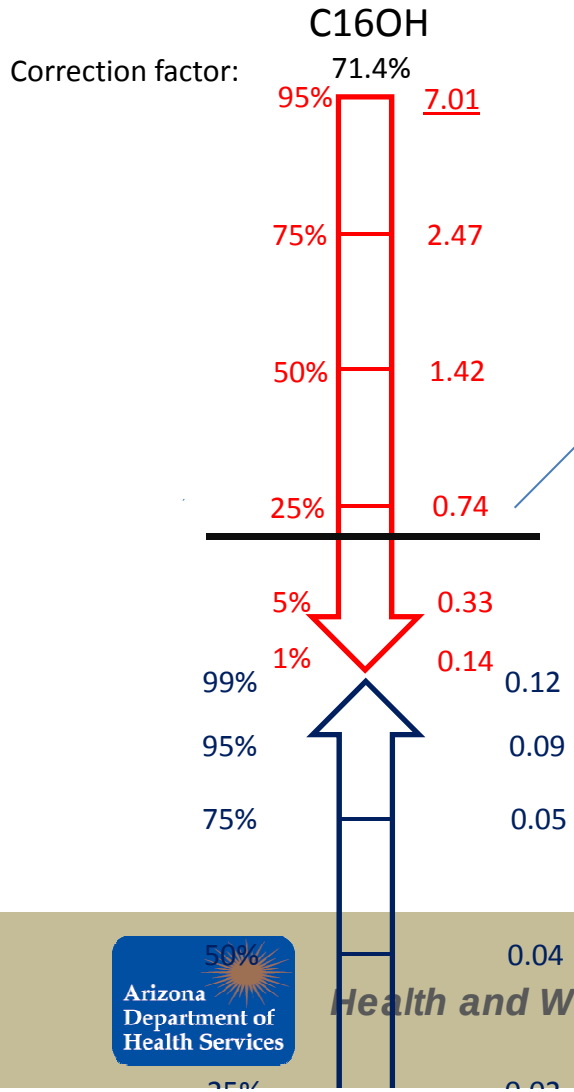
Purpose of the normalization: What would the R4S disease percentiles would looked like if we would had performed the analysis on those samples with our instruments?

I use the AZ and R4S normal percentiles for each marker and calculate a correction factor which I apply to the R4S disease percentile (for the same marker)



Challenges associated with establishing high-performing cut-offs

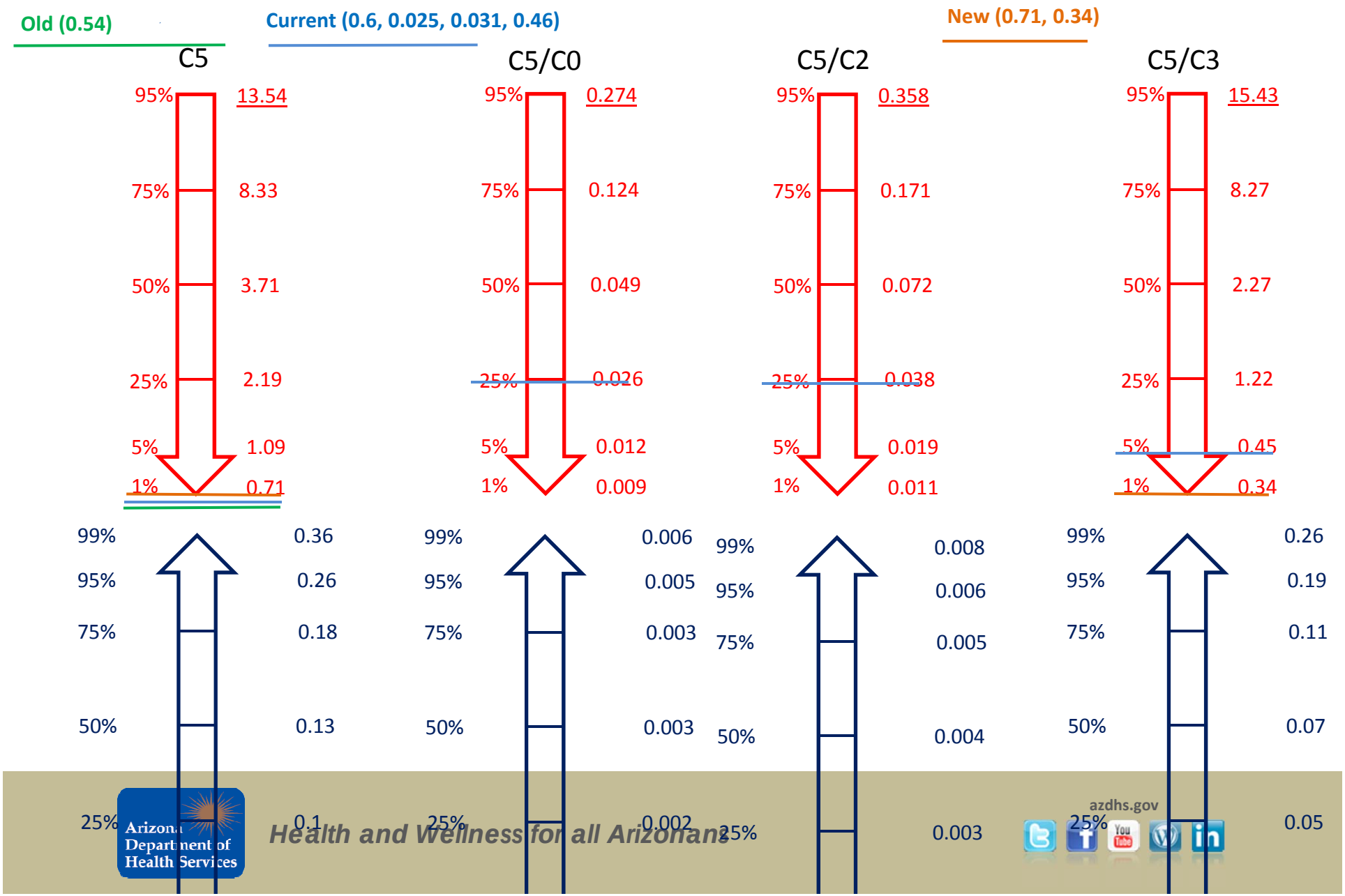
After normalizing the R4S disease percentiles, we get the same or very similar healthy-disease overlap as the R4S database



Now if we apply a cut-off, we can have an estimate of the false negatives

The spread between 1 and 99% disease percentile will be a little bit larger as those are cumulative data from several labs, but still a very good approximation.

Cutoff calculation - Isovaleric Acidemia - IVA



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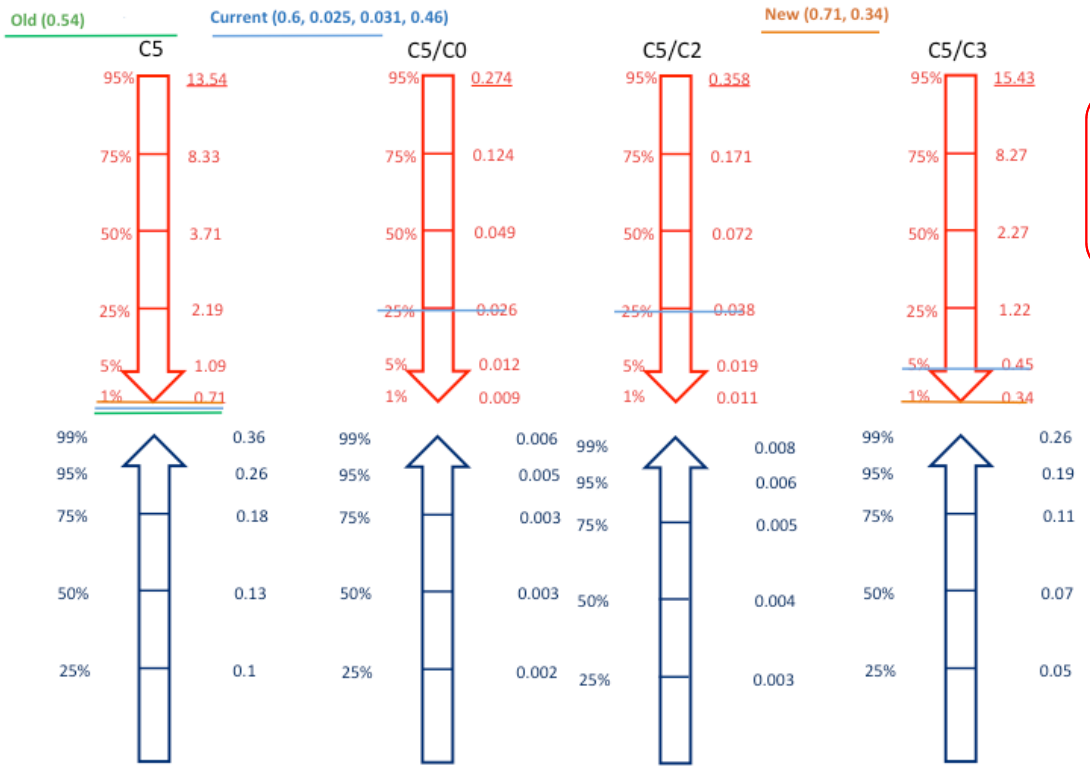

C5	C5/C0	C5/C2	C5/C3	FP/year
0.60	**0.025**	**0.031**	**0.46**	**4**
0.60	-	-	-	57
-	0.025	-	-	5.5
-	-	0.031	-	24
-	-	-	0.46	1371.5
0.71	**0.012**	**0.019**	**0.45**	**9.5**
0.71	-	-	-	22.5
-	0.012	-	-	652.5
-	-	0.019	-	597
-	-	-	0.45	1510.5
0.71	0.012	-	-	13.5
0.71	-	0.019	-	18
0.71	-	0.019	0.45	12
0.71	0.012	-	0.45	10
0.71	**-**	**-**	**0.45**	**13**
0.71	**-**	**-**	**0.34**	**16**

- The C5/C0 and C5/C2 are set too high, increasing the likelihood of false positives to >24%.
- Other evidence that they have been set too high is that they have the least amount of false positives when set as a cut-off by themselves, even less than the primary marker from which they have been derived.
- By adjusting the C5 cut-off to 0.71 (around 1% false negatives) and the C5/C3 to 0.34 (around 5% false negatives), the false positives increase slightly while the false negative rate decreases significantly to around >1% .
- The C5/C0 and C5/C2 are removed from the biomarker panel.

Cutoff calculation - Isovaleric Acidemia – IVA
IVA- confirmed cases

C5/C0 and C5/C2 have been challenged in the past

	Cut-off Threshold	<0.6	<0.025	<0.031	<0.46
Accession Number	Condition	C5	C5/C0	C5/C2	C5/C3
2013 - 29	IVA	3.83	0.170	0.210	8.15
2013 - 44	IVA	3.07	0.128	0.151	2.58
2013 - 36	IVA	0.82	0.025	0.043	1.34
2013 - 62	IVA	0.79	0.027	0.031	0.91
2013 - 88	IVA	8.43	0.364	0.432	13.60
2013 - 56	IVA	8.59	0.323	0.362	8.95
2009 - 84	IVA	13.79	0.186	0.801	12.42
2009 - 11	IVA	59.79	0.113	5.470	186.84
2009 - 34	IVA	8.99	0.383	0.490	17.63
2009 - 15	IVA	9.73	0.372	0.443	15.44

Considerations when normalizing percentiles

- Normalizing (aggregate from different laboratories) percentiles is not as accurate as working with the actual values.
- Normalizing NBS analyte values from different laboratories is a relatively easy task when compared to the the normalization challenges in other field such as proteomics

Newborn Screening

- Small number of analytes
- Different instruments, different laboratories
- Easy, standardized sample preparation
- Analyte values

vs.

Proteomics

- Hundreds of thousands of analytes
- Liquid chromatography-retention time
- Accurate mass
- Ion-mobility
- Difficult, convoluted sample preparation
- Different instruments, *different labs*
- Semi-quantitative data

K. Petritis et al.
Anal. Chem. 75 (2003) 1039-1048
Use of artificial neural networks for the accurate predictions of peptide liquid chromatography elution time in proteome analyses
Anal. Chem. 78 (2006) 5026-5039.
Improved peptide elution time prediction for reversed-phase liquid chromatography by incorporating complete peptide sequence information

AZ False positives and False Negative risk

Condition	FN risk Old	FN risk Current	FN risk Proposed	Prevalence*	# FN per M old	# FN per M current	# FN per M proposed
PKU	0.1%	2%	1%	0.000040	0.04	0.80	0.40
TYR I	80%	90%	90%	0.000010	8.00	9.00	9.00
MSUD	25%	35%	5%	0.000010	2.50	3.50	0.50
HCY	25%	20%	5%	0.000010	2.50	2.00	0.50
CUD	55%	60%	30%	0.000010	5.50	6.00	3.00
LCHAD	3%	25%	3%	0.000013	0.40	3.33	0.40
MCAD	1%	2%	1%	0.000040	0.40	0.80	0.40
VLCAD	4%	5%	6%	0.000013	0.53	0.67	0.80
BKT	7%	5%	5%	0.000010	0.70	0.50	0.50
MCD	55%	55%	5%	0.000010	5.50	5.50	0.50
MMA/PROP	17%	23%	10%	0.000030	5.10	6.90	3.00
HMG/3MCC	3%	5%	4%	0.000011	0.34	0.57	0.46
IVA	1%	24%	5%	0.000010	0.10	2.40	0.50
GA1	4%	7%	4%	0.000013	0.53	0.93	0.53
Total FN risk					32.15 per M	42.9 per M	20.5 per M
* Prevalence taken from Laboratory Medicine Practive Guidelines (if <1:100,000 then =1:100,000)							
M: Million							
FN: False Negative							

False positives:	560	126	145

The proposed cut-offs significantly decrease the False Negative risk for a small increase in false positives.

Conclusions

- It is possible to normalize the R4S database disease percentiles to allow an estimate of individual labs false negative risks
- With access to only percentiles values the accuracy of the calculations is not as good as it could be if we had access to individual values; but it still offers a good approximation
- Access to the appropriate data could allow the generation of high fidelity disease percentiles that are normalized to the data of the different laboratories which could facilitate false negative estimation vs. different cutoff scenarios

Acknowledgements

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- Co-authors: S. Bhakta, W. Slanta, D. Carr, D. Wright, S. Aponte, W. Jacox
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Thank you!

Questions/Comments/Feedback?