Issues Surrounding the NeoBase non-derivatized MS/MS Kits: Acylcarnitines FC (C0) and C3DC

What to do?

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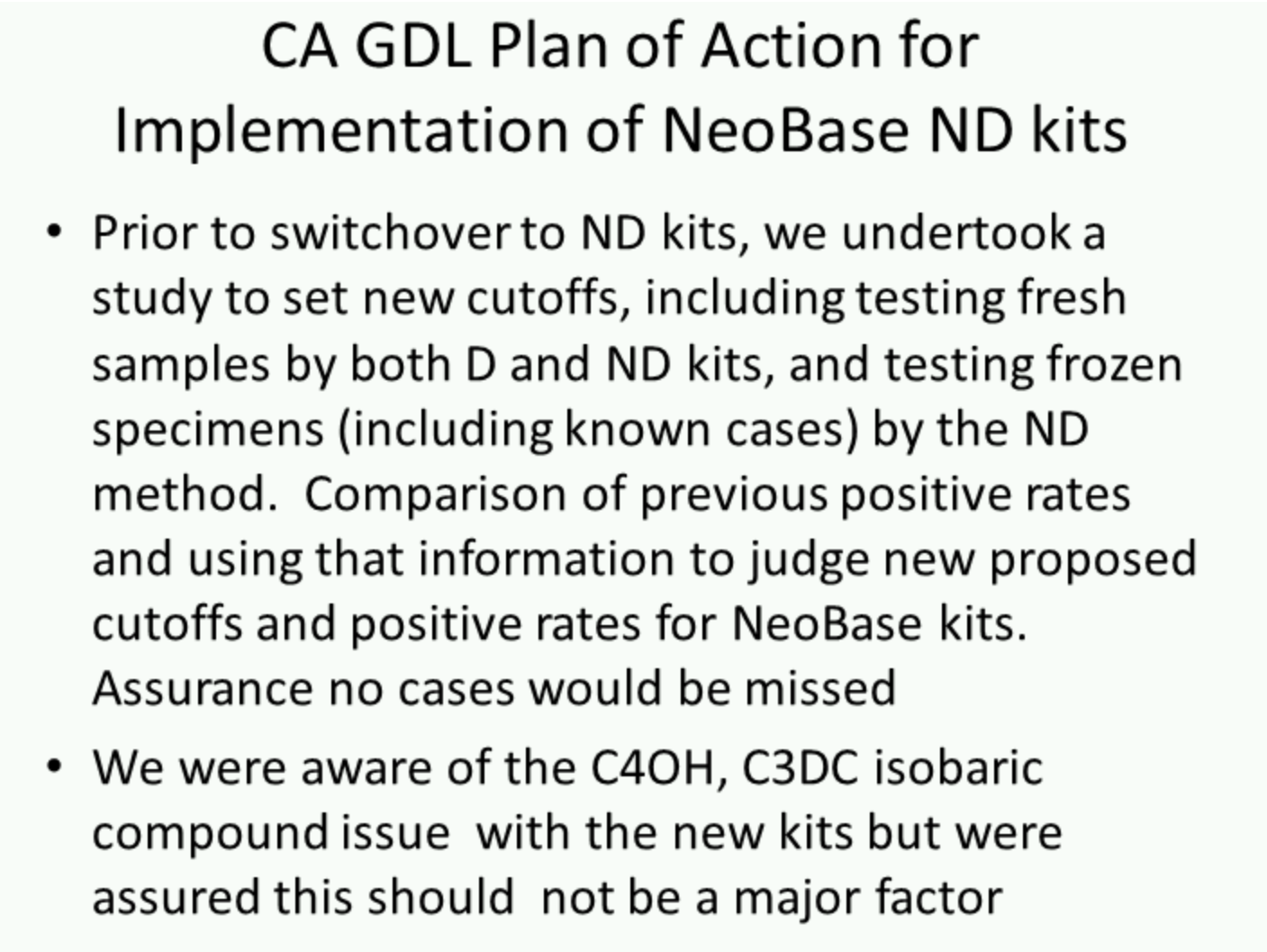


CA GDL Plan of Action for Implementation of NeoBase ND kits

Cases of Malonic Acidemia

Analyte	C3DC (DER)	C3DC NB (ND)	C3DC/C10
C03DC	4.08	0.46	5.44
C03DC	5.32	0.69	6.9
C03DC	3.3	.41	5.86
C03DC	(0.44*)	0.53	6.63

* Tested with NB on fresh sample

Cases of Malonic Acidemia

Overview:

Issues associated with introduction of the ND NeoBase tandem MS kits

- Extremely high and unacceptable number of false positives for C3DC (MAL)
- Loud screaming associated with f.p. rate for C3DC from follow up staff and metabolic specialists

- A similar very high false positive rate with free carnitine low cutoffs (C0)
- Resulting concern with missing MAL cases
- Sudden multiple failures in CDC PT for C3DC (false negatives, low values)

Overview: Issues associated with introduction of the ND NeoBase tandem MS kits

REALITY BITES

- Switch-over to NB in December 2009
- By second day we received hundreds of calls regarding excessive f.p for C3DC and FC (low)
- Due to urgency we had to implement a quick and dirty cutoff change:
  - FC (low) from 7.5 to 7.0 (prev derivitized had been **12.0**)
  - C3DC from 0.4 to 0.45 (prev derivatized had been **0.3)**

REALITY BITES

WHAT NEXT?

- IT TOOK A FEW DAYS TO CHANGE THESE CUTOFFS. IT SIGNIFICANTLY LOWERED THE F.P. RATES AND THE DECIBELS OF CALLS, BUT THEY WERE STILL TOO HIGH, PARTICULARLY C3DC.

- ALSO AS AN INTERIM CHANGE WHILE WE STUDIED FURTHER, WE AGREED TO RAISE C3DC TO 0.50

- WE ALL STARTED TAKING ANTACIDS....

WHAT NEXT?

AS LUCK WOULD HAVE IT

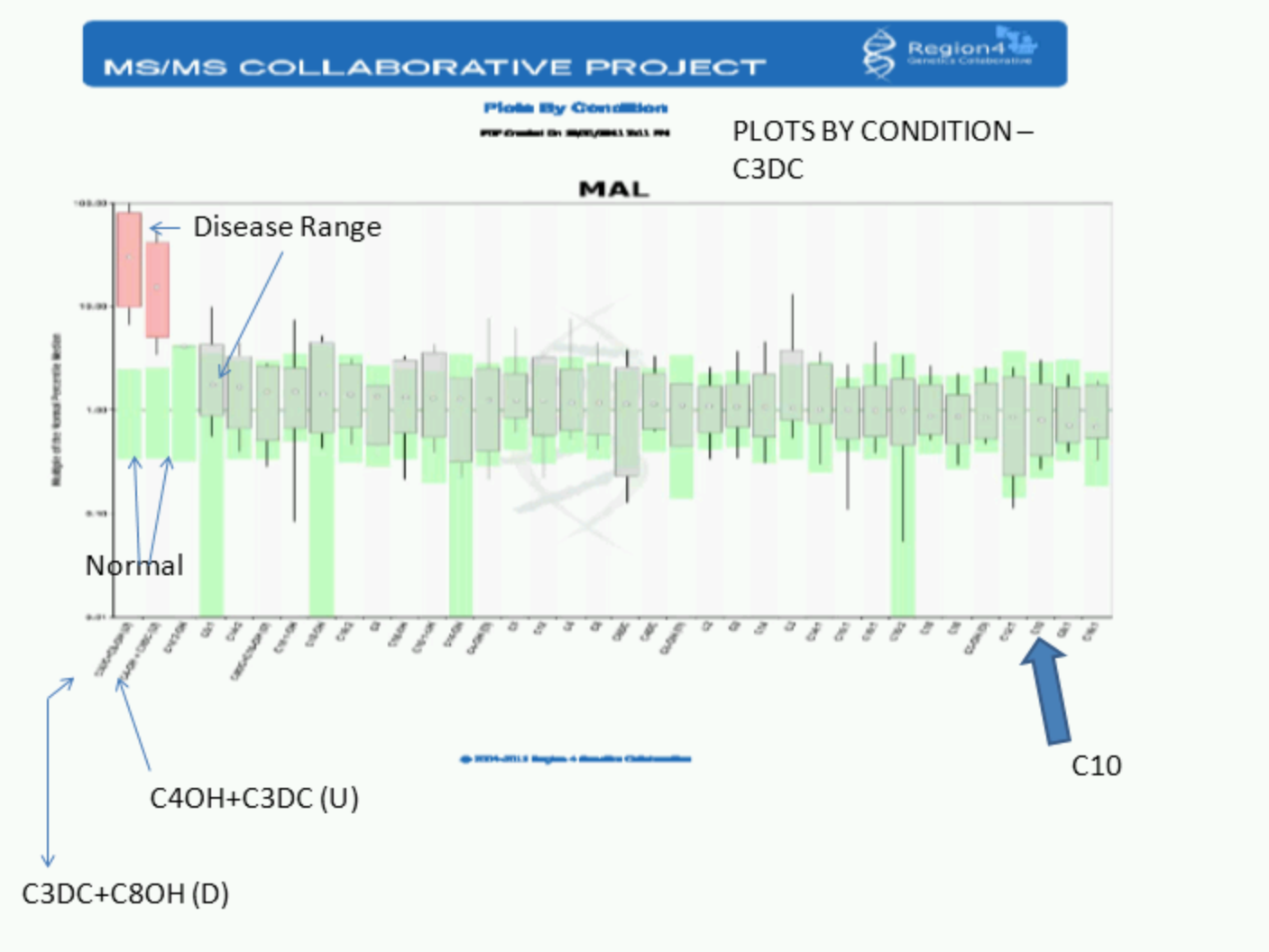
- During the few days before the new cutoffs were changed, a confirmed case of Malonic acidemia was diagnosed by a specialist. Child was already symptomatic.
- C3DC VALUE - 0.44 –Only reason we caught it was because the 0.45 cutoff change hadn't been implemented yet. And yet we were planning another increase to 0.5.
- BACK TO THE DRAWING BOARD

AS LUCK WOULD HAVE IT

CONSULTATION –C3DC

- Obviously no further cutoff changes could be considered. In fact we decided to take the risk of increasing C3DC cutoff to 0.5 temporarily due to low prevalence of the disorder while we implemented new software.
- Consulted R4S ms/ms database and Dr. Rinaldo for implementing a more sophisticated algorithm that included a ratio to keep f.p. rate down and keep C3DC cutoff lower.
- Since no good high/low ratio existed (like phe/tyr) the next best option was to find a high/normal ratio
- Looking at plots by condition in R4S, C10 appears to be a good candidate as a normal denominator for the ratio because:
  - Disease range and normal range are almost the same, and
  - The range is relatively narrow
  - Peaks of C3DC and C10 relatively close
  - Of the other analytes considered in that category (C12:1, C8:1, C16:1), C10 is the analyte run by most laboratories.

CONSULTATION –C3DC


MS/MS COLLABORATIVE PROJECT
Region4
PLOTS BY CONDITION – C3DC
MAL
Disease Range
Normal
C4OH+C3DC (U)
C3DC+C8OH (D)
C10

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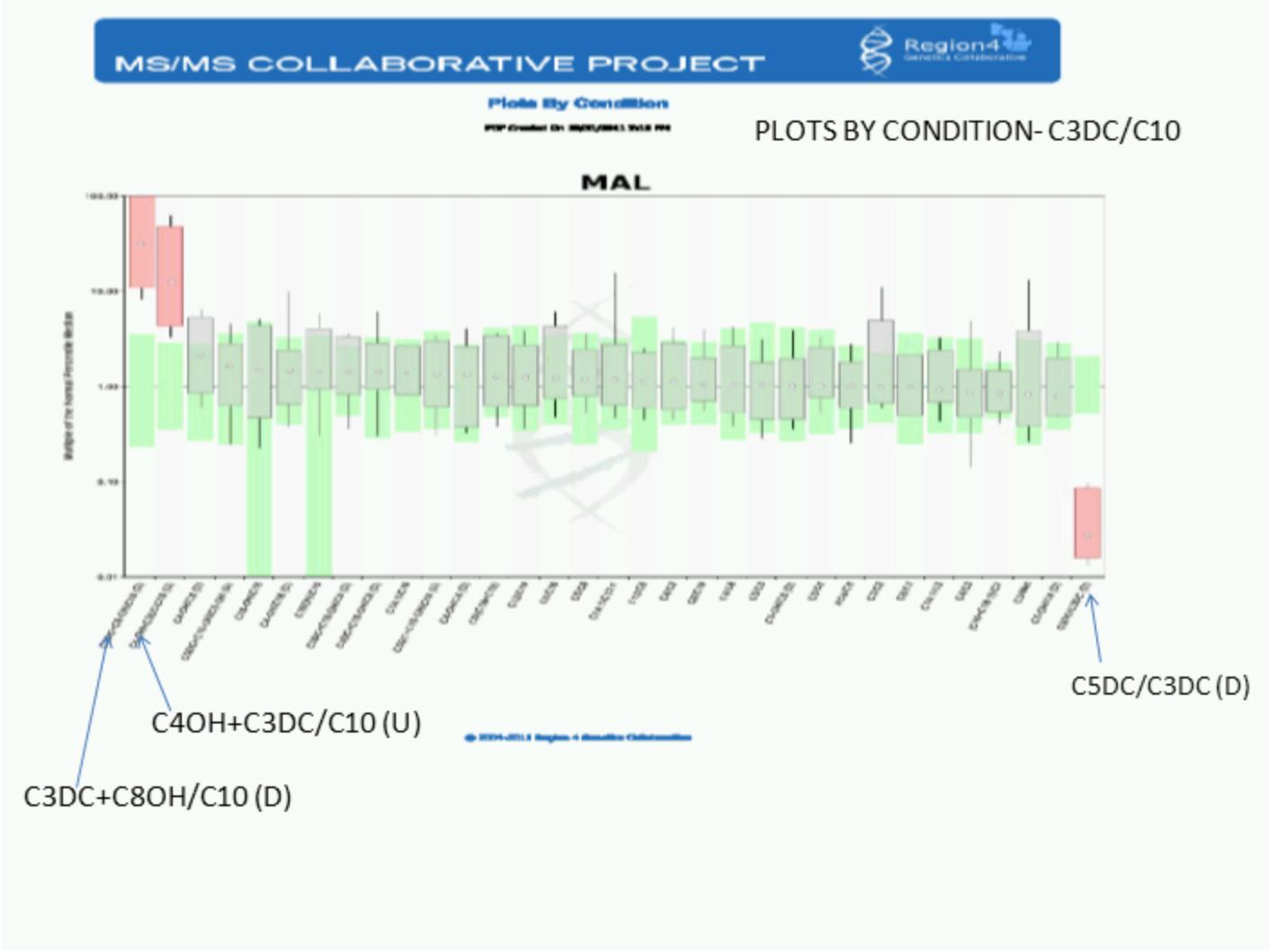
MS/MS COLLABORATIVE PROJECT
Region4
Plots By Condition
PLOTS BY CONDITION- C3DC/C10
MAL
C5DC/C3DC (D)
C4OH+C3DC/C10 (U)
C3DC+C8OH/C10 (D)

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CTD (CUD) CASES DETECTED SINCE CUTOFF LOWERED TO 7.0

CLEARLY ADDITIONAL ALGORITHM OR TOOL DEVELOPED NEEDED TO LOWER F.P. RATE FURTHER

	Case Reliability	FC Value
	Confirmed	4.4
	Confirmed	4.4
	Confirmed	5
	Confirmed	5.2
	Confirmed	5.3
	Probable	5.8
	Probable	6.1
	Probable	6.1
	Probable	6.5
	Confirmed	6.6
	Probable	6.7
	Probable	6.7
	Probable	6.7
	Confirmed	6.7
	Confirmed	6.9
	Probable	7

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Presumptive Positive Rates of CTD (CUD) and MAL

		Derivatized: < 12/12/2009	INITIAL ND IMPLEMENTATION 12/2009-1/2010	(ND) QUICK ADJUSTMENT 1/2010-7/2010	ND 2nd ADJUSTMENT 7/2010-8/2010	ND Implementation of C3DC/C10 R >8/4/2010
CTD	Cutoff (FC)	12 (D)	7.5	7.1	7	
	PP Rate	0.0208%	0.1622%	0.0554%	.03%	
MAL	Cutoff (C3DC)	0.3 (D)	0.4	0.45	0.5	0.4
	Cutoff (Ratio (C3DC/10)	--	--	--	--	5
	PP Rate	0.0055%	0.1387%	0.0868%	0.0157%	0.0136%

(D): Derivitized
(ND): Non-Derivatized

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CDC PROFICIENCY TESTING FOR C3DC

NEXT ISSUE: HOW DOES THE ND NEOBASE ISSUE AFFECT THE CDC PROFICIENCY TESTING SAMPLES FOR C3DC?

CAST OF ~~CHARACTERS~~, EXPERTS

NEXT ISSUE: HOW DOES THE ND NEOBASE ISSUE AFFECT THE CDC PROFICIENCY TESTING SAMPLES FOR C3DC?

ANALYSIS OF CHANGING THE IS FOR C3DC FROM C4 TO C5DC

AFTER CONSULTATION WITH CDC PT STAFF, IT WAS SUGGESTED THAT PART OF THE PT TESTING FAILURES, IN SPITE OF A SUCCESSFUL SCREENING ALGORITHM FOR MAL and DESPITE SOME KNOWN ISSUES WITH THE ND KIT, MIGHT BE DUE in part TO USING C4 AS OUR INTERNAL STANDARD (IS) AND WE SHOULD TRY USING C5DC AS THE IS. WE RAN 200 PT SAMPLE USING BOTH C4 and C5DC as IS

CDC Suggestions:

*Chemical structure similarity between C3DC and C5DC may result in similar ionization efficiency in the MS source - use of C5DC IS may account for this difference during semi-quantitation

*Endogenous levels of C4OH higher than C3DC, thus effect is masked -

*C5DC IS may used in derivatized assay as IS for C3DC semi-quantitation with proper algorithms. Can also be used in ND assay for more robust values.

* 4 confirmed MAL cases sent to CDC blinded with controls – all easily detected

Confirmed Cases of Malonic Aciduria Tested by NeoBase with two different IS

Case No.	C10	C3DC (C4IS)	C3DCR (C5DC IS)	R03D10 (C4 IS)	R03DR10 (C5DC IS)
Case 1	0.09	0.49	1.96	5.44	21.78
Case 2	0.1	0.69	3.23	6.90	32.30
Case 3	0.07	0.41	1.54	5.86	22.00
Case 4	0.08	0.53	2.08	6.63	26.00

ANALYSIS OF CHANGING THE IS FOR C3DC FROM C4 TO C5DC

Changes in CDC PT results when changing internal standard

Period	No. of Specimen	Expected		C3DC with C4 IS		C3DC with C5DC IS	
		Positive	Negative	False Positive	False Negative	False Positive	False Negative
2010-Q1	40	0	40	8	0	14	0
2010-Q3	40	8	32	8	**8**	13	1
2010-Q4	40	8	32	0	**8**	1	**0**
2011-Q1	40	8	32	0	**8**	0	**0**
2011-Q3	40	0	40	16	no positives sent	17	**0**

Changes in CDC PT results when changing internal standard

Summary

- Excessive false positive rates for C3DC and FC in ND Neobase kits
- C3DC requires more sophisticated algorithm than a simple cutoff to adequately deal with screening for MAL. C3DC + C3DC/C10 works.
- The low readings have led to multiple P.T. failures (false negative) among labs using ND.
- Switch to a C5DC IS for C3DC can correct the PT testing issue.
- The possibility exists that PT testing in the future may need to include either determination and/or ratio testing if these problems continue.
- FC problem can be ameliorated by refined lower cutoffs to a point, but requires further study.

Summary