Establishing and Monitoring Cutoffs and Reference Ranges

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Cutoffs / Reference Ranges / Reference Intervals

- Endogenous: cannot be controlled (Age and Birthweight)
- Exogenous: can be controlled (Feeding status)
- Genetics and/or ethnicity: population dependent factor
- Laboratory: pre-analytical, analytical, and post-analytical factors affect the results of all analytes
- Statistical approaches: method can affect test interpretation
- Evaluation population
  - Heterogeneity
  - Subpopulations
  - Sufficient size

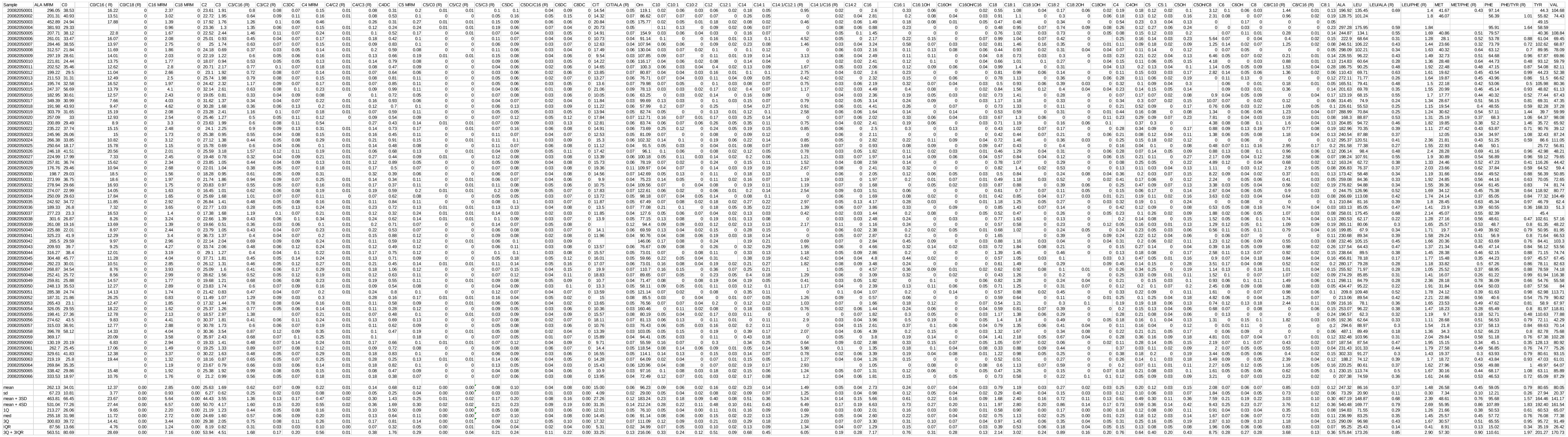
Establishing Cutoffs for Tandem Mass Spectrometry

Amino Acid and Acylcarnitine Disorders

- Spring 2008
- Two new Waters Quattro Micro Tandem Mass Spectrometers
- PerkinElmer Neogram Kit
- Instrument and Method Validation/Verification

- Cutoffs and Reference Ranges
  - Routine Patient Specimens
  - Kit Control Material
  - CDC NSMBB Control and Proficiency Testing Material
  - Confirmed Positive and Negative Patient Specimens

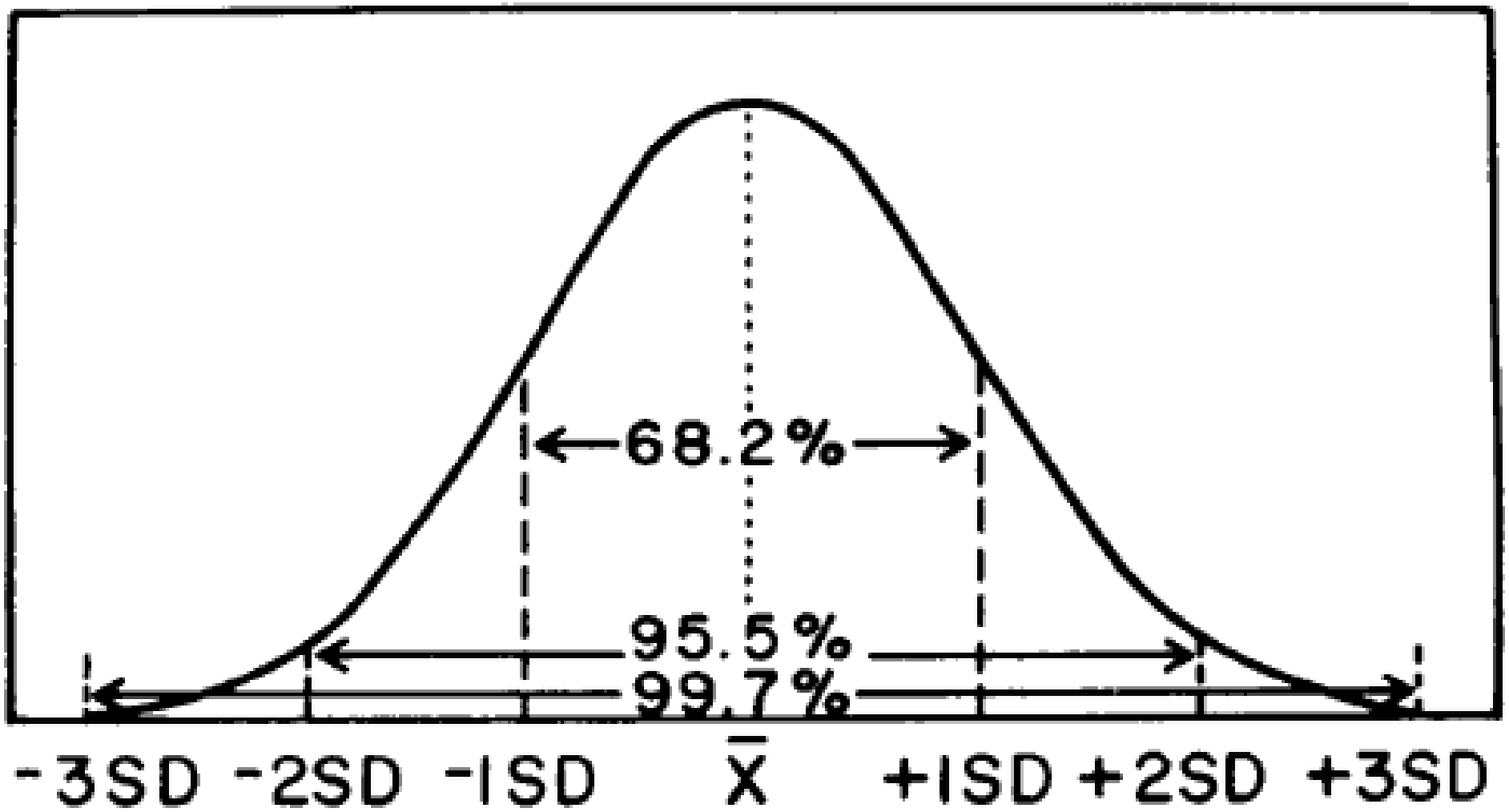
Lots of Data



- Thousands of specimens with dozens of data points
- Lack of specific software tools

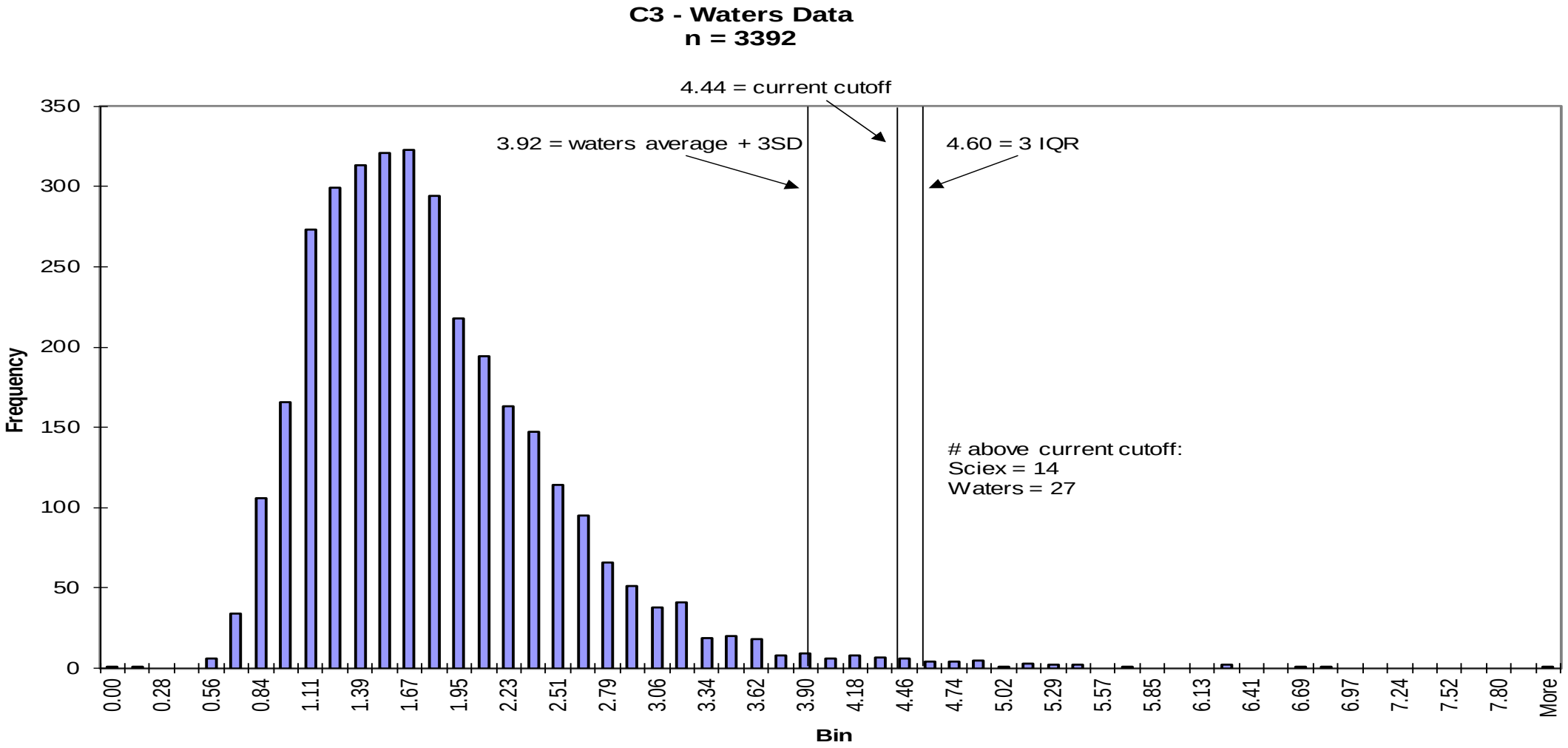
Reviewing Individual Analytes

Using Basic Analysis Tools



Reviewing Individual Analytes Continued

Using Basic Analysis Tools



Setting Preliminary Cutoffs

Analyte	Region 4		Current		Proposed 1 - 99/99.9%ile		Proposed 2	
	Lower range	Upper Range	UWL	UCL	UWL	UCL	UWL	UCL
C3	4.700	5.500	4.440	6.790	4.21	6.15	5.00	9.00

- Preliminary cutoffs established based on
  - statistical analysis
  - consultation with specialists and colleagues
- Challenges
  - Population size
  - Subpopulations
  - Different methods/instrumentation
  - Obtaining specimens
  - Biological variants

Finalizing Initial Cutoffs

DATE	Plate #	Sp# to be removed	Replaced Confirmed SP#	DISORDER
2/4/2009	033P3	[redacted]	[redacted]	MCAD
				3MCC
				PROP
				GAI
				VLCAD
				HCY
				MSUD
				SCAD
				MAL
				PKU

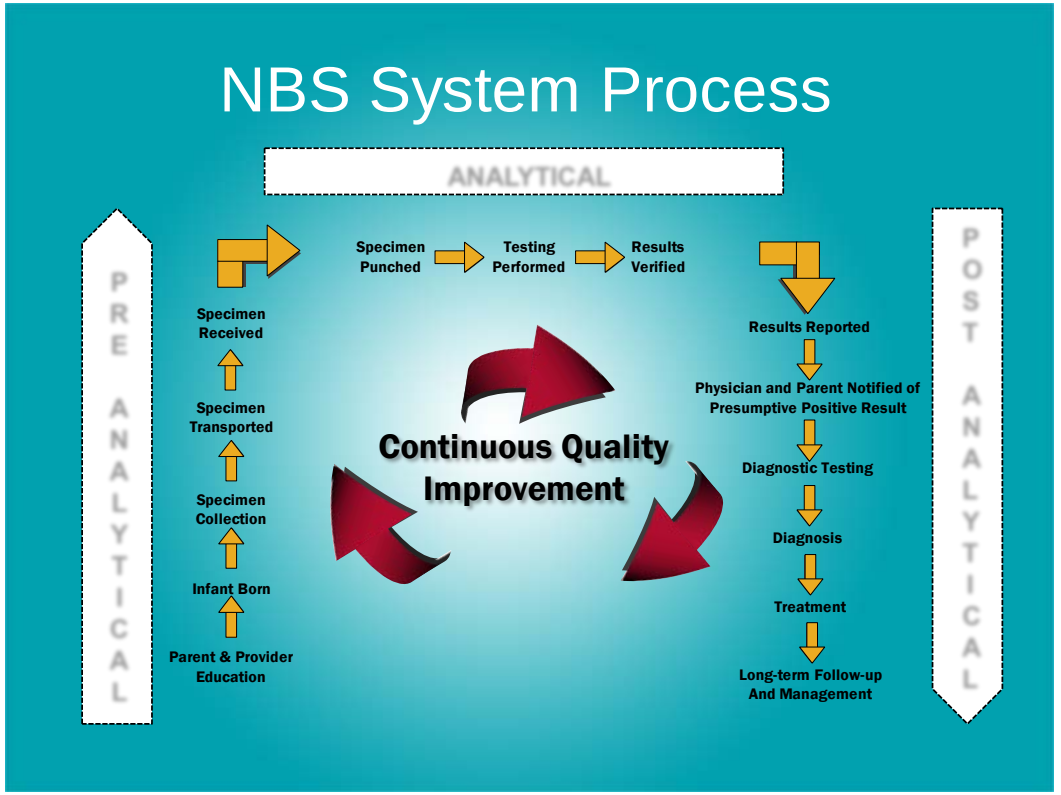
- Cutoffs are validated by running known positive and negative specimens
- Ensure results are classified in the expected categories
- Confirm with specialists

Sample	[redacted]					
C0	44.25	69.18	45.66	51.64	64.04	38.04
C8	0.07	**12.1**	0.09	0.19	0.13	0.11
C10:1	0.04	**1.04**	0.06	0.1	0.06	0.14
C6	0.04	**2.49**	0	0.12	0.06	0.04
C8/C10 (R)	0.97	**17.73**	0.73	1.14	1.84	0.47
C8/C16 (R)	0.04	**3.79**	0.02	0.05	0.07	0.01
C14	0.22	0.33	0.27	0.47	0.12	**3.76**
C14:1	0.12	0.09	0.16	0.34	0.07	**4.48**
C16:1	0.14	0.1	0.29	0.29	0.09	**1.24**
C14:2	0.05	0.07	0.04	0.12	0.05	**0.47**
C14:1/C16 (R)	0.07	0.03	0.05	0.1	0.04	**0.56**
C14:1/C12:1 (R)	2.92	0.77	2.21	1.74	2.04	**12.77**
C4	0.23	0.53	0.28	0.33	0.33	0.15
C5	0.14	0.17	0.15	0.19	0.37	0.08
C3	3.6	2.72	**15.63**	**6.25**	1.32	1.25
C3/C2 (R)	0.13	0.12	**1.47**	**0.24**	0.03	0.1
C3/C16 (R)	2.06	0.85	**4.57**	1.83	0.73	0.16

Continuous Quality Monitoring

Making Adjustments to Cutoffs

- Routine review of assay performance
- As the number of specimens screened increases, more biological variation is found
- Adjustments are required when the laboratory determines that the reference intervals for a test procedure are inappropriate for the laboratory's patient population.



Monitoring Analyte Performance

Improved Statistical Tools

Data Set

Statistics	
N:	101628
Mean:	2.76
Median:	2.65
Std Dev:	0.95
Min:	0.25
Max:	10.50

Cut-Off	
Absolute:	7
Percentage:	99.9%
n SD:	4.46

Std Dev:	
2SD:	4.65
3SD:	5.60
4SD:	6.55
5SD:	7.49

Detection Rates	
Within limits:	101527
Outside limits:	101
False positives:	101
False negatives:	0

Percentiles	
1.0%	1.04
10.0%	1.66
50.0%	2.65
90.0%	3.99
99.0%	5.52
99.5%	5.98
99.9%	7.00

Data Set

Statistics	
N:	101628
Mean:	2.76
Median:	2.65
Std Dev:	0.95
Min:	0.25
Max:	10.50

Cut-Off	
Absolute:	7.5
Percentage:	99.95%
n SD:	4.99

Std Dev:	
2SD:	4.65
3SD:	5.60
4SD:	6.55
5SD:	7.49

Detection Rates	
Within limits:	101576
Outside limits:	52
False positives:	52
False negatives:	0

Percentiles	
1.0%	1.04
10.0%	1.66
50.0%	2.65
90.0%	3.99
99.0%	5.52
99.5%	5.98
99.9%	7.00

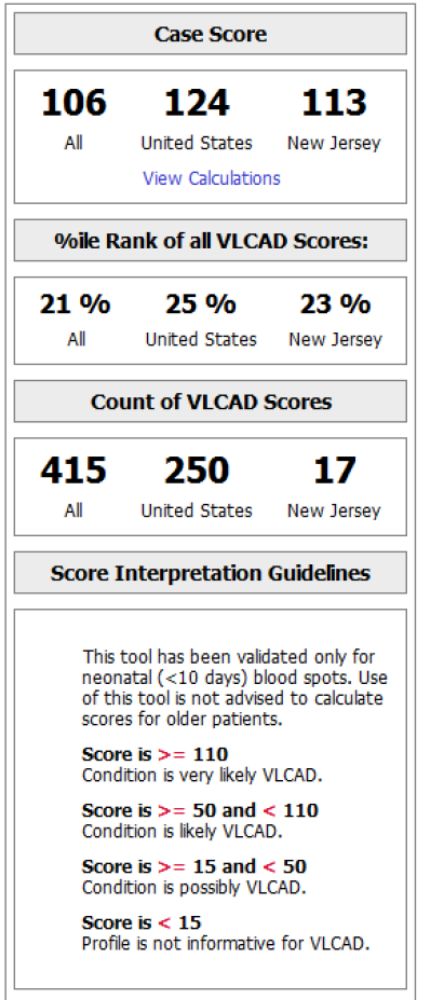
	Target Range	Cutoffs	Peer Percentiles							
			N	1%ile	10%ile	25%ile	50%ile	75%ile	90%ile	99%ile
C16	6.04 - 7.74	7.00	113	4.73	5.62	6.66	7.46	8.20	9.30	12.22
C16	6.04 - 7.74	7.50	113	4.73	5.62	6.66	7.47	8.20	9.30	12.22

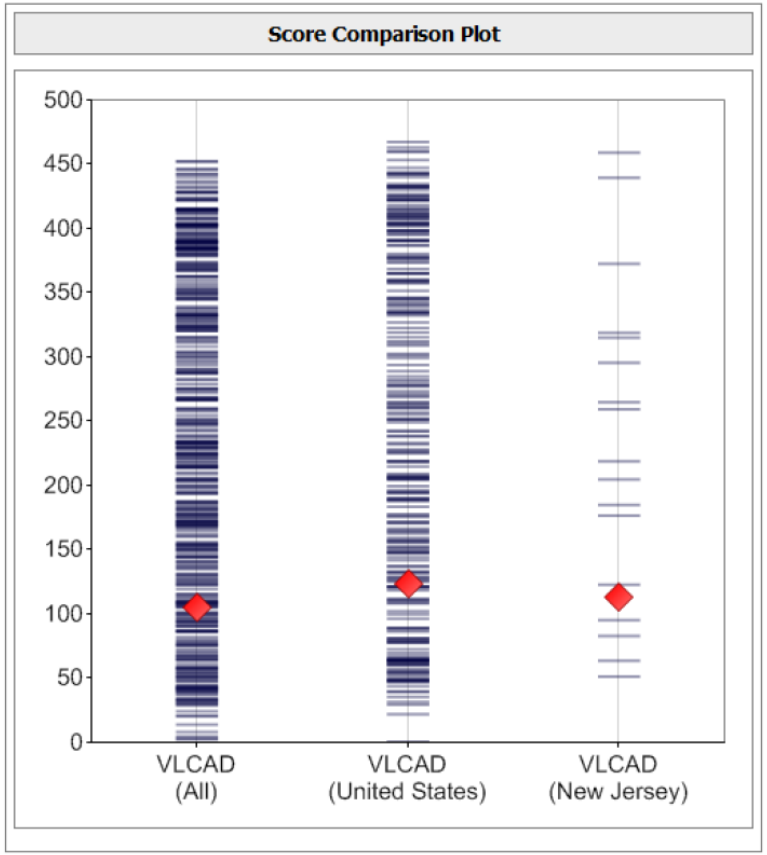
Interpretations Agree

And still get false positives

- Both R4S and laboratory algorithm called specimen as Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)
- Baby was cleared after diagnostic testing

C14:1	**0.89**
C14	**0.85**
C16:1	0.41
C14:2	0.19
C14:1/C16 (R)	**0.28**
C14:1/C12:1 (R)	1.57



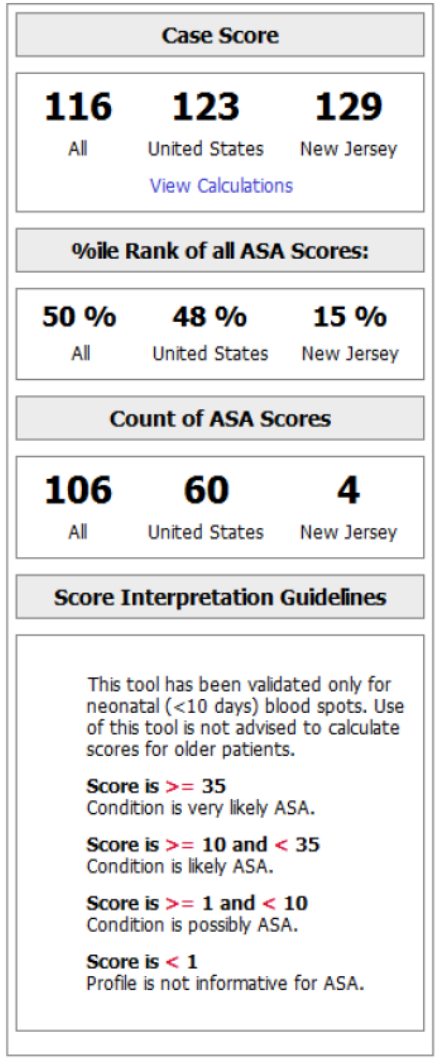


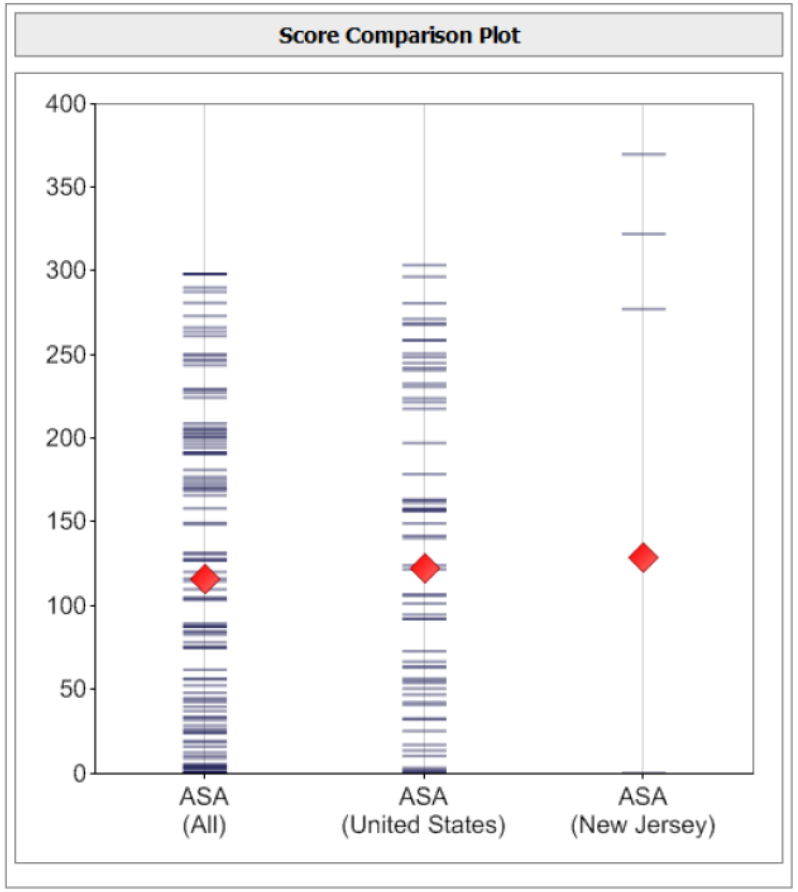
Interpretations Disagree

Program Needs to Adjust Range

- Laboratory citrulline cutoff (100umol/L) did not flag for referral
- Baby was diagnosed with Argininosuccinic aciduria (ASA)
- Program adjusted cutoff
- Retrospective R4S review identified newborn as very likely ASA

CIT (umol/L)	Diagnosis
282	CIT
126	ASA
1170	CIT
143	ASA
121	ASA
149	CIT
81	ASA





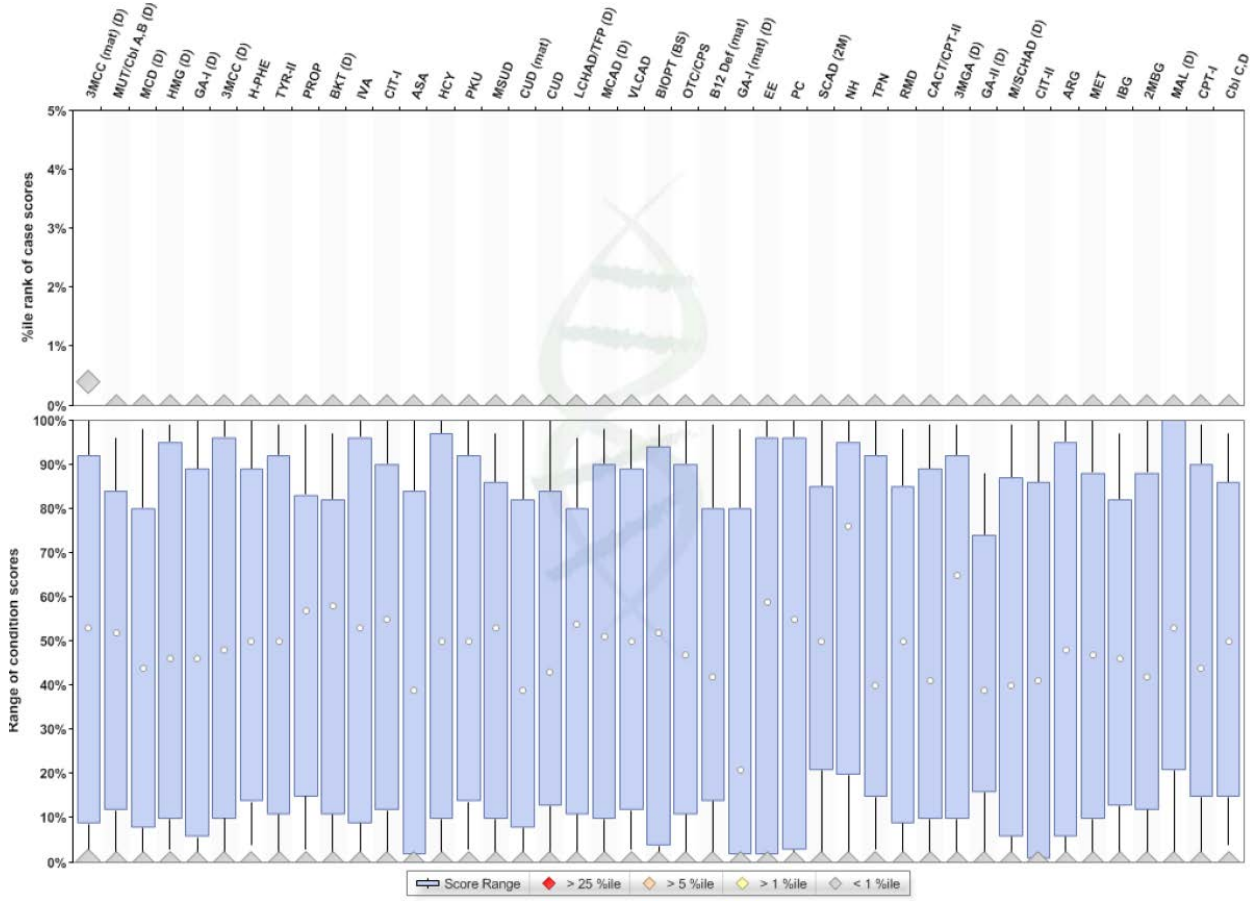
Interpretations Disagree

Laboratory algorithm correct

C5OH	**0.78**
C6DC	0.08
C6DC	0.08
C5DC	0.11
C5DC/C16	0.03
C5:1	0.01

C5OH	**0.81**	**0.85**
C6DC	0.1	0.1
C6DC	0.1	0.1
C5DC	0.1	0.09
C5DC/C16 (R)	0.06	0.05
C5:1	0.03	0.09

- Laboratory flagged initial specimen as borderline
- Repeat specimen was borderline and baby referred
- Baby was diagnosed with 3-hydroxy-3-methylglutaryl-CoA lyase (HMG)
- Retrospective R4S was not informative



Case Score

2	1	8
All	United States	New Jersey

View Calculations

%ile Rank of all HMG Scores:

0 %	0 %	0 %
All	United States	New Jersey

Count of HMG Scores

33	14	6
All	United States	New Jersey

Score Interpretation Guidelines

This tool has been validated only for neonatal (<10 days) blood spots. Use of this tool is not advised to calculate scores for older patients.

Score is >= 95
Condition is very likely HMG.

Score is >= 55 and < 95
Condition is likely HMG.

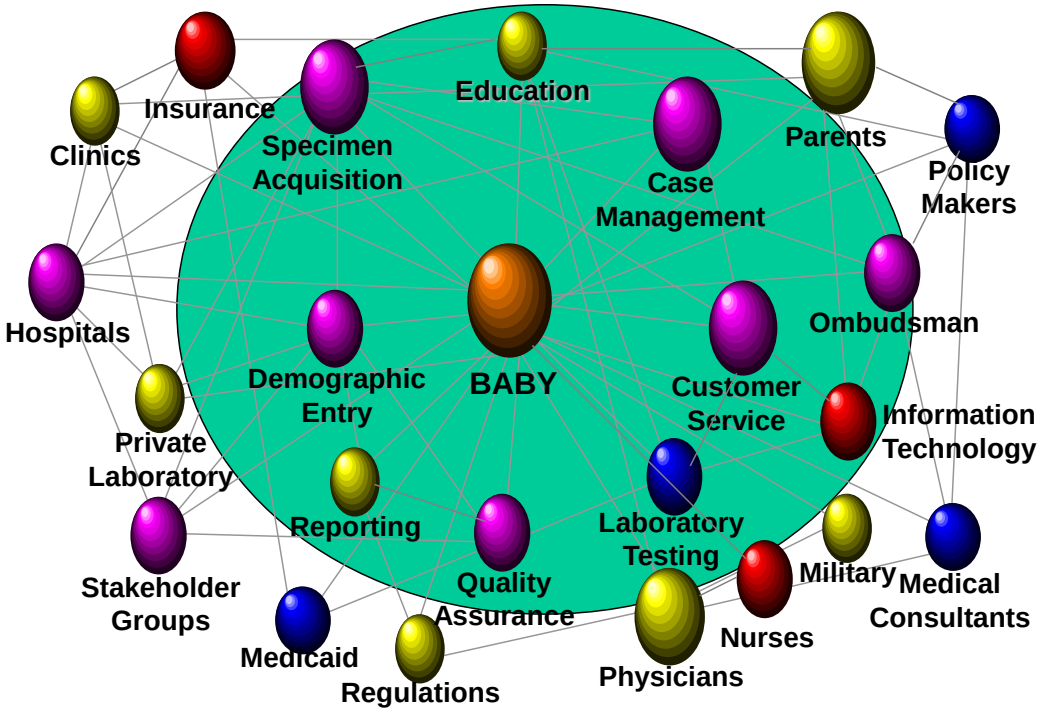
Score is >= 40 and < 55
Condition is possibly HMG.

Score is < 40
Profile is not informative for HMG.

Working as a System, Not Just a Laboratory

Case Review

- Routine meetings between Laboratory and Follow-up
- Consultation with subspecialist groups
- Technical assistance and collaboration with colleagues



Going Forward

In summary

- Setting and monitoring cutoffs has many challenges
  - Laboratory diversity
  - Volumes of data
  - Biological variability
  - Case definitions
- No one tool or methodology covers all the regulatory requirements, addresses good laboratory practices, or tackles all the challenges
- Multidisciplinary and collaborative approach is best to identify newborns at highest risk