Acylcarnitine Analysis using the Perkin Elmer Neobase Kit

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- **2007**

  Work on an in-house method for acylcarnitines (Waters, Quattro Micro)

  However.....

  - Newborn Screening at Alder Hey using PE Kit (Neogram)

  - ? Usage and stability of CIL Acylcarnitine/Amino Acid Internal Standard

- **MCADD Screening introduced July 2008**

  Full scan analysis required as part of newborn screening follow-up

  Decision made to develop a diagnostic service for acylcarnitines using the PE *non-derivatised Neobase Kit.*

- **1st July 2009**

  First diagnostic samples reported on by Alder Hey

The Assay

- Non-Derivastised.
- Kit contains Internal Standards and QC for :

  C0, C2, C3, C4, C5, C5DC, C6, C8, C10, C12, C14, C16 and C18

  (and amino acids)

1. Reconstitute Internal Standard in Extraction Solution (stable for 30 days).
2. Working solution prepared daily.
3. One 3.2mm bloodspot punch per well.
4. 100 ul working solution added (IS and extraction solution).
5. Plate covered and incubated for 45 min at 45^{o}C with shaking.
6. MS/MS Analysis.

Developing the Knowledge

- Comparisons of numerical data and ‘interpretation’ with current provider.
- Collation of in-house population data.
- CDC quantitative EQA.
- ERNDIM Interpretative Scheme.
- Case-based learning exercise.

Introduced a tiered approach :

- Cut-offs for referral based on comparison data and population limits.
- Results either reported as normal * or sent to external laboratory for analysis.
- Review of results.

- An audit 9 months after this service was introduced showed we were only sending away 39% of the samples we had been previously. About half of these were being reported as ‘normal’.

- Post Mortem samples, urine and plasma sent away for analysis.

Developments :

Introduced use of C3/C2 and C16/C2 ratios.

Further ERNDIM Interpretative Samples.

Revised **population limits.**

Collection of post-mortem data.

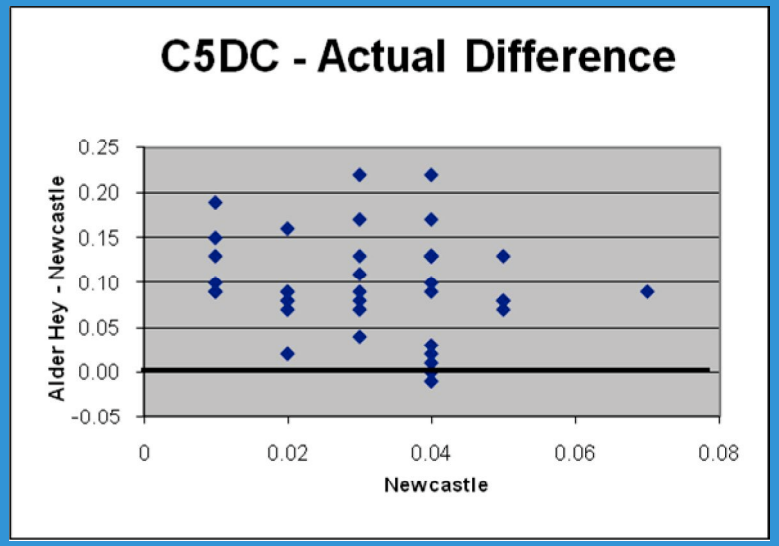
A continuing learning curve and adding to knowledge.....

Lessons from EQA

- **Positive bias for C5DC :**

- Neobase contains an IS for C5DC.

Target	Neobase Group	Alder Hey
0.03	0.05	0.07
0.02	0.11	0.11
0.03	0.11	0.21
2.1	2.18	3.51
0.08	0.06	0.06
0.03	0.05	0.11
0.2	0.11	0.22
2.1	2.26	4.32
0.02	0.07	0.15



- Also high for Neobase kit QC.

- Alder Hey Population Range 0.04 – 0.26 (CDC cut-off 0.35)
- Potential for over interpretation rather than missing a case.

Lessons from EQA

Significant positive bias seen for C4-OH (TV= 0.07, Alder Hey = 0.47) :

- Positive bias only seen for 1 out of 5 samples, and the Neobase group mean was also high.
- This sample contained C3DC.
- **Isobaric Interference between C4-OH and C3DC.**

  - C4-OH and C3DC share the same m/z ratio of 248.1 on the non-derivastised assay therefore both contribute to the intensity at 248.
  - The two cannot be distinguished and an elevated level would need to be confirmed using a derivatised assay.

- **Also get isobaric interference between e.g. C4DC in MMA and C5-OH.**
- **? high C5 may interfere in C4-OH/C3DC (M+2 isotopic interference)**

Lessons from EQA

Negative Bias for C8 and C10 :

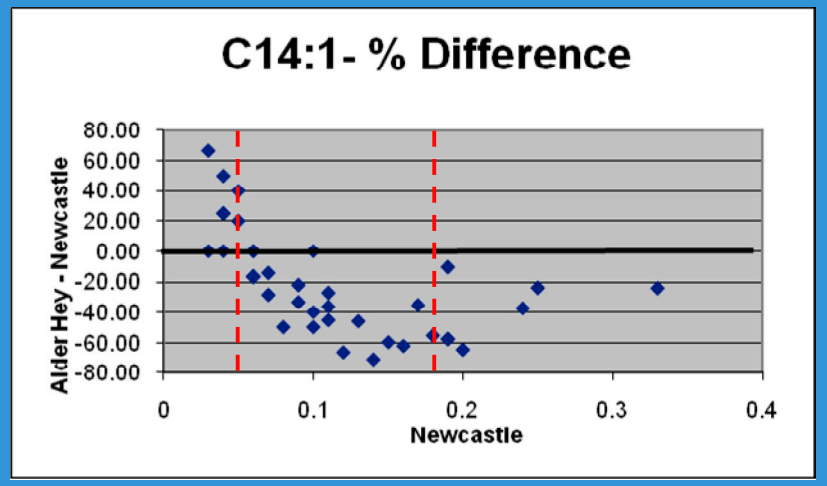
- Noticeable on CDC but would not have missed MCADD.
- C8 good on NEQAS although C10 does show negative bias.

- **For Newborn Screening instructed to calibrate C10 with C8 IS.**
  - ? Rationale.
  - Do other labs do this ?

Lessons from EQA

? Concentration dependent bias for C14:1 :

Target Value	Alder Hey
0.03	0.12
0.06	0.13
0.04	0.19
7.56	6.37



- Neobase mean on target at low levels. High level Neobase mean lower.
- ? Issue of sensitivity.
- Possible false negatives but ? ↑↑ in disease.
- ? Drug interference in C14:1.
- ? Low risk of over-interpretation ? other acylcarnitines always high in VLCAD.

Lessons from EQA

‘Cut-Offs’....Significant Limits v. Population Limits :

- Using population ranges rather than ‘significant limits’ means Alder Hey ‘cut-offs’ tend to be lower than CDC and other labs, however in general ‘interpretation’ agrees with the consensus.

- ? More potential for over interpretation and referral rather than missing a significant finding using population limits rather than significant limits.

- Cases of carnitine deficiency have also been correctly identified.

	Population Range
C0	11.7 – 55.8
C2	2.28 – 27.4
C3	0.27 – 1.87
C4	0.10 – 0.52
C4-OH	0.03 – 0.32
C5	0.04 – 0.25
C5:1	0.00 – 0.05
C5DC	0.04 – 0.26
C5-OH	0.19 – 0.74
C6	0.02 – 0.55
C6DC	0.02 – 0.17
C8	0.01 – 0.09
C8:1	0.02 – 0.35
C10	0.02 – 0.17
C10:1	0.03 – 0.15
C10:2	0.00 – 0.04
C12	0.01 – 0.12
C12:1	0.04 – 0.19

	Population Range
C14	0.04 – 0.19
C14:1	0.04 – 0.16
C14:2	0.01 – 0.04
C14-OH	0.00 – 0.02
C16	0.35 – 2.44
C16:1	0.02 – 0.20
C16:1-OH	0.01 – 0.10
C16-OH	0.01 – 0.10
C18	0.16 – 0.93
C18:1	0.39 – 1.80
C18:1-OH	0.01 – 0.04
C18:2	0.11 – 0.52
C18-OH	0.00 – 0.02
C3/C2	0.04 – 0.23
C3/C16	0.25 – 3.08
C8/C10	0.33 – 1.24
C16/C2	0.03 – 0.45

Normal QC

		Alder Hey
C0	98 – 166	**122 – 146**
C2	56.8 – 95.6	**55.2 – 71.8**
C3	8.9 – 14.9	**9.3 – 12.1**
C4	2.17 – 3.69	**2.37 – 2.86**
C4-OH		**0.08 – 0.11**
C5	0.75 – 1.31	**0.85 – 1.09**
C5:1		**0.01 – 0.03**
C5DC	0.43 – 0.83	**0.95 – 1.93**
C5-OH		**0.64 – 0.91**
C6	0.49 – 0.89	**0.59 – 0.75**
C6DC		**0.05 – 0.10**
C8	0.43 – 0.83	**0.47 – 0.62**
C8:1		
C10	0.64 – 1.16	**0.72 – 0.88**
C10:1		**0.04 – 0.11**
C10:2		**0.00 – 0.02**
C12	1.47 – 2.47	**1.65 – 2.02**
C12:1		**0.12 – 0.16**
C14	1.41 – 2.37	**1.75 – 2.24**
C14:1		**0.07 – 0.10**
C14:2		**0.01 – 0.03**
C14-OH		**0.00 – 0.01**
C16	9.7 – 16.5	**10.2 – 13.2**
C16:1		**0.07 – 0.10**
C16:1-OH		**0.09 – 0.13**
C16-OH		**0.01 – 0.03**
C18	1.94 – 3.26	**2.23 – 2.99**
C18:1		**1.39 – 1.88**
C18:1-OH		**0.22 – 0.35**
C18:2		**0.34 – 0.54**
C18-OH		**0.01 – 0.03**

Abnormal QC

		Alder Hey
C0	209 – 357	**257 – 328**
C2	130.7 – 219.5	**129 – 162**
C3	21.2 – 36.0	**21.9 – 28.6**
C4	5.45 – 9.21	**6.18 – 7.40**
C4-OH		**0.09 – 0.16**
C5	1.81 – 3.17	**2.01 – 2.66**
C5:1		**0.01 – 0.04**
C5DC	1.03 – 1.91	**2.96 – 4.70**
C5-OH		**0.66 – 0.90**
C6	1.26 – 2.30	**1.45 – 1.89**
C6DC		**0.10 – 0.19**
C8	1.10 – 2.06	**1.21 – 1.63**
C8:1		
C10	1.63 – 1.95	**1.77 – 2.25**
C10:1		**0.07 – 0.12**
C10:2		**0.00 – 0.02**
C12	3.82 – 6.46	**4.19 – 5.23**
C12:1		**0.21 – 0.32**
C14	3.51 – 5.87	**4.47 – 5.33**
C14:1		**0.04 – 0.14**
C14:2		**0.01 – 0.03**
C14-OH		**0.01 – 0.03**
C16	23.8 – 39.8	**25.7 – 30.6**
C16:1		**0.07 – 0.11**
C16:1-OH		**0.17 – 0.26**
C16-OH		**0.02 – 0.07**
C18	3.84 – 6.48	**4.52 – 5.60**
C18:1		**1.29 – 1.79**
C18:1-OH		**0.61 – 0.87**
C18:2		**0.36 – 0.49**
C18-OH		**0.02 – 0.05**