The Products, Impact, and Future Applications of the Region 4 Stork (R4S) Collaborative Project

Piero Rinaldo, MD, PhD

Professor of Laboratory Medicine

T. Denny Sanford Professor of Pediatrics

Mayo Clinic, Rochester, MN

U.S. Department of Health and Human Services

Discretionary Advisory Committee on Heritable Disorders in Newborns and Children

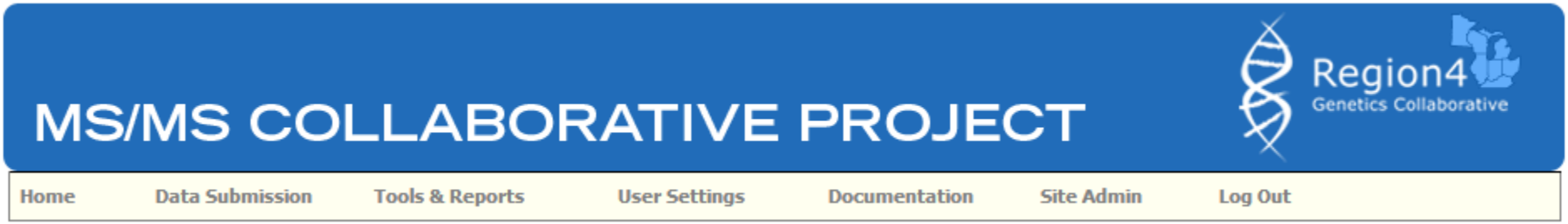
September 11th, 2014

Outline

- Origin and evolution of the Region 4 Stork (R4S) collaborative project
- The impact of R4S productivity and post-analytical interpretive tools
- Applicability of R4S beyond MS/MS (the “100/100” vision)
- Brief overview of CLIR 2.0 (4Q14)

Outline

- **Origin and evolution of the Region 4 Stork (R4S) collaborative project**



www.clir-r4s.org

Origin of the R4S Project

- **Region 4 Stork (R4S) started as a regional laboratory quality improvement project of expanded newborn screening by tandem mass spectrometry (7 state programs)**

- **R4S was selected as one of three projects of a Regional Genetics collaborative funded by the Health Resources and Services Administration (2004-2012)**

Region4
Genetics Collaborative

- **In May 2012 the R4S database became part of the *Newborn Screening Translational Research Network*, which is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development**

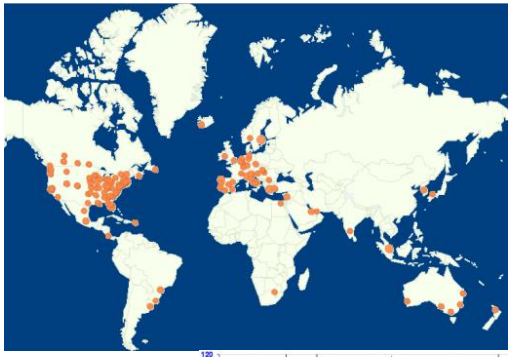
NBSTRN
Newborn Screening
Translational Research
Network

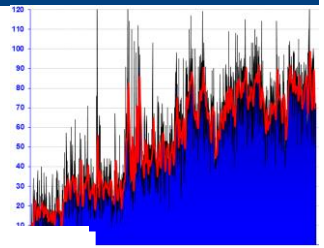
Evolution of R4S Project (2004-2014)

- **Worldwide participation and utilization (Sep 2014)**

66 States

235 Programs





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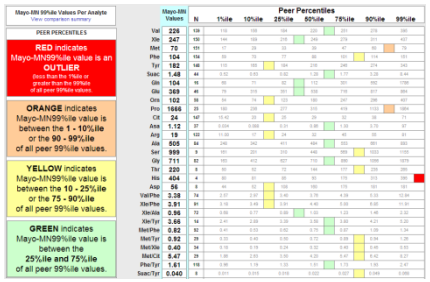
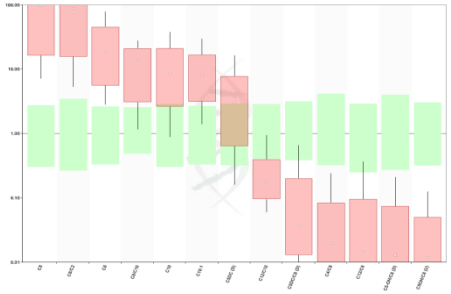
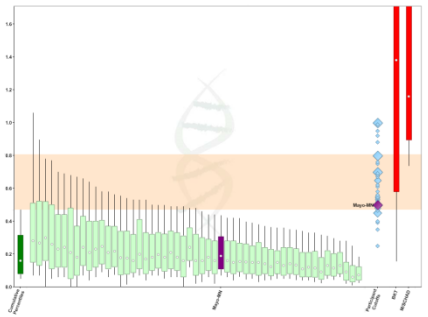
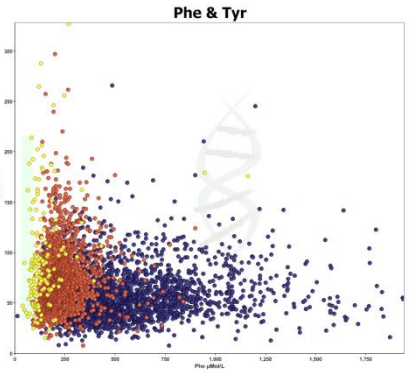
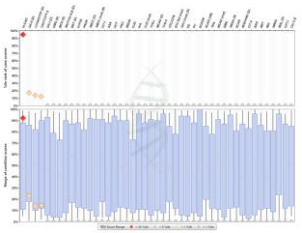
- The impact of R4S productivity and post-analytical interpretive tools

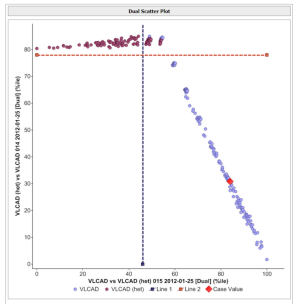


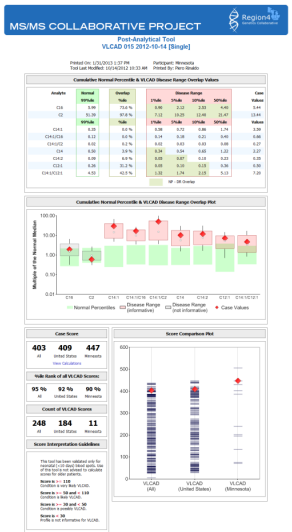












MAYO CLINIC

U.S. Department of Health and Human Services

Discretionary Advisory Committee on Heritable Disorders in Newborns and Children

The Impact of R4S

Process	Current NBS	R4S
Collaboration	Limited	Worldwide

ARTICLE

Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: A worldwide collaborative project

David M. S. McHugh[1], Cynthia A. Cameron, PhD[2], Jose E. Abdenur, MD[3], Mahera Abdulrahman, MD, PhD[4], Ona Adair, PhD[5],
Shahira Ahmed Al Nuaimi, BSc[4], Henrik Åhlman, MSc[6], Jennifer J. Allen, RN, BSN[7], Italo Antonozzi, MD[8], Shaina Archer, MSc[9],
Sylvia Au, MS[10], Christiane Auray-Blais, PhD[11], Mei Baker, MD[12], Fiona Bamforth, MD[9], Kinga Beckmann[13], Gessi Bentz Pino, MS[1],
Stanton L. Berberich, PhD[14], Robert Binard, BS[15], François Boemer, PharmD, PhD[16], Jim Bonham, PhD[17], Nancy N. Breen, MT[18],
Sandra C. Bryant, MS[1], Michele Caggana, ScD[19], S. Graham Caldwell[20], Marta Camilot, PhD[21], Carlene Campbell[22], Claudia Carducci, MS[8],
Rohit Cariappa, PhD[23], Clover Carlisle[24], Ubaldo Caruso[25], Michela Cassanello, PhChem[25], Ane Miren Castilla[26],
Daisy E. Castiñeiras Ramos[27], Pranesh Chakraborty, PhD[28], Ram Chandrasekar, PhD[29], Alfredo Chardon Ramos[30], David Cheillan, PhD[31],
Yin-Hsiu Chien, MD, PhD[32], Thomas A. Childs[33], Petr Chrastina, MSc[34], Yuri Cleverthon Sica, MSc[35], Jose Angel Cocho de Juan, PhD[27],
Maria Elena Colandre, PhD[36], Veronica Cornejo Espinoza, MSc[37], Gaetano Corso, MD[38], Robert Currier, PhD[39], Denis Cyr, MSc[11],
Noemi Czuczy, MSc[40], Oceania D'Apolito, PhD[38], Tim Davis, BS[41], Monique G. de Sain-Van der Velden, PhD[42],
Carmen Delgado Pecellin, PhD[43], Iole Maria Di Gangi, PhD[44], Cristina Maria Di Stefano, MD[45], Yannis Dotsikas, PhD[46],
Melanie Downing, MSc[17], Stephen M. Downs, PhD[47], Bonifacio Dy, MD[48], Mark Dymerski[49], Inmaculada Rueda, MD[50], Bert Elvers[51],
Roger Eaton, PhD[52], Barbara M. Eckerd[53], Fatma El Mougy, MD[54], Sarah Eroh[55], Mercedes Espada, PhD[26], Catherine Evans, PhD[56],
Sandy Fawbush, RN, BSN[57], Kristel F. Fijolek[53], Lawrence Fisher[28], Leifur Franzson, PhD[58], Dianne M. Frazier, PhD[59], Luciana R. C. Garcia[60],
Maria Sierra Garcia-Valdecasas Bermejo, PhD[43], Dimitar Gavrilov, MD, PhD[1], Rosemarie Gerace, BSc[61], Giuseppe Giordano, PhD[44],
Yolanda González Irazabal[62], Lawrence C. Greed, BSc[63], Robert Grier, PhD[64], Elyse Grycki, MS[1], Xuefan Gu, PhD[65],
Fizza Gulamali-Majid, PhD[66], Arthur F. Hagar, PhD[67], Lianshu Han, MD[65], W. Harry Hannon, PhD[68], Christa Haslip[69],
Fayza Abdelhamid Hassan, MD[54], Miao He, PhD[70], Amy Hietala[71], Leslie Himstedt, BSMT (ASCP)[55], Gary L. Hoffman[13],
William Hoffman, BS[41], Philis Hoggatt[72], Patrick V. Hopkins[22], David M. Hougaard, MD[73], Kerie Hughes[2], Patricia R. Hunt[74],
Wuh-Liang Hwu, MD[32], June Hynes[75], Isabel Ibarra-González, MSc[76], Cindy A. Ingham, RN, BSN[77], Maria Ivanova, PhD[78], Ward B. Jacox[79],
Catharine John, PhD[80], John … …sper, PhD[83], Brenda Klopper[84],
Dimitris Katakouzinos … …D[88], Ronald Koneski[74],
Viktor Kožich, MD, PhD[34], … …ncarlo la Marca, Pharm Sc[91],
Marcia Lavochkin[92], Soo… …e[94], Barbara Lesko, MT[18],
Barry Lewis, MD[63], Carol … …, MD[97], Fred Lorey, PhD[39],
Yannis L. Loukas, P… …Shawn Manos, BS[100],
Sandrine Marie, Ph… …etrich Matern, MD[1],
Stephanie K. Mayfield Gibso… …BA[71], Julie McClure, MPH[59],
James J. McGill, MBBS[103], Christine D. McKeever[33], Barbara McNeilly[104], Mark A. Morrissey, PhD[19], Paraskevi Moutsatsou, PhD[105],
Eleanor A. Mulcahy, RNC[106], Dimitris Nikoloudis, MSc[107], Bent Norgaard-Pedersen, MD[73], Devin Oglesbee, PhD[1], Mariusz Oltarzewski, PhD[108],
Daniela Ombrone[109], Jelili Ojodu, MPH[110], Vagelis Papakonstantinou, PhD[85], Sherly Pardo Reoyo, MD[30], Hyung-Doo Park, MD, PhD[93],
Marzia Pasquali, PhD[111], Elisabetta Pasquini, MD[91], Pallavi Patel[112], Kenneth A. Pass, PhD[113], Colleen Peterson[100],
Rolf D. Pettersen, PhD[114], James J. Pitt, PhD[115], Sherry Poh, MSc[80], Arnold Pollak, MD[83], Cory Porter[49], Philip A. Poston, PhD[116],
Ricky W. Price, BSc[117], Cecilia Queijo, BS[95], Jonessy Quesada, MD[118], Edward Randell, PhD[75], Enzo Ranieri, PhD[61],
Kimiyo Raymond, MD[1], John E. Reddic, PhD[20], Alejandra Reuben[118], Charla Ricciardi, BS[119], Piero Rinaldo, MD, PhD[1],
Jeff D. Rivera, PhD[120], Alicia Roberts, MS[121], Hugo Rocha, MSc[122], Geraldine Roche, MSc[102], Cheryl Rochman Greenberg, MD[123],
José María Egea Mellado, PhD[124], María Jesús Juan-Fita, PhD[124], Consuelo Ruiz[125], Margherita Ruoppolo, MD[109], S. Lane Rutledge, MD[121],
Euijung Ryu, PhD[1], Christine Saban, PhD[31], Inderneel Sahai, MD[52], Maria Isabel Salazar Garcia-Blanco[62], Pedro Santiago-Borrero, MD[30],
Andrea Schenone, PhD[36], Roland Schoos, PhD[16], Barb Schweitzer, RN[126], Patricia Scott[24], Margretta R. Seashore, MD[127],
Mary A. Seeterlin, PhD[128], David E. Sesser[129], Darrin W. Sevier[57], Scott M. Shone, PhD[112], Graham Sinclair, PhD[130],
Victor A. Skrinska, PhD[131], Eleanor L. Stanley, BS, MT (ASCP)[128], Erin T. Strovel, PhD[132], April L. Studinski Jones, MS[1],
Sherlykutty Sunny, BS[19], Zoltan Takats, PhD[40], Tijen Tanyalcin, MD, PhD[133], Francesca Teofoli[21], J. Robert Thompson, BSc[123],
Kathy Tomashitis, MNS[20], Mouseline Torquado Domingos[35], Jasmin Torres[48], Rosario Torres[125], Silvia Tortorelli, MD, PhD[1],
Sandor Turi, MD, PhD[82], Kimberley Turner, RN[134], Nick Tzanakos[115], Alf G. Valiente, PhD[37], Hillary Vallance, MD[130],
Marcela Vela-Amieva, MD[135], Laura Vilarinho, PhD[122], Ulrika von Döbeln, MD, PhD[6], Marie-Francoise Vincent, MD, PhD[101],
B. Chris Vorster, FCPath[84], Michael S. Watson, PhD[136], Dianne Webster, PhD[87], Sheila Weiss, MS[41], Bridget Wilcken, MD[96],
Veronica Wiley, PhD[96], Sharon K. Williams, MS[137], Sharon A. Willis, BS, MT (ASCP)[129], Michael Woontner, PhD[15], Katherine Wright[138],
Raquel Yahyaoui, MD[50], Seiji Yamaguchi, MD[88], Melissa Yssel, MB ChB, FC Path(SA) Chem[139], and Wendy M. Zakowicz, BS[79]

247 co-authors
(in alphabetical order)

Genet Med 2011;13:230-254

The Impact of R4S

Process	Current NBS	R4S
Collaboration	Limited	Worldwide (230+)
Peer comparison	Sparse (feared?)	On demand, up to date

Cutoff Comparison Tool

Mayo-MN 99%ile Values Per Analyte
View comparison summary

PEER PERCENTILES

RED indicates Mayo-MN99%ile value is an **OUTLIER**
(less than the 1%ile or greater than the 99%ile of all peer 99%ile values.

ORANGE indicates Mayo-MN99%ile value is between the **1 - 10%ile** or the **90 - 99%ile** of all peer 99%ile values.

YELLOW indicates Mayo-MN99%ile value is between the **10 - 25%ile** or the **75 - 90%ile** of all peer 99%ile values.

GREEN indicates Mayo-MN99%ile value is between the **25%ile and 75%ile** of all peer 99%ile values.

	Mayo-MN Values	N	Peer Percentiles 1%ile	10%ile	25%ile	50%ile	75%ile	90%ile	99%ile
Val	226	139	118	156	184	220	251	278	395
Xle	247	150	144	189	216	249	279	311	437
Met	70	151	17	29	33	39	47	60	79
Phe	104	154	59	70	77	88	101	114	151
Tyr	182	148	115	165	184	216	246	274	343
Suac	1.48	44	0.52	0.63	0.82	1.28	1.77	3.28	8.44
Gln	104	16	68	71	82	112	301	592	1786
Glu	369	46	79	315	351	538	716	817	864
Orn	102	58	54	74	123	180	247	296	407
Pro	1666	25	180	236	277	315	419	1133	1864
Cit	24	147	15.42	20	25	29	32	38	71
Asa	1.12	37	0.034	0.088	0.31	0.86	1.30	3.70	97
Arg	19	122	11.00	17	24	32	45	55	81
Ala	505	84	248	342	411	484	553	661	893
Ser	999	9	161	201	310	448	569	1033	1155
Gly	711	82	163	412	627	710	890	1056	1879
Thr	220	8	50	52	72	144	177	235	285
His	404	4	80	81	85	93	175	313	395
Asp	56	8	44	52	108	160	175	181	181
Val/Phe	3.38	74	2.57	2.97	3.40	3.76	4.39	5.33	12.84
Xle/Phe	3.91	91	3.18	3.49	3.91	4.40	5.00	6.85	11.91
Xle/Ala	0.96	72	0.68	0.77	0.89	1.03	1.23	1.46	2.32
Xle/Tyr	3.66	14	2.41	2.89	3.39	3.58	3.80	4.21	5.20
Met/Phe	0.82	92	0.41	0.53	0.62	0.75	0.87	1.09	1.34
Met/Tyr	0.92	29	0.33	0.40	0.50	0.72	0.89	0.94	1.26
Met/Xle	0.40	34	0.18	0.19	0.24	0.32	0.40	0.45	0.53
Met/Cit	5.47	29	1.86	2.63	3.50	4.20	5.47	6.42	8.27
Phe/Tyr	1.61	118	0.96	1.19	1.33	1.51	1.73	1.93	2.47
Suac/Tyr	0.040	8	0.011	0.015	0.018	0.022	0.027	0.049	0.068

The Impact of R4S

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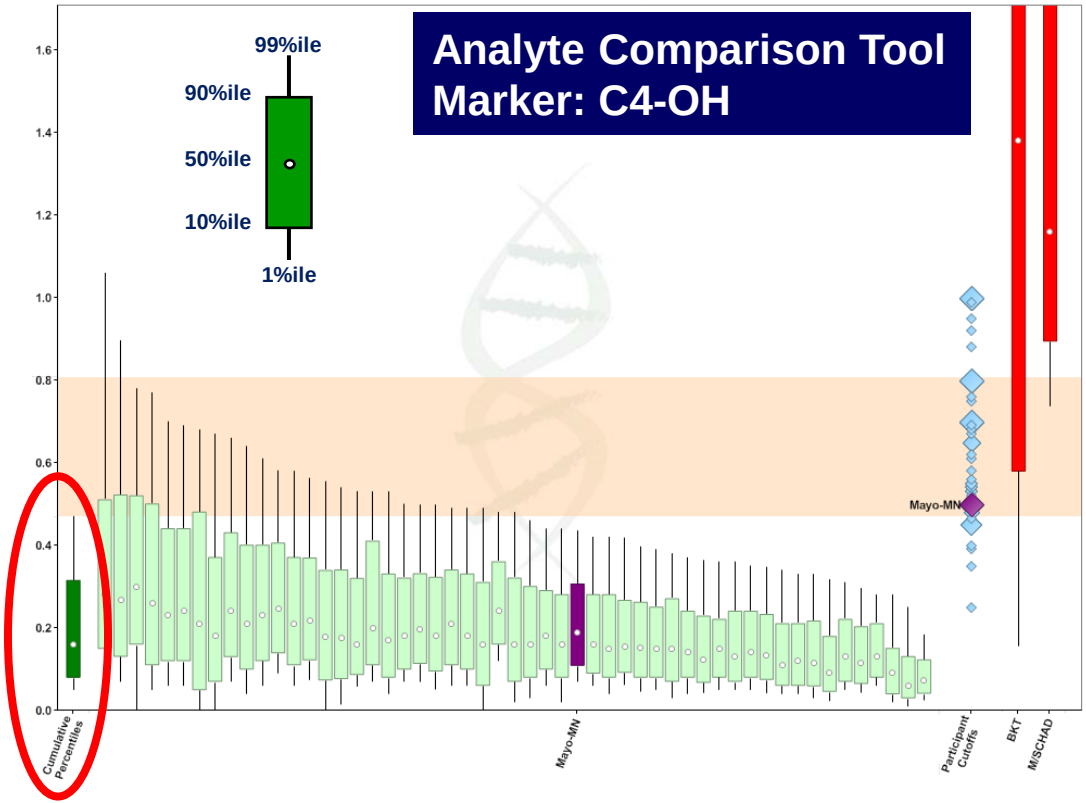
Definition of “normal”	<X and/or >Y	Cumulative percentiles



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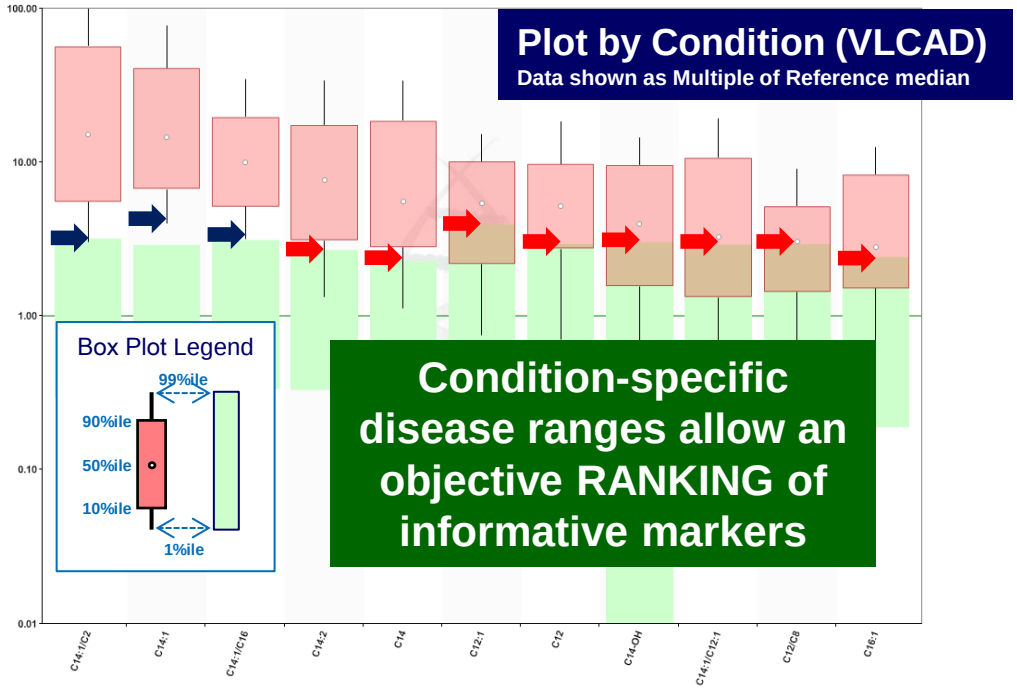

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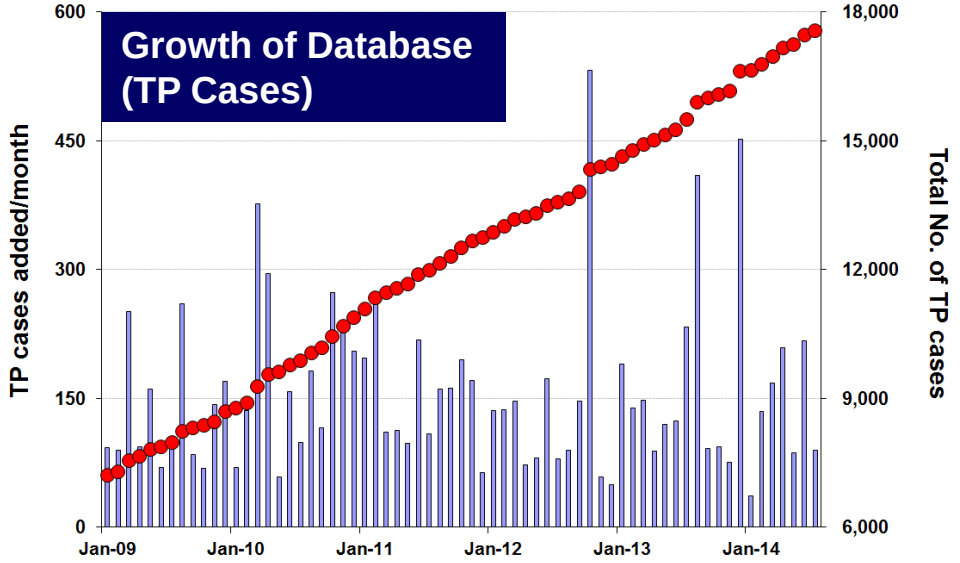
Definition of “abnormal”	Cutoff values	Dis. range (no overlap)
Clinical validation	Static	Constantly evolving



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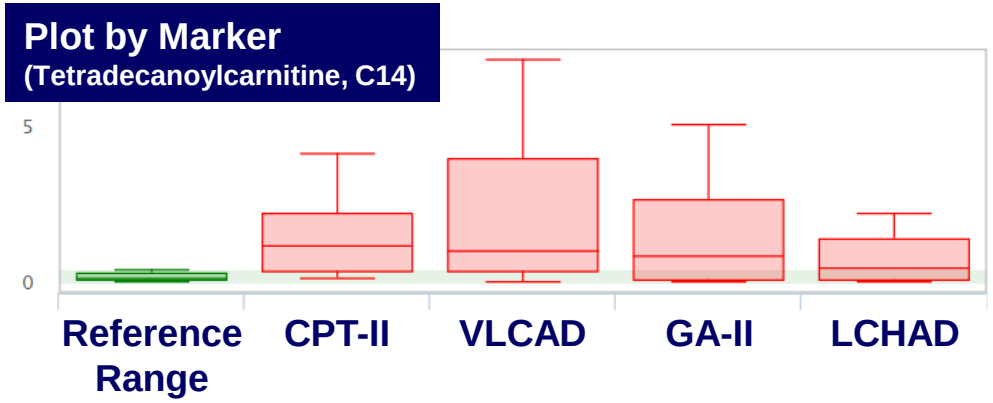
Clinical validation	Static	Dynamic
Disease ranges	None	Condition-specific



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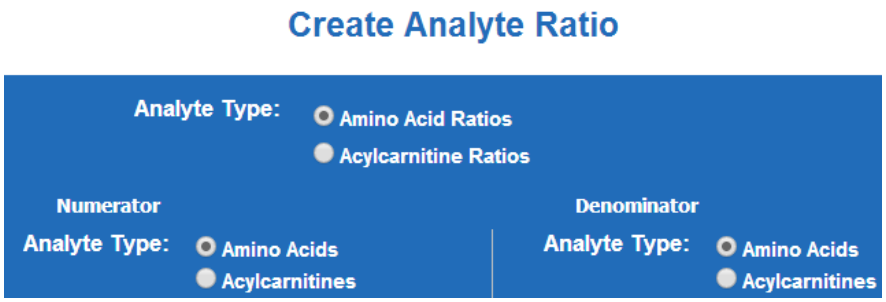
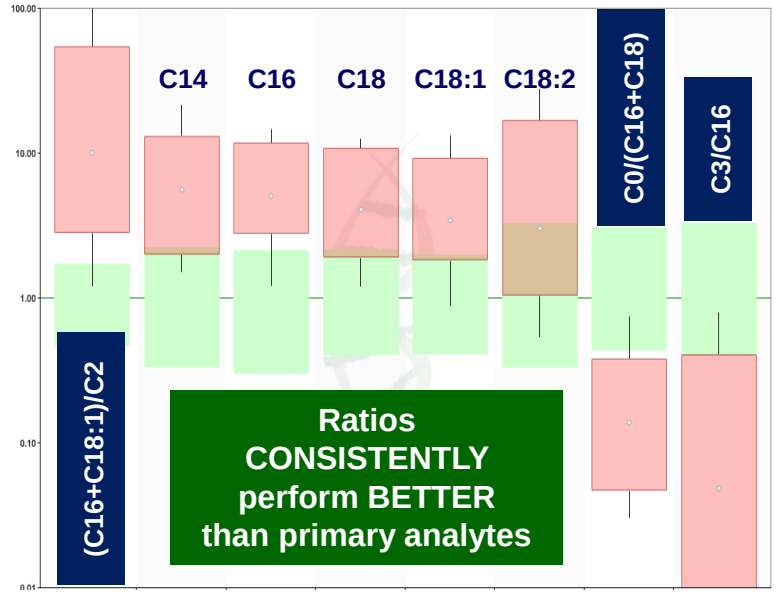
Clinical validation	Static	Dynamic
Disease ranges	None	Condition-specific
Utilization of Ratios	Minimal	Extensive



A Tale of Three Cases with CPT-II def.

	AC Species	TP Case	FP Case	FN Case	Median Cutoff	Lab Count
(nmol/mL)	C14				0.68	(121)
	C16				7.24	(130)
	C18:2				0.67	(78)
	C18:1				3.00	(118)
	C18				2.05	(108)
						(47)
						(21)
						(59)



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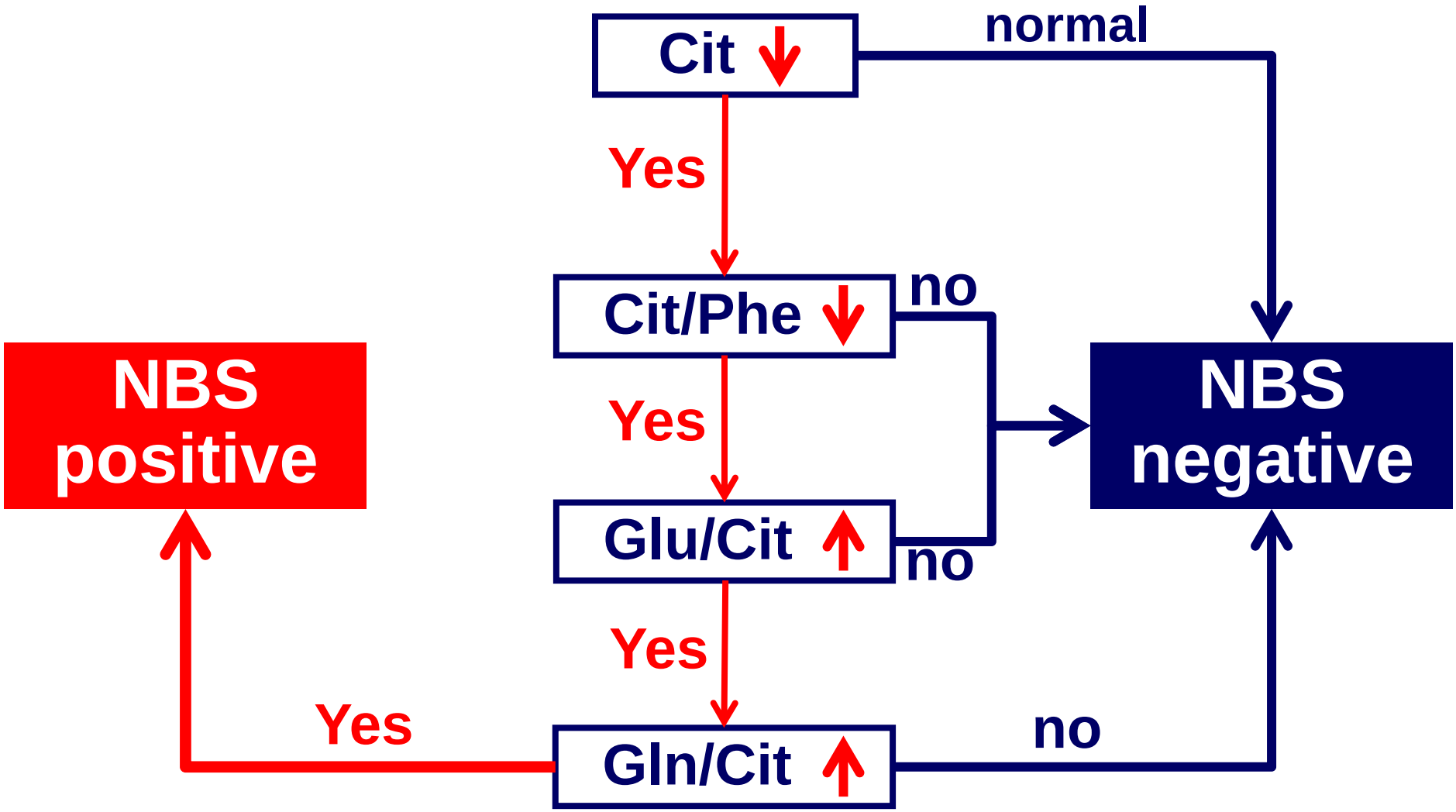
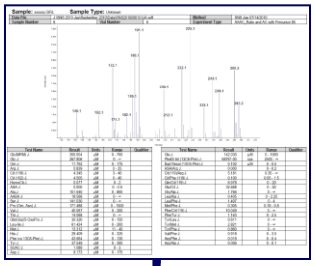
Disease ranges	None	Condition-specific
Utilization of Ratios	Arbitrary, limited	Extensive (effort)
Algorithms	Sequential	Parallel (tools)

SEQUENTIAL Algorithm (LOW Citrulline)



PARALLEL Algorithm



Cit

Cit/Phe

Glu/Cit

Gln/Cit

R4S Post-Analytical Interpretive Tool

SCORE

NOT Informative

INFORMATIVE

NBS Negative (OTC/CPS)

NBS POSITIVE (OTC/CPS)

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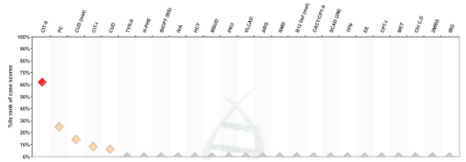
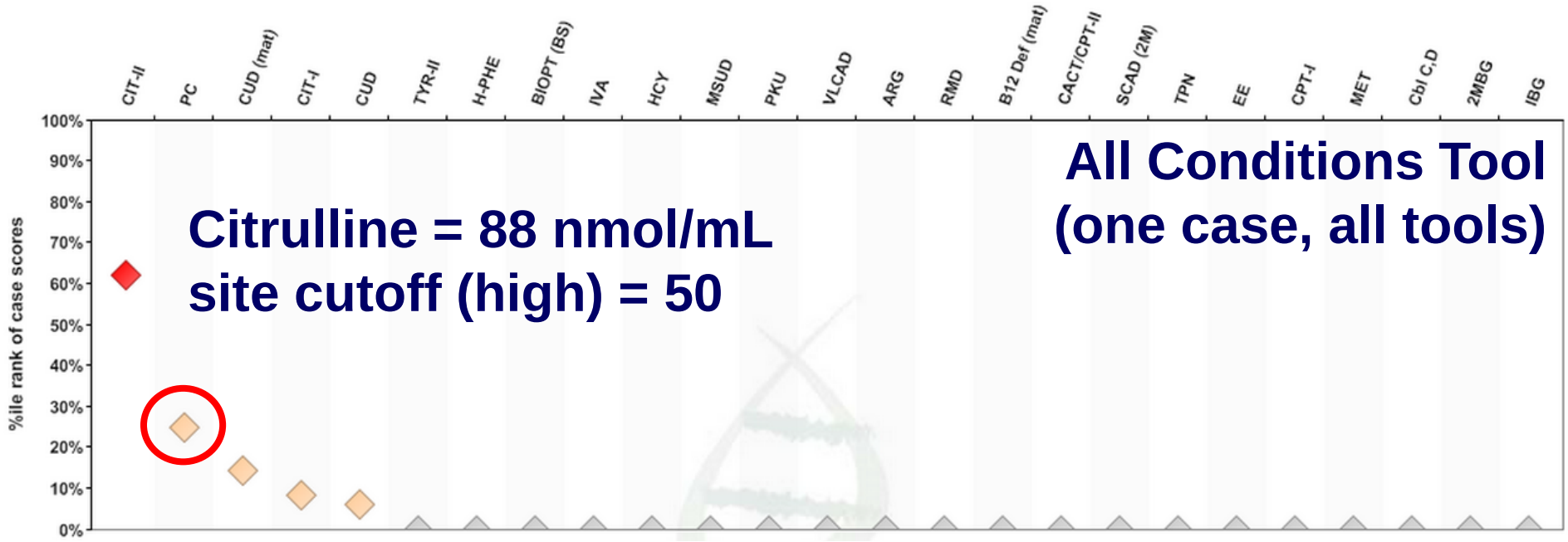
Disease ranges	None	Condition-specific
Utilization of Ratios	Arbitrary, limited	Extensive (effort)
Algorithms	Sequential	Parallel (tools)
Differential diagnosis	If thought of…	“Built-in”



“Built-in” Differential Dx



What is “PC”?

(Pyruvate carboxylase deficiency)

R4S Post-Analytical Tools

- **The R4S tools can provide a clinically useful answer to three basic questions:**
  - **YES or NO (one condition)**
  - **ONE or the OTHER (differential dx)**
  - **Pick ONE out of a GROUP (many conditions)**

MS/MS COLLABORATIVE PROJECT

Region4 Genetics Collaborative

Post-Analytical Tool
VLCAD 015 2012-10-14 [Single]

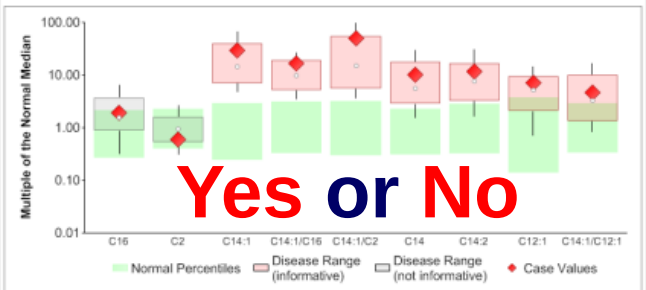
Printed On: 1/31/2013 1:37 PM
Tool Last Modified: 10/14/2012 10:33 AM
Participant: Minnesota
Printed By: Piero Rinaldo

Cumulative Normal Percentile & VLCAD Disease Range Overlap Values

Analyte	Normal 99%ile	Overlap %ile	Disease Range 1%ile	5%ile	10%ile	50%ile	Case Values
C16	5.99	73.6 %	0.90	2.12	2.53	4.40	5.44
C2	51.39	97.8 %	7.12	10.25	12.40	21.47	13.44
	99%ile	%ile	1%ile	5%ile	10%ile	50%ile	Values
C14:1	0.35	0.0 %	0.58	0.72	0.86	1.74	3.59
C14:1/C16	0.12	0.0 %	0.14	0.18	0.21	0.40	0.66
C14:1/C2	0.02	0.2 %	0.02	0.03	0.03	0.08	0.27
C14	0.50	3.9 %	0.34	0.54	0.65	1.22	2.27
C14:2	0.09	6.9 %	0.05	0.07	0.10	0.23	0.35
C12:1	0.26	31.2 %	0.05	0.10	0.15	0.36	0.50
C14:1/C12:1	4.53	42.5 %	1.32	1.74	2.15	5.13	7.20

NP - DR Overlap

Cumulative Normal Percentile & VLCAD Disease Range Overlap Plot



Yes or No

Case Score

403	409	447
All	United States	Minnesota

View Calculations

%ile Rank of all VLCAD Scores:

95 %	92 %	90 %
All	United States	Minnesota

Count of VLCAD Scores

248	184	11
All	United States	Minnesota

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.

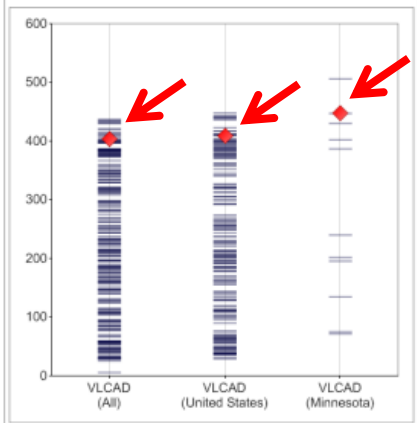
Score is >= 110
Condition is very likely VLCAD.

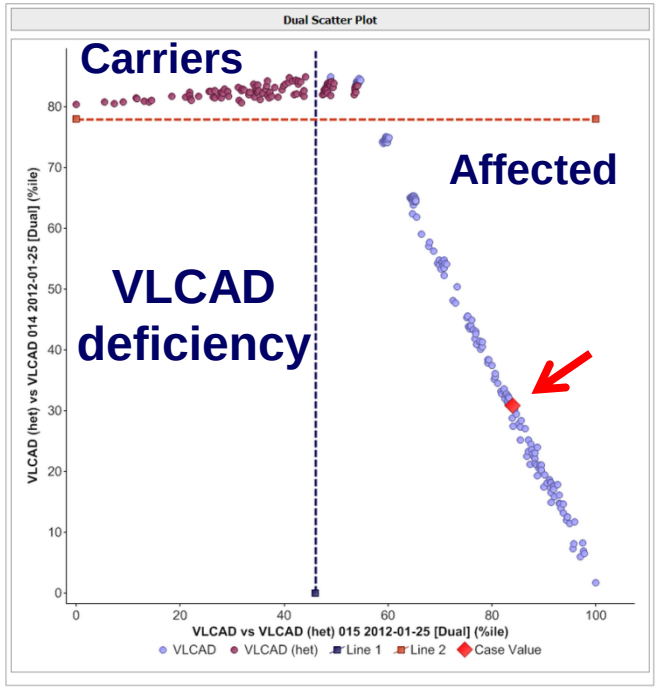
Score is >= 50 and < 110
Condition is likely VLCAD.

Score is >= 30 and < 50
Condition is possibly VLCAD.

Score is < 30
Profile is not informative for VLCAD.

Score Comparison Plot





One or Another

Molecular Genetics and Metabolism 111 (2014) 484–492

Contents lists available at ScienceDirect

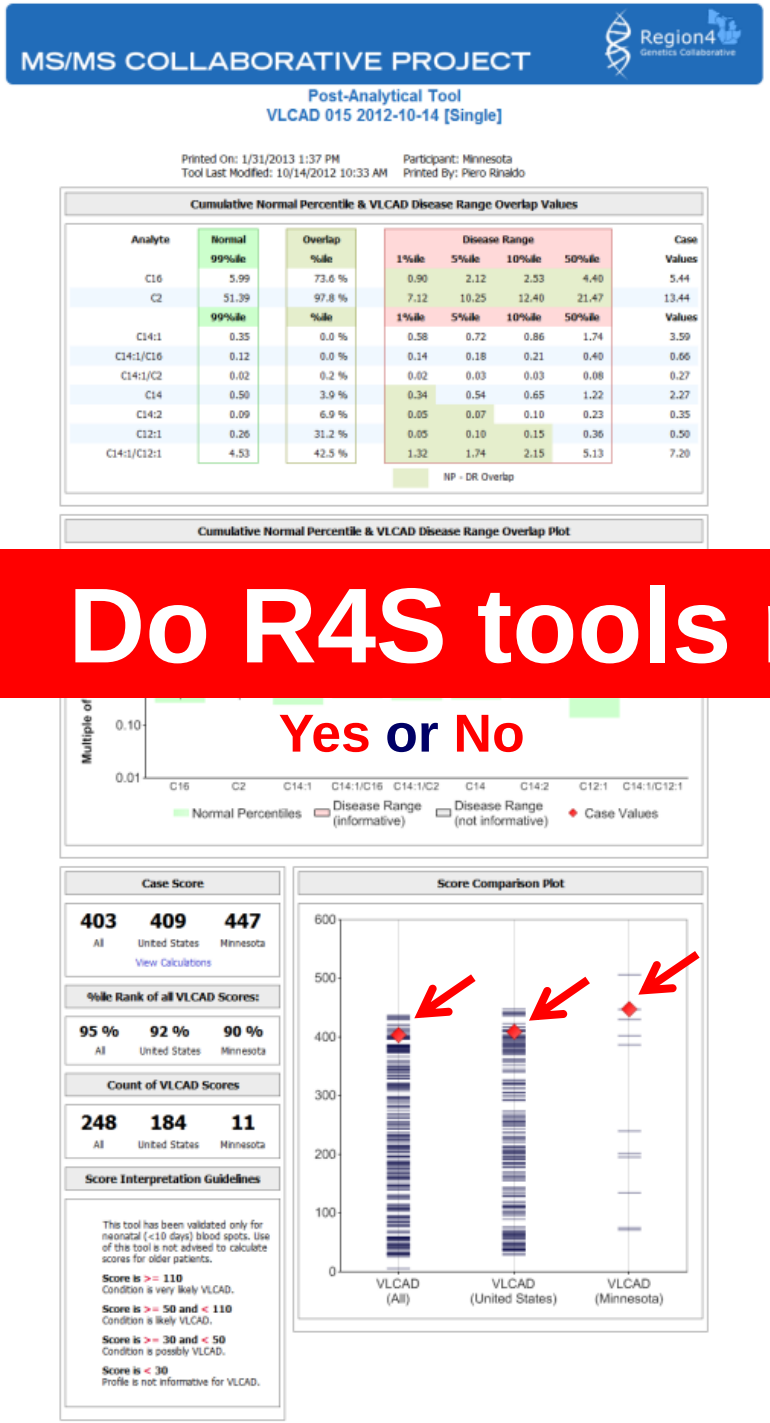
Molecular Genetics and Metabolism

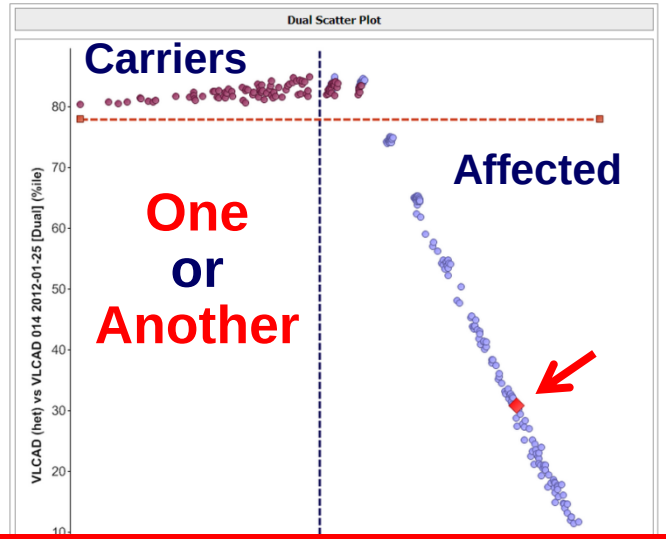
journal homepage: www.elsevier.com/locate/ymgme

Infants suspected to have very-long chain acyl-CoA dehydrogenase deficiency from newborn screening

J. Lawrence Merritt II [a,*], Sverre Vedal [b], Jose E. Abdenur [c], Sylvia M. Au [d], Bruce A. Barshop [e], Lisa Feuchtbaum [f], Cary O. Harding [g], Cheryl Hermerath [h], Fred Lorey [f], David E. Sesser [h], John D. Thompson [i], Arthur Yu [d]

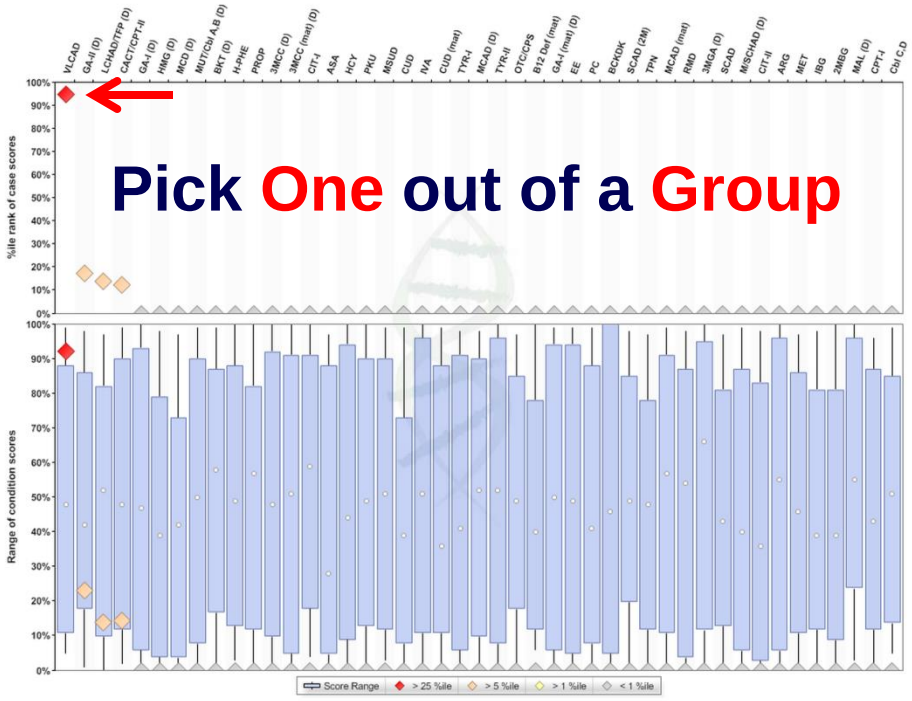
When we focused upon the HET subgroup, of the 27 cases calculated, 23 were predicted to be heterozygotes, 4 were inconclusive, and none were predicted to be VLCADD.



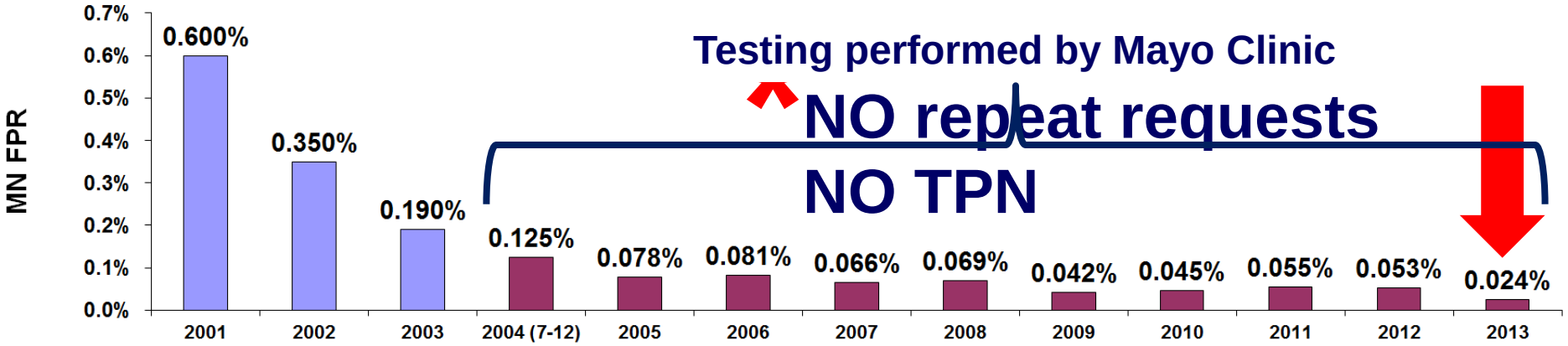


Do R4S tools make any difference?

Yes or No



MN Performance by MS/MS (2004-13)

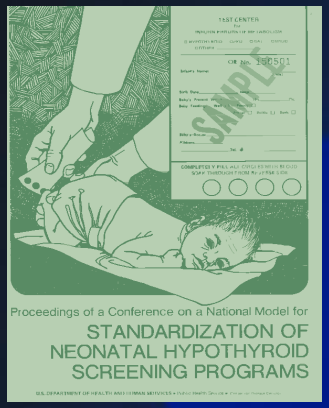


Period	**2013**	
Births	**71,207**	
Abnormal cases	**55***	**(N=28)**
True positives	**38**	**USA**
False positives	**17**	**AVERAGE**
		7.1
FPR	**0.024%**	**0.51%**
PPV	**69%**	**18%**

Outline

- **Origin and evolution of the Region 4 Stork (R4S) collaborative project**

- **Applicability of R4S beyond MS/MS (the "100/100" vision)**



"...it's very important in the deliberations that are carried out here that all bear in mind that there are things other than hypothyroid being tested on the blood spot specimen."

"...there will be other tests that will be as important."

Dr. Robert Guthrie
August 22, 1979
State University of New York at Buffalo

Courtesy of Harry Hannon

CDC

Partial List of Candidate Conditions for Expansion of Newborn Screening

Uniform Panel **87**

- Fabry disease (X-linked)
- Gaucher disease
- Krabbe disease
- Metachrom. Leukodystr. (MLD)
- Pseudo MLD
- MPS I
- MPS II
- MPS IIIA
- MPS VI
- Mucolipidosis type II/III
- Multiple sulphatase deficiency
- Niemann–Pick disease type A/B
- Pompe disease

- **Fragile X**
- **Friedreich's ataxia**
- **LSD**
- **Proximal UCDs**
- **SLO**
- **SMA**
- **Toxoplasmosis**
- **Wilson disease**

Partial List of Candidate Conditions for Expansion of Newborn Screening

Uniform Panel

100+

- ALD (X-linked)
- CDG Ib
- CMV
- Creatine defects
- DMD
- G6PD
- HIV
- Fam. Hypercholesterol.

- ALD carriers
- Zellweger sdr
- Other DPBs
- CRT (X-linked)
- CRT carriers
- GAMT
- AGAT

How Can We Possibly Do THAT?

- **Multiplexing might be a compelling necessity (the traditional "one condition-one test" model is no longer feasible)**
- **Evidence of analytical robustness and reproducibility will be scrutinized more**
- **Implementation may require in-depth clinical validation (higher expectations driven by evidence review process)**
- **Performance metrics must exceed GREATLY historical standards (0.1%-0.5% per condition)**

Minnesota NBS Performance in DBS

(N=209,432; Period: 2008-2010)

	Conditions UP	Conditions ST	FPR	MN FP/wk	CA (560k)	USA (4.2M)
IEM (MS/MS)	20	22	0.05%	0.7	5.4	40.4
Biotinidase	1	0	0.09%	1.2	9.7	72.7
CAH	1	1	0.11%	1.5	11.8	88.8
Hypothyroidism	1	0	0.21%	2.9	22.6	169.6
Cystic Fibrosis	1	0	0.34%	4.7	36.6	274.6
Galactosemia	1	2	0.06%	0.8	6.5	48.5
Hbpathies	3	(1)	0.02%	0.3	2.2	16.2
TOTAL	28	26	0.88%	12	95	711

NBS Performance by MS/MS

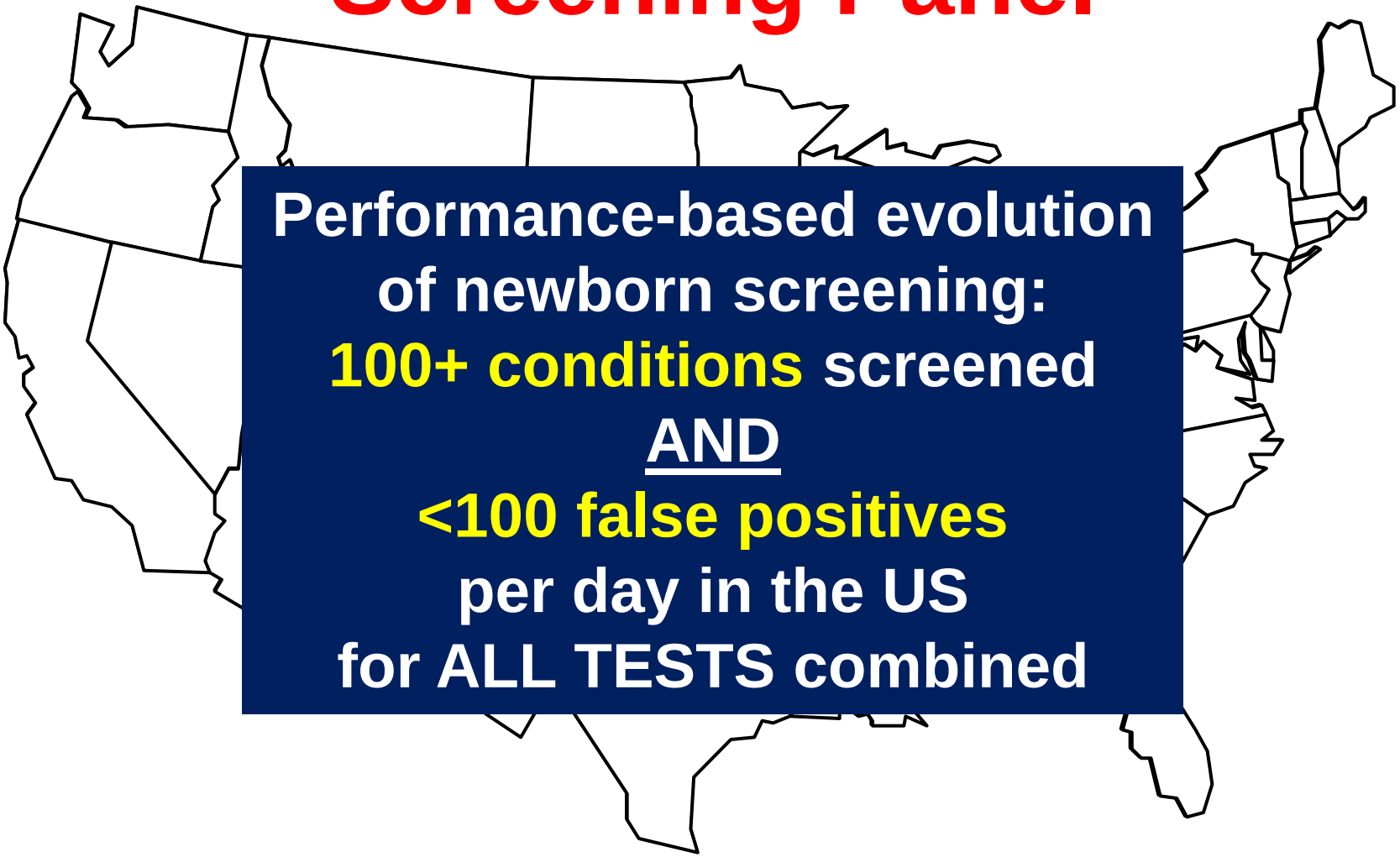
(US Average FPR, N=28)

	Conditions UP	Conditions ST	FPR	MN FP/wk	CA (560k)	USA (4.2M)
IEM (MS/MS)	20	22	0.51%	7.0	54.9	411.9
Biotinidase	1	0	0.09%	1.2	9.7	72.7
CAH	1	1	0.11%	1.5	11.8	88.8
Hypothyroidism	1	0	0.21%	2.9	22.6	169.6
Cystic Fibrosis	1	0	0.34%	4.7	36.6	274.6
Galactosemia	1	2	0.06%	0.8	6.5	48.5
Hbpathies	3	(1)	0.02%	0.3	2.2	16.2
TOTAL	28	27	1.34%	19	145	1083

Impact of Improved Performance

	Conditions UP	Conditions ST	MN FP/wk	CA (560k)	USA (4.2M)
IEM (MS/MS)	20	22	1.4	10.8	80.8
Biotinidase	1	0	1.2	9.7	72.7
CAH	1	1	1.4	10.8	80.8
Hypothyroidism	1	0	1.4	10.8	80.8
Cystic Fibrosis	1	0	1.4	10.8	80.8
Galactosemia	1	2	0.8	6.5	48.5
Hbpathies	3	(1)	0.3	2.2	16.2
SCID	1	(1)	1.4	10.8	80.8
TOTAL	29	27	9	72	541

A Personal View of the Evolution of the Recommended Uniform Screening Panel



Performance-based evolution of newborn screening:
100+ conditions screened
AND
<100 false positives
per day in the US
for ALL TESTS combined

False Positives:
The Dark Side of Newborn Screening

- Recall and repeat analysis (2nd, 3rd, 4th…)
- Disruption of care (premature, sick newborns)
- ER visit(s), admission(s)
- Confirmatory testing ($$$)
- Referral to multiple specialists, 2nd opinions
- Disruption of family life, and work schedule
- Impact on extended family life (stress)

In the current (and future) health care climate, reduction of false positives is an ABSOLUTE requirement in parallel to any expansion of the recommended panel

How to Improve Performance?

T. Murner, *Appeal to fools* (1512)

- **Adopt "top" screening**
- **Increase frequency of testing**
- **Find new and better markers**
  - **Succinylacetone for Tyrosinemia type I**
- **Better clinical validation of cutoff values**

or

- **Do more with what is being done ALREADY**

R4S Elsewhere

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Open

Postanalytical tools improve performance of newborn screening by tandem mass spectrometry

Patricia L. Hall, PhD[1], Gregg Marquardt, MSS[1], David M.S. McHugh[1], Robert J. Currier, PhD[2], Hao Tang, PhD[2], Stephanie D. Stoway, BS[1] and Piero Rinaldo, MD, PhD[1]

Purpose: The purpose of this study was to compare performance metrics of postanalytical interpretive tools of the Region 4 Stork collaborative project to the actual outcome based on cutoff values for amino acids and acylcarnitines selected by the California newborn screening program.

Methods: This study was a retrospective review of the outcome of 176,186 subjects born in California between 1 January and 30 June 2012. Raw data were uploaded to the Region 4 Stork Web portal as .csv files to calculate tool scores for 48 conditions simultaneously using a previously unpublished functionality, the tool runner. Scores for individual target conditions were deemed informative when equal or greater to the value representing the first percentile rank of known true-positive cases (17,099 cases in total).

Results: In the study period, the actual false-positive rate and positive predictive value were 0.26 and 10%, respectively. Utilization of the Region 4 Stork tools, simple interpretation rules, and second-tier tests could have achieved a false-positive rate as low as 0.02% and a positive predictive value >50% by replacing the cutoff system with Region 4 Stork tools as the primary method for postanalytical interpretation.

Conclusion: Region 4 Stork interpretive tools, second-tier tests, and other evidence-based interpretation rules could have reduced false-positive cases by up to 90% in California.

Genet Med advance online publication 29 May 2014

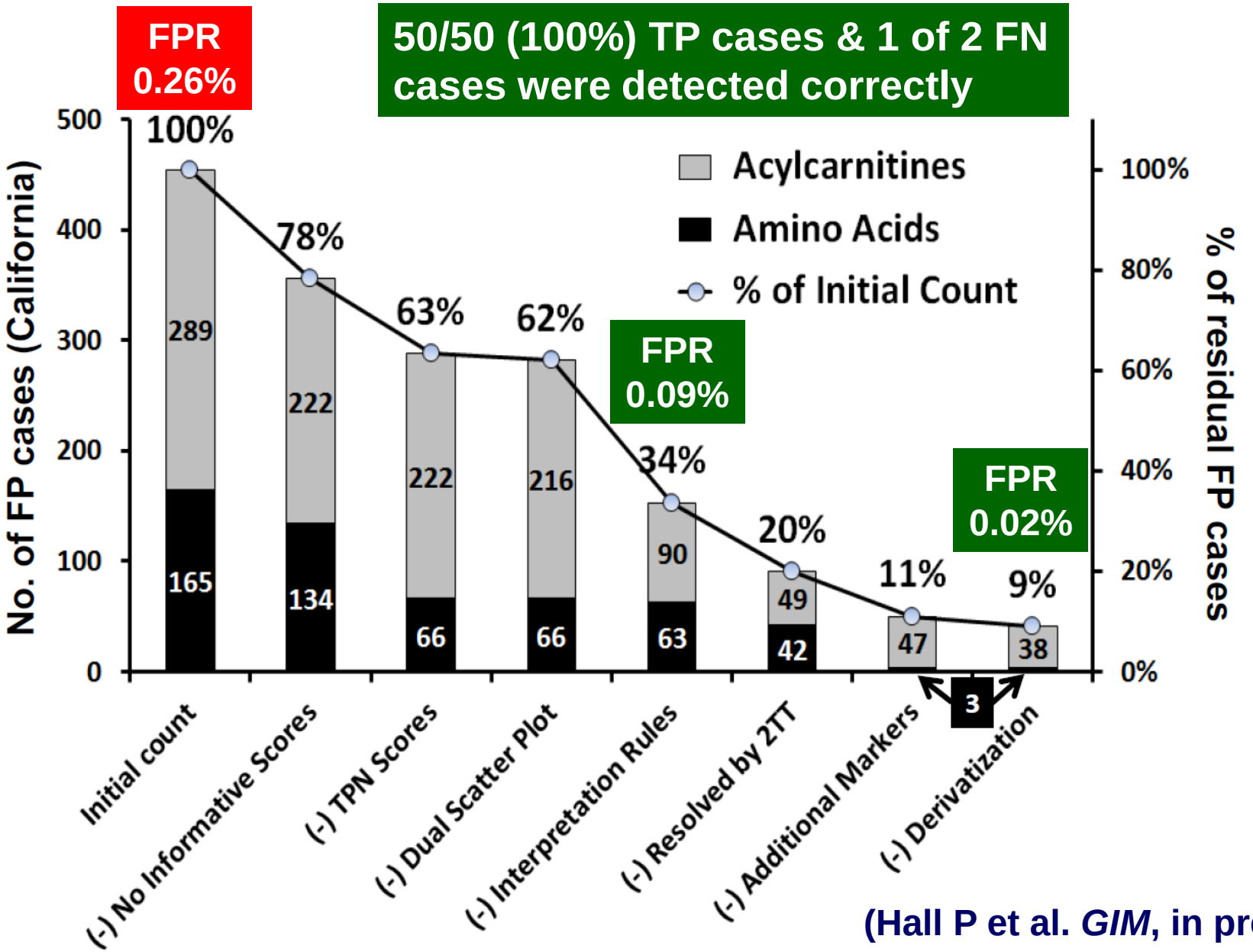
Key Words: cutoff values; newborn screening; postanalytical interpretive tools; second-tier test; tandem mass spectrometry

R4S Elsewhere

- **Comparison between actual (cutoff values) and estimated outcome (R4S tools) using a high-throughput functionality (tool runner)**
- **Data from California DOPH (Jan-Jun 2012)**
- **176,186 newborns after exclusion criteria**

(Hall P et al. *GIM*, in press)

California False Positives



(Hall P et al. *GIM*, in press)

Impact of Improved Performance

(Goal: each test FPR ≤0.1%)

	Conditions UP	Conditions ST	FPR	MN FP/wk	CA (560k)	USA (4.2M)
IEM (MS/MS)	20	22	0.02%	0.3	2.2	16.2
Biotinidase	1	0	0.09%	1.2	9.7	72.7
CAH	1	1	0.10%	1.4	10.8	80.8
Hypothyroidism	1	0	0.10%	2.9	22.6	80.8
Cystic Fibrosis	1	0	0.10%	4.7	36.6	80.8
Galactosemia	1	2	0.06%	0.8	6.5	48.5
Hbpathies	3	(1)	0.02%	0.3	2.2	16.2
SCID	1	(1)	0.10%	1.4	10.8	80.8
			PER DAY	~1	<10	<70
TOTAL	29	28	0.60%	8	64	481

Currently Nominated Conditions

Uniform Panel

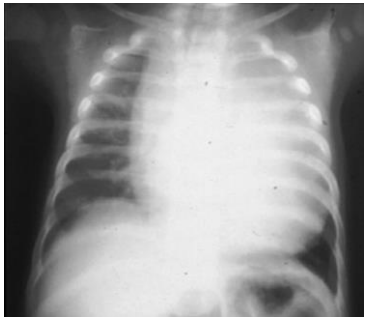
58 ?

- Fabry disease
- Gaucher disease
- Krabbe disease
- Metachrom. Leukodystr. (MLD)
- Pseudo MLD
- MPS I
- MPS II
- MPS IIIA
- MPS VI
- Mucolipidosis type II/III
- Multiple sulphatase deficiency
- Niemann–Pick disease type A/B
- Pompe disease

- Fragile X
- Friedreich's ataxia
- LSD
- Menkes disease
- SLO
- SMA
- Toxoplasmosis
- Wilson disease

Pompe Disease (Condition)

- **Incidence**
  - **1:40,000 by clinical ascertainment (US)**
- **Timing of clinical onset**
  - **Continuum of disease spectrum (all ages)**
  - **Median age at onset between 1.5 - 4 months of age**
- **Severity of disease**
  - **Death due to cardio-respiratory failure in the first year of life in the infantile form**

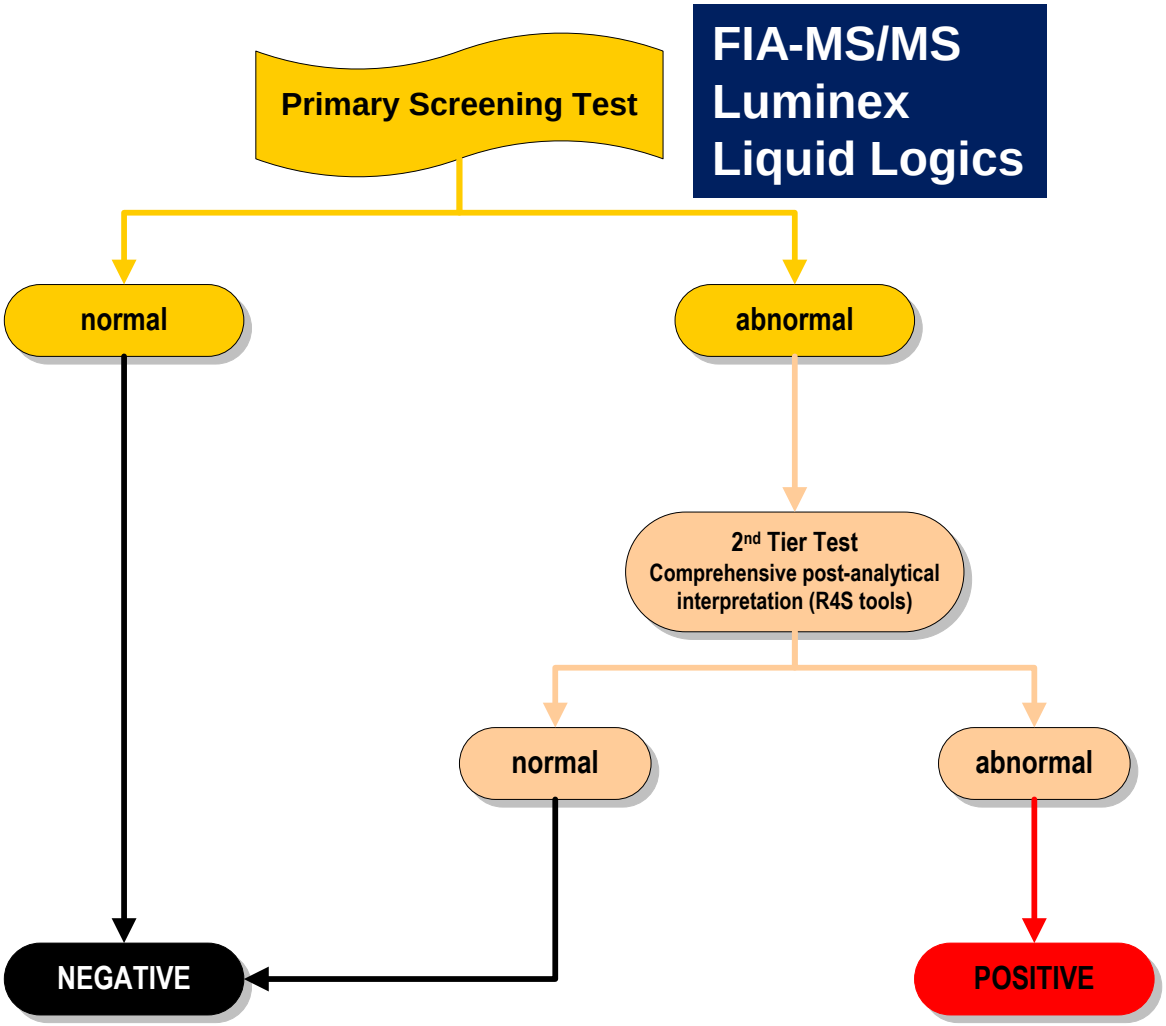


Pompe Disease (Test)

- **Screening test(s) to be used**
  - Three alternative platforms under evaluation
- **Modality of screening**
  - Dried blood spots (DBS)
- **Risks**
  - Detection of late-onset cases
  - Interference by neutral maltase (pseudo-deficiency)
- **Clinical validation**
  - First pilot study in Taiwan (2008)
- **Performance metrics**
  - Preliminary evidence of high false positive rate

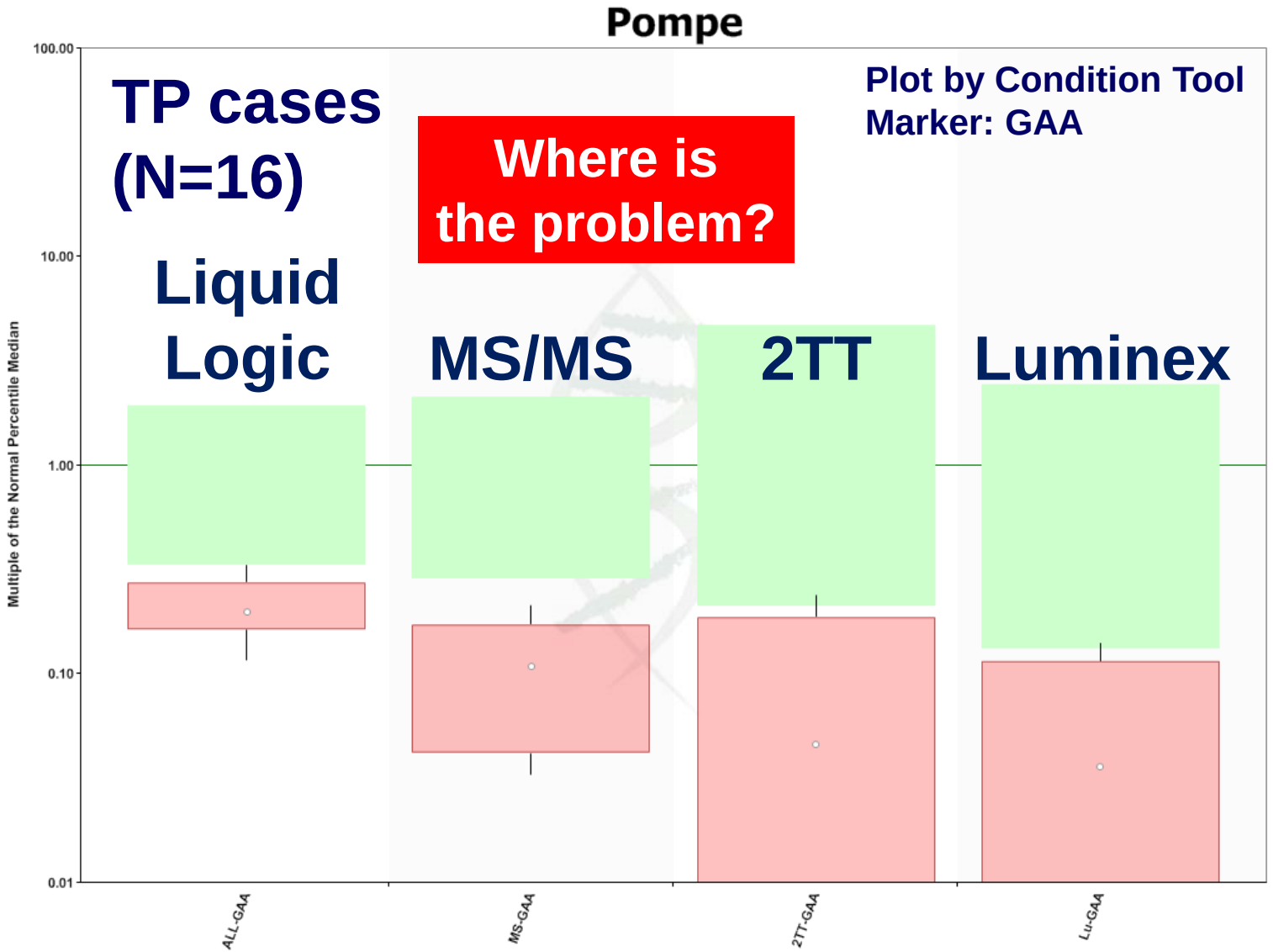
FIA-MS/MS
Luminex
Liquid Logics

LSD Pilot Study



Principal Investigator:
Dietrich Matern, MD, PhD

Method Comparison



Pilot Study for Pompe Disease

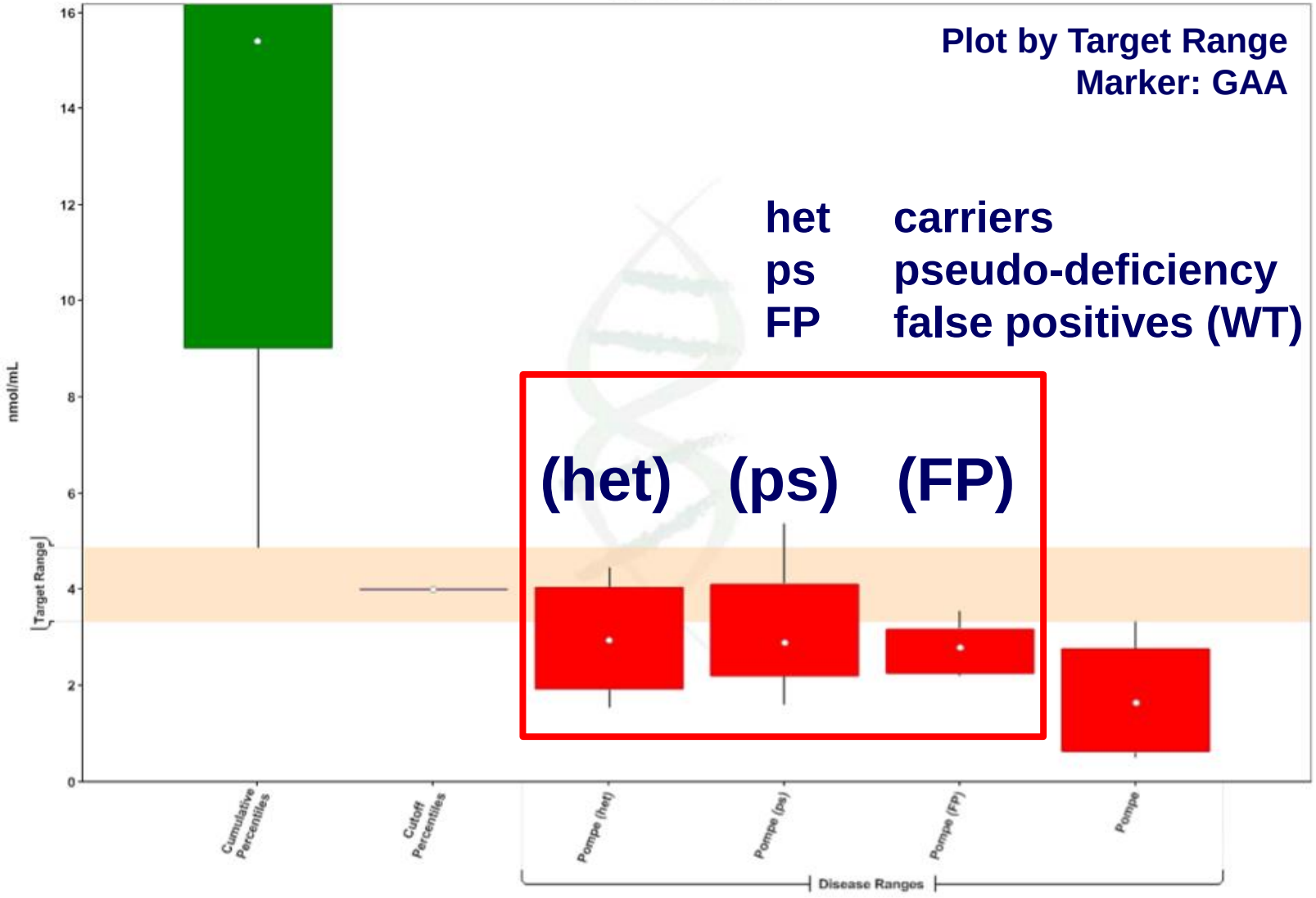
	Luminex	MS/MS	Liquid Logic
Samples tested:	99,856	99,925	90,713
GAA abnormal (1st Tier):	457 (0.46%)	588 (0.59%)	330 (0.33%)
GAA activity low (2nd Tier)	37 (0.04%)	40 (0.04%)	36 (0.04%)

Molecular genetic analysis

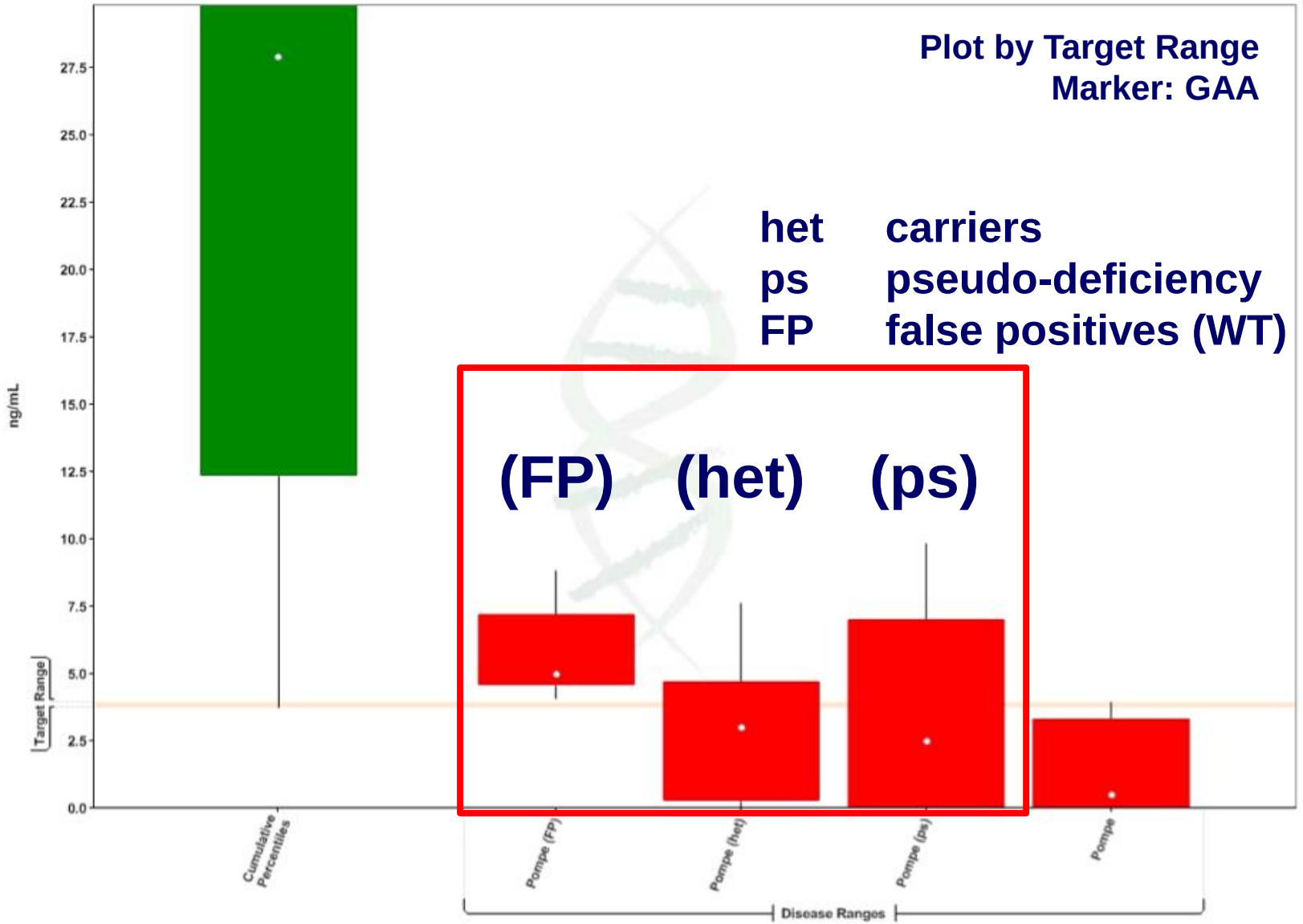
- 5 True Positives (likely late onset)
- 26 Pseudo deficiency
- 5 Carriers
- 4 No mutations

} = FALSE POSITIVES

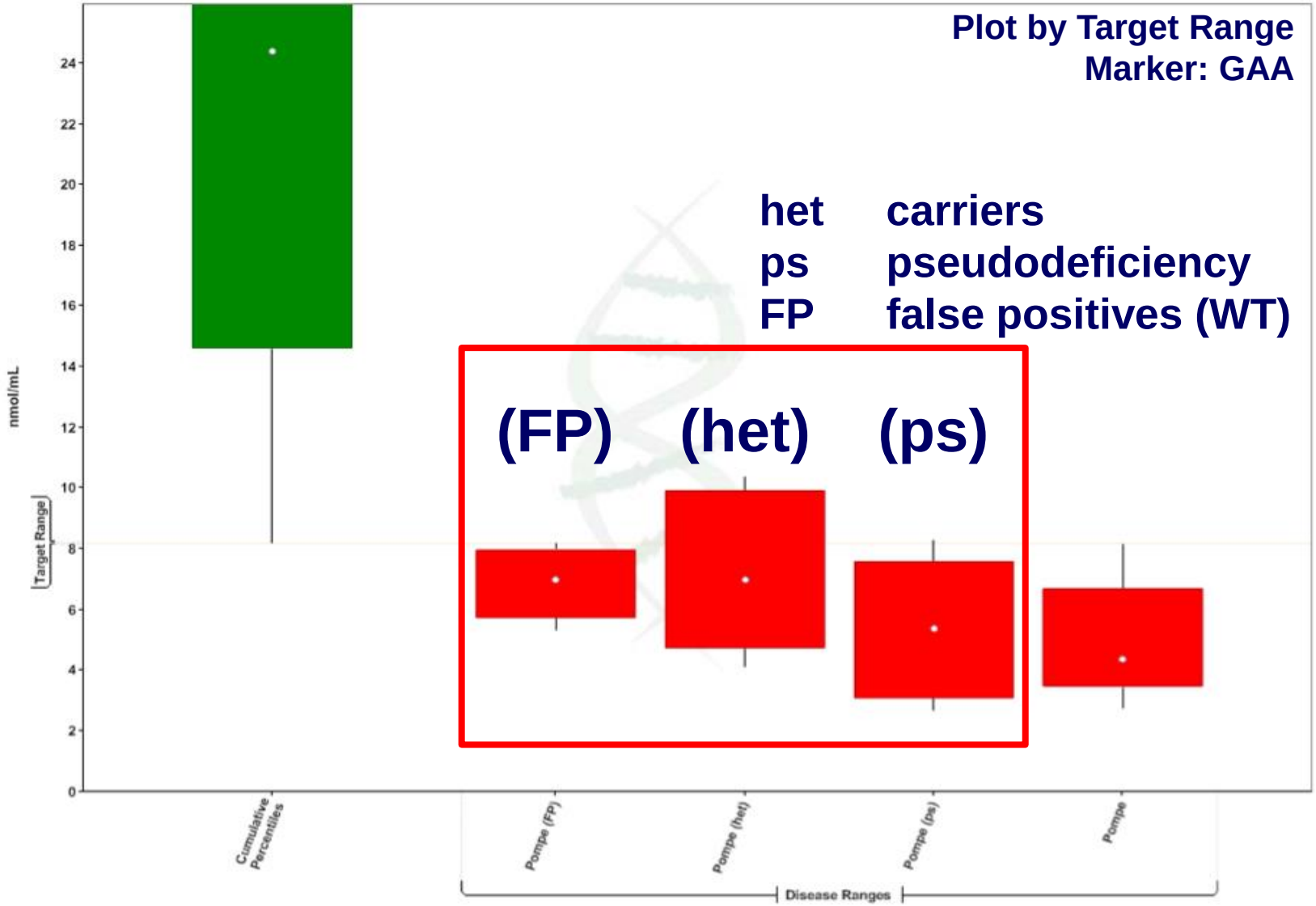
Pompe False Positives (MS/MS)



Pompe False Positives (Luminex)



Pompe False Positives (Liquid Logic)



Pilot Study for Pompe Disease

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Samples tested:	99,856	99,925	90,713
GAA abnormal (1st Tier):	457 (0.46%)	588 (0.59%)	330 (0.33%)
GAA activity low (2nd Tier)	37 (0.04%)	40 (0.04%)	36 (0.04%)
Molecular genetic analysis	5 True Positives (likely late onset) 26 Pseudo deficiency 5 Carrier 4 No mutation	= FALSE POSITIVES	
FPR:	0.032%	0.035%	0.034%
Positive PV:	13.5%	12.5%	13.9%

Pilot Study for Pompe Disease

	Luminex	MS/MS	Liquid Logic
Samples tested:	99,856	99,925	90,713
GAA abnormal (1st Tier):	457 (0.46%)	588 (0.59%)	330 (0.33%)
GAA activity low (2nd Tier)	37 (0.04%)	40 (0.04%)	36 (0.04%)

FPR:	0.032%	0.035%	0.034%
Positive PV:	13.5%	12.5%	13.9%

Pilot Study for Pompe Disease

	Luminex	MS/MS	Liquid Logic
Samples tested:	99,856	99,925	90,713
GAA abnormal (1st Tier):	457 (0.46%)	588 (0.59%)	330 (0.33%)
GAA activity low (2nd Tier)	37 (0.04%)	40 (0.04%)	36 (0.04%)
Abnormal per R4S Tools	4 (0.004%)	5 (0.005%)	11 (0.011%)
FPR (R4S):	0.000%	0.000%	0.007%
Positive PV:	100%	100%	45.5%

Currently Nominated Conditions

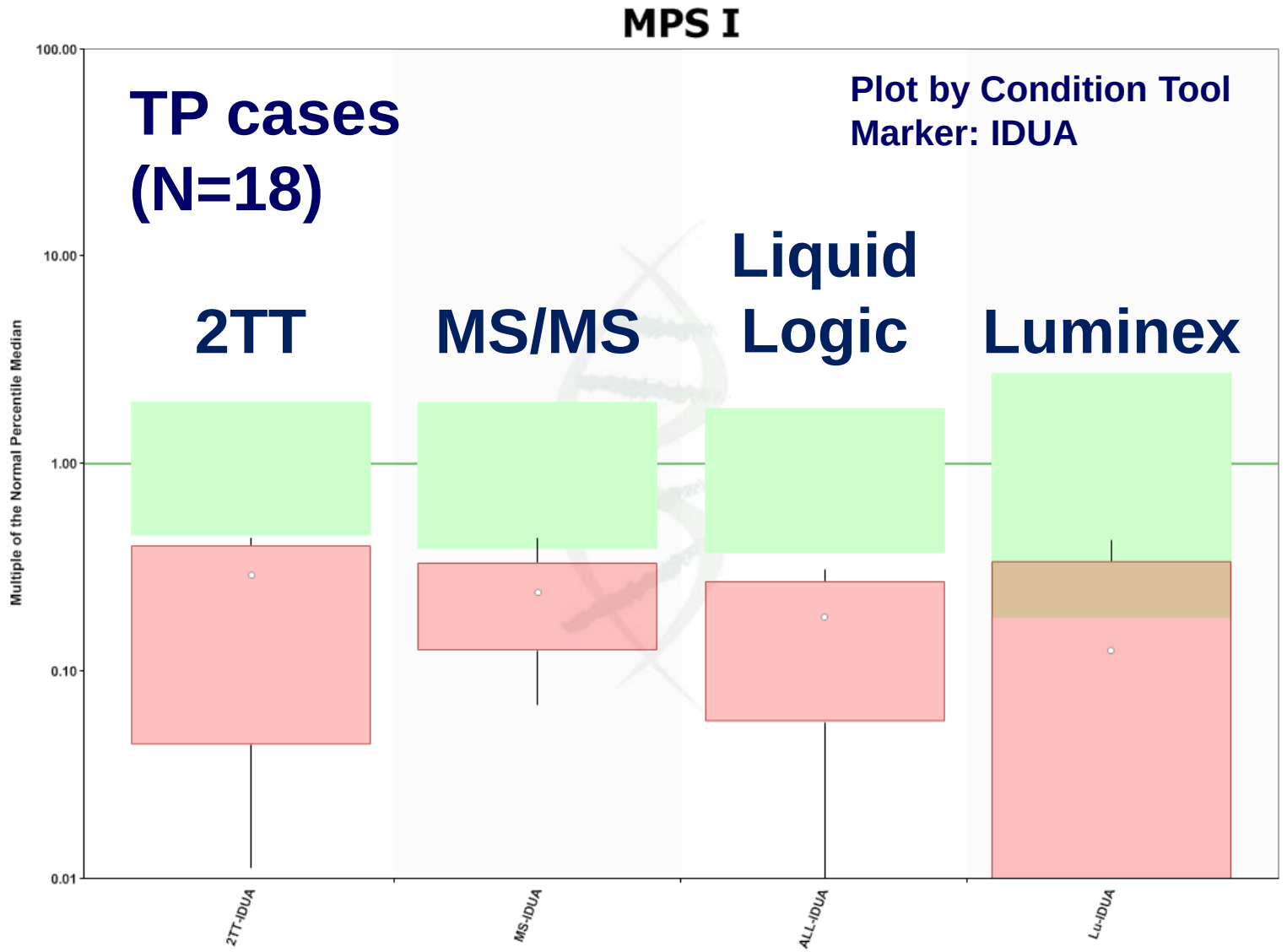
Uniform Panel

59 ?

- Fabry disease
- Gaucher disease
- Krabbe disease
- Metachrom. Leukodystr. (MLD)
- Pseudo MLD
- MPS I
- MPS II
- MPS IIIA
- MPS VI
- Mucolipidosis type II/III
- Multiple sulphatase deficiency
- Niemann–Pick disease type A/B
- Pompe disease

- Fragile X
- Friedreich's ataxia
- LSD
- Menkes disease
- SLO
- SMA
- Toxoplasmosis
- Wilson disease

Method Comparison



Pilot Study for MPS-I

	Luminex	MS/MS	Liquid Logic
Samples tested:	99,856	99,925	90,713
IDUA abnormal (1st Tier):	397 (0.59%)	590 (0.40%)	182 (0.18%)
IDUA activity low (2nd Tier)	113 (0.11%)	135 (0.14%)	90 (0.09%)
Abnormal per R4S Tools	18 (0.018%)	20 (0.020%)	19 (0.019%)
FPR (R4S):	0.002%	0.004%	0.006%
Positive PV:	89%	80%	74%

Pilot Study for X-ALD

	MS/MS
Samples tested:	**99,925**
LPCs elevated (1st Tier):	**2,245 (2.25%)**
LPCs elevated (2nd Tier)	**12 (0.012%)**
Abnormal per R4S Tools	**2 (0.002%)**
FPR (R4S):	**0.000%**
Positive PV:	**100%**

Performance of R4S Tools for RUSP Candidate Conditions

Condition	N	FPR	PPV
Pompe	100K[1]	0.007%[2]	46%[2]
MPS-I	100K[1]	0.006%[2]	74%[2]
X-ALD	100K[1]	0.000%	100%
OTC/CPS	432K[3]	0.003%	31%
RMD	680K[4]	0.000%	100%

[1] NICHD pilot study (P.I., Dietrich Matern)
[2] Worst performance of the 3 tests
[3] MN prospective experience 2009-2013
[4] MN prospective experience 2004-2013

Impact of Improved Performance

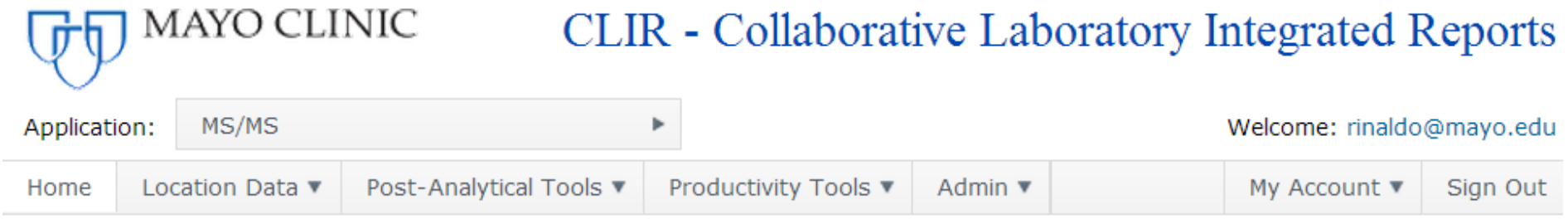
(Goal: each test FPR ≤0.1%)

	Conditions UP	Conditions ST	FPR	MN FP/wk	CA (560k)	USA (4.2M)
IEM (MS/MS)	20	22	0.02%	0.3	2.2	16.2
Biotinidase	1	0	0.09%	1.2	9.7	72.7
CAH	1	1	0.10%	1.4	10.8	80.8
Hypothyroidism	1	0	0.10%	1.4	10.8	80.8
Cystic Fibrosis	1	0	0.10%	1.4	10.8	80.8
Galactosemia	1	2	0.06%	0.8	6.5	48.5
Hbpathies	3	(1)	0.02%	0.3	2.2	16.2
SCID	1	(1)	0.10%	1.4	10.8	80.8
6 NEW Targets	6	4	0.016%	0.1	0.5	4.0
TOTAL	34	32	PER DAY	~1	<10	<70

UNCHANGED!

Outline

- **Origin and evolution of the Region 4 Stork (R4S) collaborative project**
- **The impact of R4S productivity and post-analytical interpretive tools**

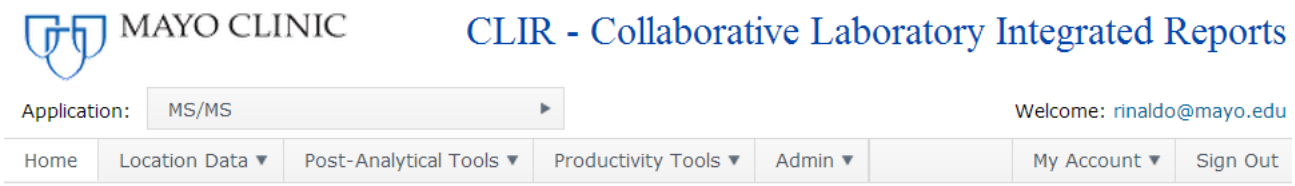


- **Brief overview of CLIR 2.0 (4Q14)**

The Evolution of R4S/CLIR

- R4S is based on a multivariate pattern recognition software we have named CLIR, Collaborative Laboratory Integrated Reports
- Version 2.0 in under development within the Mayo IT infrastructure and will include several upgrades and new functionalities:
  - Single repository of all markers (application neutral)
  - Collection of individual reference cases, not cumulative percentiles
  - Collection of covariate information for all cases (BW, GA, age)
  - Ability to create and apply complex ratios and equations
  - Adjustment of results for one or more covariates to reduce the overlap between reference and disease ranges

R4S 2.0 Goes “Big Data”



Participating sites	**55**
Countries	**24**
Data allocation	**Repository**
Ref. data points	**108,870,757**
Covariates	**4 (for NBS)**
Cov. data points	**3,766,621**
TP cases	**6,827**
TP data points	**489,618**
FP cases	**194**

Is R4S Applicable to Other Tests?

- New tests and/or platforms in newborn screening
- Old screening tests (with poor performance)
- Other tests generating numerical data, especially if/when combined in complex profiles
  - Biochemical Genetics
  - Pediatric laboratory medicine, basic and esoteric
  - Collaborative research projects
  - Clinical trials

Minnesota NBS Performance in DBS

(N=209,432; Period: 2008-2010)

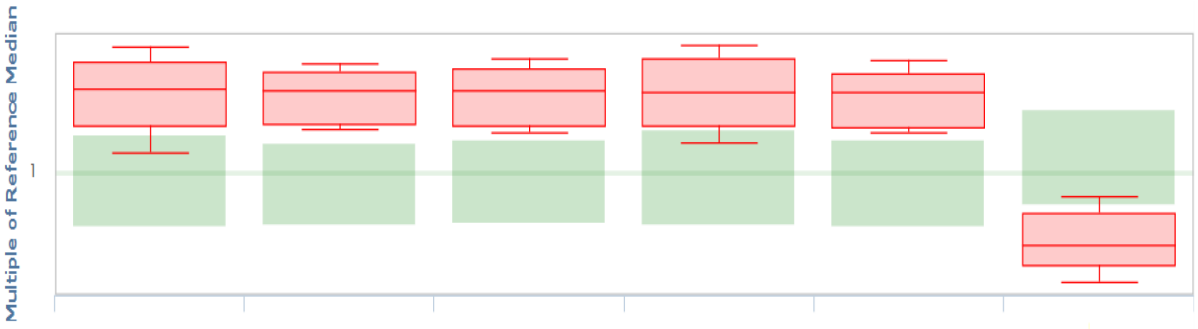
	Conditions UP	Conditions ST	FPR	MN FP/wk	CA (560k)	USA (4.2M)
IEM (MS/MS)	20	22	0.05%	0.7	5.4	40.4
Biotinidase	1	0	0.09%	1.2	9.7	72.7
CAH	1	1	0.11%	1.5	11.8	88.8
Hypothyroidism	1	0	**0.21%**	2.9	22.6	**169.6**
Cystic Fibrosis	1	0	**0.34%**	4.7	36.6	**274.6**
Galactosemia	1	2	0.06%	0.8	6.5	48.5
Hbpathies	3	(1)	0.02%	0.3	2.2	16.2
TOTAL	**28**	**26**	**0.88%**	12	95	711

R4S for Other NBS Tests

Condition	Marker	TN	TP	FP
Hypothyroidism	TSH	60,598	191	139
Cystic Fibrosis	IRT	60,598	239	109
CAH	17OHP	60,598	96	410
Galactosemia	GALT	60,598	113	91
Biotinidase	BIOT	60,598	0	0
SCID	TRECs	60,598	0	0

Data provided by Bob Currier and Hao Tang, courtesy of the California Department of Public Health

Could R4S Improve the Specificity of CH screening?



INFORMATIVE
Score CH Tool

Evaluation	Rules	Adjust.	TP	FP	Delta
Count			191	139	0

R4S tools could have prevented 58% of 139 false positive cases based on TSH cutoff alone

Conclusions

- From an analytical perspective, future expansions of the NBS panel should be driven by (much) better specificity
- "Old" tests should be improved as well, best if done first
- The goal of 100+ conditions causing <100 FP cases/day in the US is likely attainable with increased reliance on post-analytical interpretive tools based on large scale data sharing and worldwide collaboration
- R4S has provided a blueprint for future activities. CLIR 2.0 website (https://clir.mayo.edu/) go live in 4Q2014
- Got profiles?
- Built-in adjustment(s) for 1+ covariates could be an alternative to conventional reference ranges (age-matched)
- To participate in CLIR, send e-mail to rinaldo@mayo.edu

Thank You for Your Attention

"Today the only thing that is permanent is change"

Charles H. Mayo, MD (1919)