ERNDIM

QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

Acylcarnitines in DBS

Charles Turner-London

ERNDIM Workshop, 21st & 22nd November 2017, Manchester, UK

Acylcarnitine analysis
ERNDIM Qualitative QA scheme

2002 ERNDIM users canvassed

Over 30 laboratories expressed an interest

By the dispatch date, 4th April 2003 45 laboratories registered

Problems of blood volume and sample transport

scheme for plasma/serum excluded

Blood spots from real clinical cases

Education & Interpretation

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Acylcarnitine measurement in blood spots ERNDIM Qualitative QA scheme

2003-2009 London Charles Turner/Neil Dalton

Grew from 45 to >120 registered participants (2017:122)

2010 split between two centres, 60+ participants in each centre

Heidelberg: Claus-Dieter Langhans

2017 Third centre added, 40+ participants in each centre

Zurich: Ralph Fingerhut

Acylcarnitine measurement in blood spots ERNDIM Qualitative QA scheme

Country (s)	Participants
FRANCE, UNITED KINGDOM	15
GERMANY	10
ITALY	9
BELGIUM, SPAIN	6
NETHERLANDS	5
CANADA, TURKEY	4
ARGENTINA, AUSTRALIA, CHINA, SWITZERLAND	3
CZECH REPUBLIC, ISRAEL, MALAYSIA, PORTUGAL	2
AUSTRIA, BRAZIL, BULGARIA, CHILE, CROATIA, ESTONIA, GREECE, HONG KONG S.A.R., INDIA, IRELAND, KINGDOM of SAUDI ARABIA, LEBANON, LITHUANIA, LUXEMBOURG, MEXICO, MOROCCO, NEW ZEALAND, POLAND, REPUBLIC OF SINGAPORE, RUSSIA, SLOVAKIA, SLOVENIA, SOUTH AFRICA, SULTANATE OF OMAN, TAIWAN, UNITED ARAB EMIRATES	1

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Acylcarnitine measurement in blood spots ERNDIM QA scheme London

Evelina London Children's Hospital

Patients

- Presenting to the the metabolic service at Guy's Hospital (now Evelina London Childrens Hospital, St Thomas's)
- Diagnoses confirmed by enzymology or DNA analysis
- Monitoring/diagnostic blood sampling – informed consent (patient/parent) obtained for use of excess blood for quality control purposes – authorised by the Guy's & St Thomas' Hospital Ethical Committee
- Increasing numbers of adult samples
- Scheme participants encouraged to submit samples
  - 7 donors so far

Acylcarnitine measurement in blood spots
ERNDIM QA scheme

Samples

Lithium heparin anti-coagulated whole blood
40µl aliquots spotted onto Perkin Elmer 226 paper
Dried for 24h at RT before packing and despatch
Any delay stored dry at –80^{o}C

Current requirement for 1 circulation:
2.0-3.0 ml liquid whole blood
50+ dried blood spots

Reporting

Basic clinical information is given with each sample

Users are asked to analyse the sample in the normal way of their laboratory

Report as they would to a non-specialist paediatrician but within a structured report format

- **Major analytical findings (qualitative)***
- **Relevant quantitative data (optional)**
- **The most likely diagnosis (one only)***
- **Other possible diagnoses (if applicable)***
- **Further investigations required to confirm/clarify the diagnosis***
- **Clinical information/advice**
- **Any additional comments**

Report by email: website reporting as for DPT scheme is coming…..

ERNDIM Qualitative schemes: scoring and “Critical Error”

Scoring

four point system (+1, +2, + 3, +4) as used in the DPT schemes. In this system a maximum of two points each is given for analytical results and interpretation, with the latter including suggestions for further testing/ actions. The total score achievable for a single circulation of three samples is twelve and twenty four for the whole sample set of six samples per year.

To obtain satisfactory performance a score of 16/24 or more should be achieved on two returns without critical error. The SAB has agreed that this will increase to 17/24 (70%) in 2018

“critical error” is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient

A sample can only be defined as generating a critical error if a competent laboratory would be expected to find the key metabolite(s) and the interpretation is clear. Critical errors have to be agreed by the whole ERNDIM Scientific Advisory Board, not just the scheme advisor/organiser

Example scoring

Sample from a patient with methylmalonic acidaemia e.g London 2017.A: 12y old female, renal failure, episode of hyperammonaemia & acidosis, currently well

Extremely raised C3 carnitine, borderline C4DC (methylmalonylcarnitine)

- **Major analytical findings (qualitative)***
  - Must comment on raised propionyl carnitine. If borderline C4DC is reported as unequivocally normal and excluding MMA then a point will be lost
- **Relevant quantitative data (optional)**
  - And/or highlight quantitatively raised C3 or ratios

+2 for analytical findings

- **The most likely diagnosis (one only)***
  - Methylmalonic acidaemia alone if C4DC is considered high or second line tests already performed on DBS, propionic acidaemia acceptable if
- **Other possible diagnoses (if applicable)***
  - Methylmalonic acidaemia is included as a possible diagnosis and

+1 for interpretation

- **Further investigations required to confirm/clarify the diagnosis***
  - Further investigations would clarify e.g MMA/3OHpropionate/methyl citrate on blood spot, plasma, urine organic acid analysis. A good response might include plasma ammonia, total homocysteine, amino acids and mutation analysis of appropriate genes.

+1 for interpretation: can be clarified in clinical advice/further comments

ERNDIM Qualitative schemes: scoring and “Educational sample”

Educational Samples:

Samples where the metabolite abnormality is insufficiently clear for a competent laboratory to be reasonably expected to suggest the correct diagnosis on first sight.

May be an unusual profile for a known disorder or a newly described disorder : circulated to alert laboratories to the variability in possible profiles e.g. GA-1 non secretor

Educational samples are agreed by the Scientific Advisory Board and excluded from the scoring for the year.

If a large number of laboratories fail to identify a particular sample it alerts the advisor to widespread difficulty and may result in a sample being declared an “educational sample”.

If it is considered that the abnormality could have been detected, but was not part of normal practice on first circulation, it would be expected that laboratories would change their practice and a sample deemed “educational” on first circulation might be scored on a subsequent round

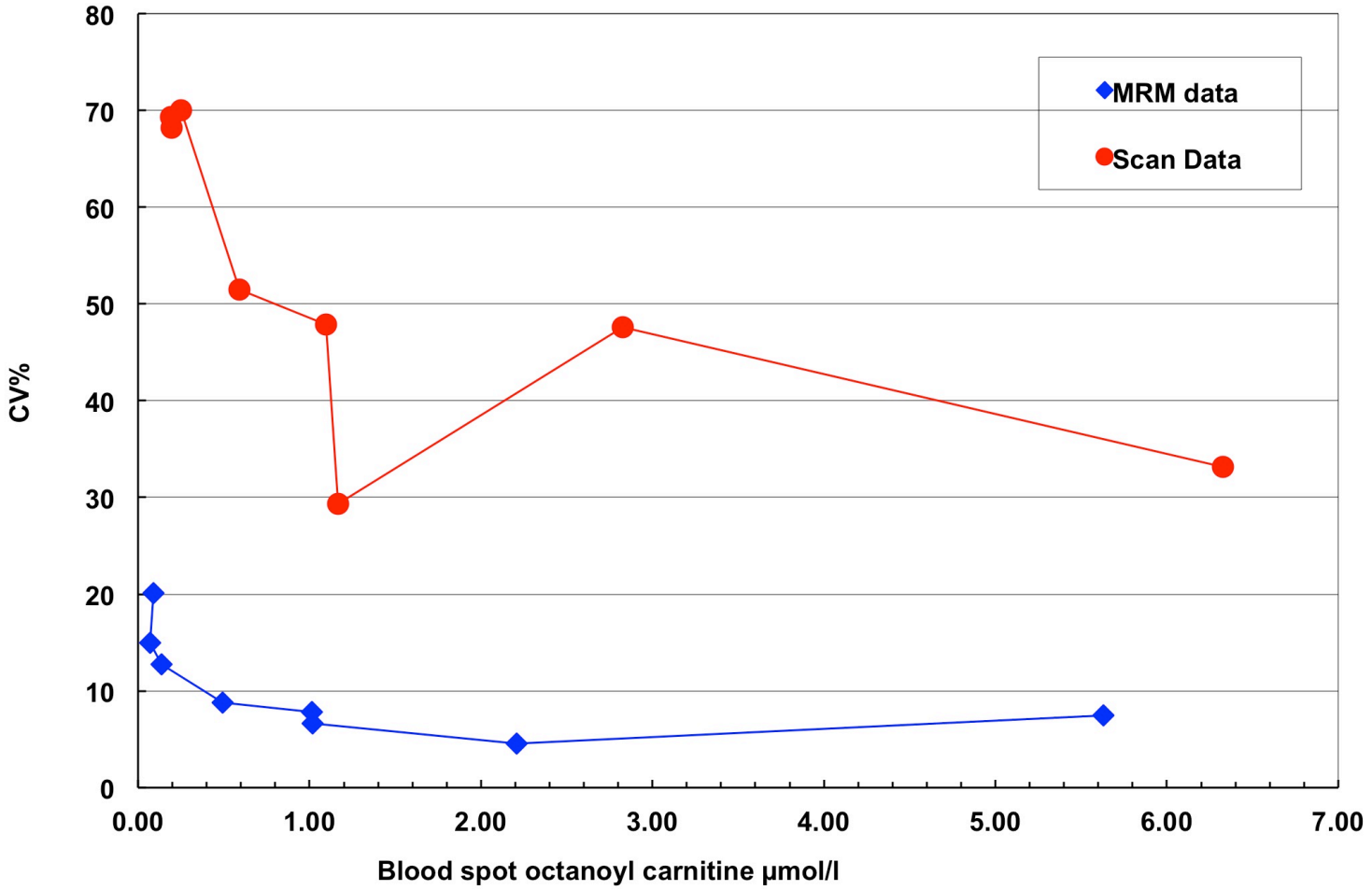
Acylcarnitine analysis

Electrospray MSMS of butylated or underivatised methanolic extracts

- Flow injection analysis
- Precursor ion scan, m/z 85

- MRM acquisitions for specific acylcarnitines & quantitation

Why use MRM acquisition for quantitation?



Acylcarnitine analysis

- MRM acquisitions also allow secondary specific product ions for confidence and separation of isobarics
  - m/z 162/102 for free carnitine

- Increasing sensitivity of modern MSMS instruments brings low abundance product ions into range
  - m/z 276/199 for C5DC
  - m/z 262/145 for C5OH

Acylcarnitine analysis

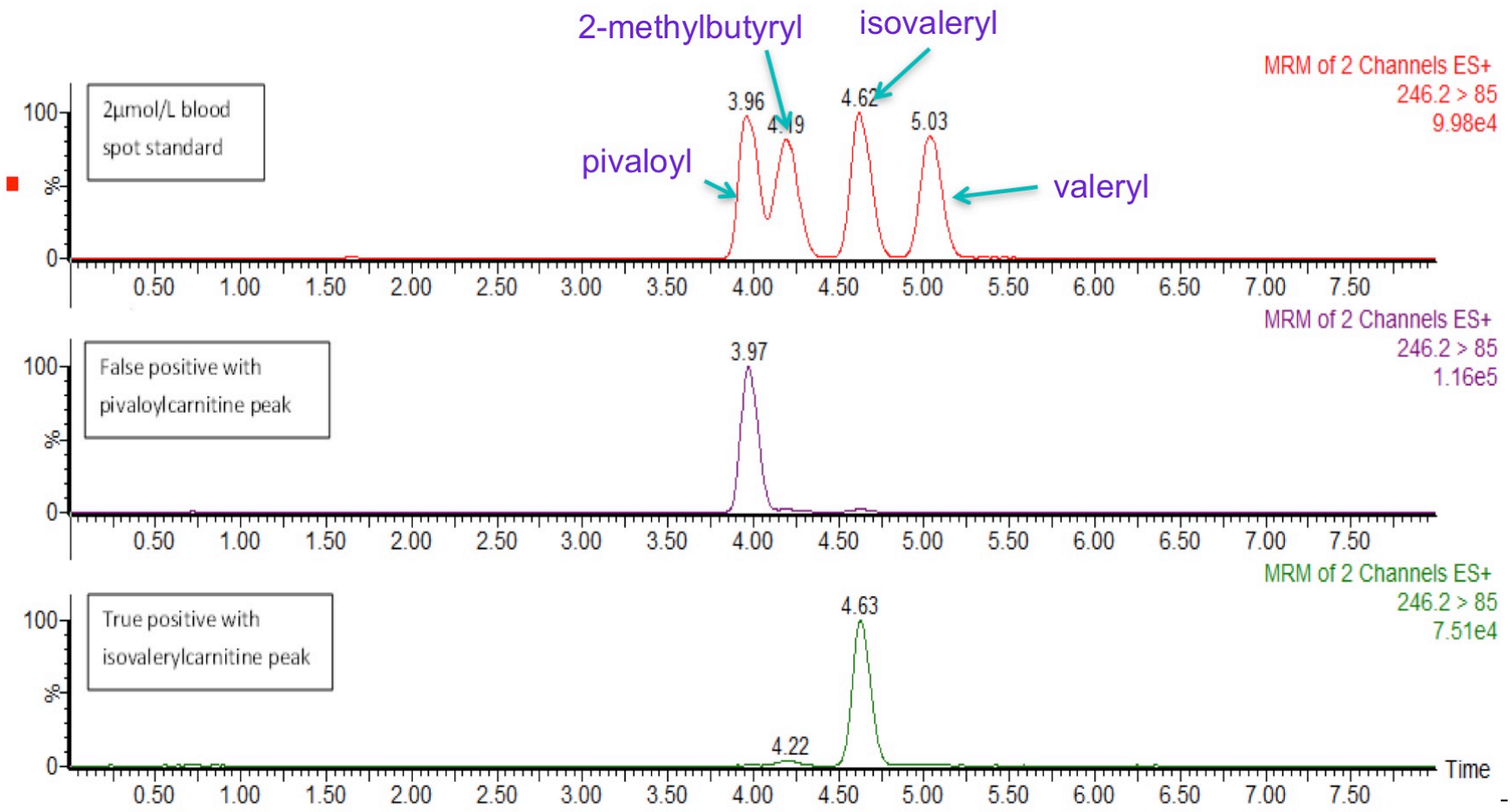
- Increasing use of chromatography in 1st or 2nd line test
  - MMA, methylcitrate, 3OHpropionate, total homocysteine
  - Chromatographic separation of isobarics e.g.C5
- Incorporation into multiplex assays

C5 isobarics: second line test for newborn screening

with thanks to Dr Rachel Carling

viapath

Chromatographic separation of the isomers



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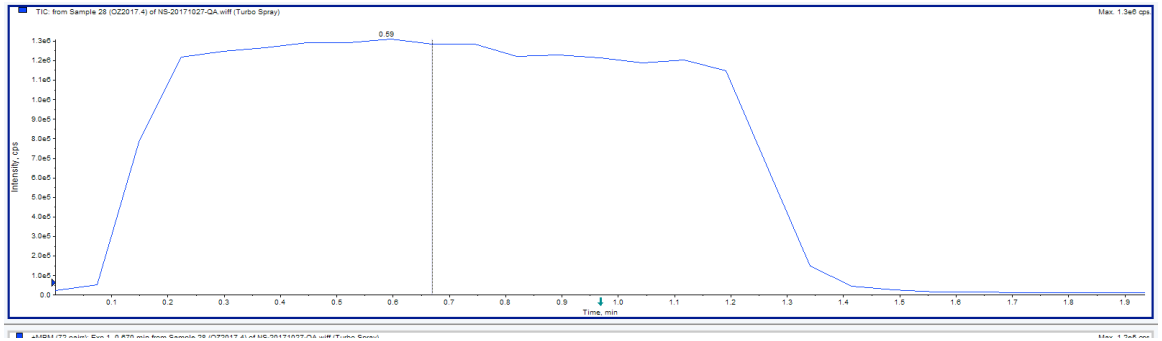
Discussion Points

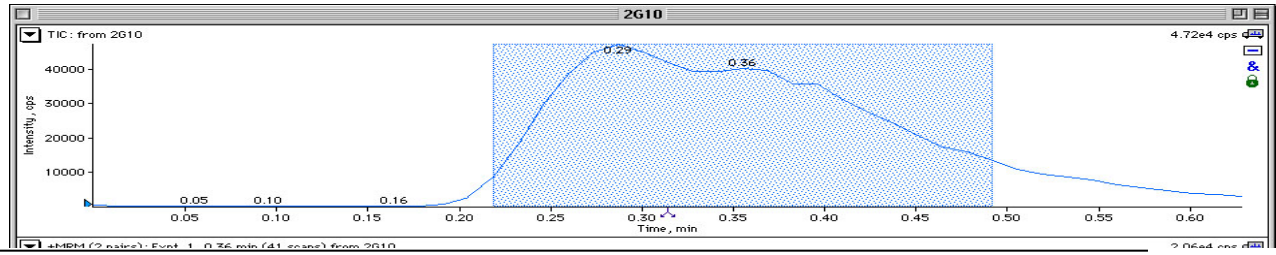
Precursor Ion Scan vs MRM

Flow injection vs Chromatography

Inject-inject time (how many scans across the peak)

“Flat top” TIC vs smooth peak





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Acylcarnitine measurement in blood spots
ERNDIM QA scheme
Methodological aspects: Underivatised v Butylation

Advantages of derivatised	Advantages of underivatised
Isobaric acylcarnitines: e.g. C4DC & C5OH m/z 374.2 & 318.3 butylated: both m/z 262.3 underivatised Sensitivity – dicarboxylic acylcarnitines Familiarity	Reduced sample transfers No corrosive reagents Less preparative equipment Less steps to QC No hydrolysis: improved C0 assay Rapid blood spot/plasma analysis Simplifies secondary assays on initial extract Isobaric acylcarnitines/interferents e.g. glutamate/C2 m/z260, C2 m/z204 underivatised OHC10/C5DC m/z388 derivatised, C5DC m/z276 underivatised

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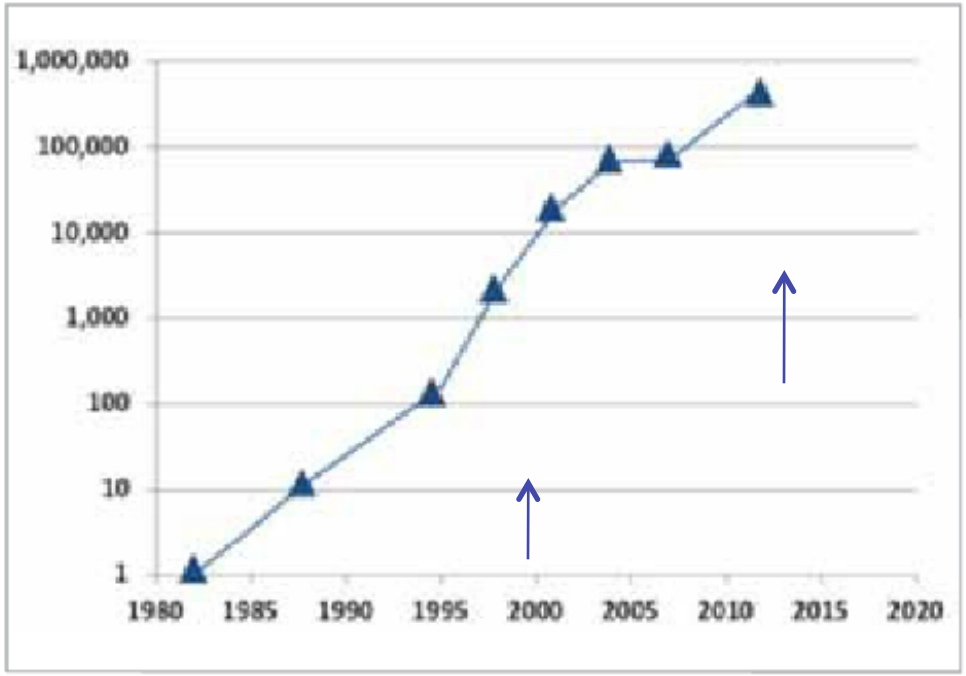
Acylcarnitine measurement in blood spots ERNDIM QA scheme

Instrument sensitivity has increased >2000fold since late 1990's: 2-3 fold increase for dicarboxylics by butylation very small by comparison

The history of sensitivity at AB SCIEX



Acylcarnitine measurement in blood spots ERNDIM QA scheme

Butylation v direct assay

1st circulation 2003 34/35 – butylation 1/35 – underivatised (3%)

19th circulation London 2012 34/44-butylation 10/44-underivatised (23%)

27th circulation London 2016 39/57 – butylation 16/57 – underivatised (29%)

20/57 kit methods (9/39 derivatised 11/16 underivatised)

PE, Chromsystems, Cambridge Isotopes

No obvious diagnostic advantage or disadvantage of either sample prep, or kit reagents has been evident from the ERNDIM London scheme

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Discussion Points

Butylation vs Underivatised

Samples circulated 2003-2016 London

Diagnosis	No of samples	Primary acylcarnitine reported	% Correct diagnosis
Normal	15		84
Normal CRF	1		63
MCADD	13	C8, C10:1, C6, C8/C10	**98**
MMA (mutase)	7	C3, C4DC	92 (94, 100,93,100, **66,** 100,90 **)**
MMA (Epimerase)	1	C3, C4DC	**49***
MMA (Cobalamin A,B,C)	3	C3, C4DC	93
PA	7	C3	94 (98, 95, 98 **75,** 98,100,100)
VLCADD	6	C14:1	82 (**50,** 95, 100, **41*,** 92, 95)
GA-1	5	C5DC	96 **(0.0*, 98, 89, 92, 100)**
MADD	5	C5, C6, C8, C10. C12, **C5DC,**ratios	**62** (61, 51*,49,**61**,84)
CTD	4	low C0	80
LCHADD	4	C18OH, C16OH, C16:1OH	92
IVA	3	C5	99 (98,100,100)
CPT-2	3	Low C2, ^C16+C18:1/C2	**41 (37*, 69, 48*)**
Malonic	2	C3DC	**(18*, 52)**
CPT-1	1	C0, C16	95
3-MCC	1	C5OH	85
HMGCoA Lyase	1	C5OH, C6DC	79
Beta ketothiolase	1	C5OH, C5:1	79
Holocarboxylase synthase	1	C5OH, C3	100 ^C5OH, 68 HCS

*Declared educational samples

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Samples circulated 2003-2016 Heidelberg

Diagnosis	No of samples	Primary acylcarnitine reported	% Correct diagnosis
Normal profile	7		**83**
Glutaric aciduria type I	6	C5DC	**97**
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	6	C8, C6, C10, C10:1	**91 (45, mild form)***
Long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	5	C16OH, C18:1OH, C18-OH	**82 (61, mild form)***
Methylmalonic aciduria (mut 0)	4	C3, C4DC	**93**
Propionic acidaemia	4	C3	**100**
Isovaleric aciduria	3	C5	**100**
Cobalamin A deficiency	2	C3, C4DC	**98**
Multiple acyl-CoA dehydrogenase (MAD) deficiency	1	C6, C8, C10	**94**
Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	1	(C14, C14:1)	**24 (compensated)***
3-hydroxy-3-methylglutaryl-CoA lyase deficiency	1	C5OH, C6DC	**91**
3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency	1	C5OH	**85**

*Declared educational samples

ERNDIM QA scheme: Educational value
Malonic acidemia
Samples 4b (2004), 9c (2007)

4b 20/42

4b 6 month old female, currently well. History of acute collapse at 4 days of age with apnoea, profound metabolic acidosis (pH 7.1 HCO3 2), hypoglycaemia, left ventricular hypertrophy.

20/42 malonic aciduria (malonyl CoA carboxylase deficiency)

- ^C3DC, m/z 248 underivatised, m/z 360 butylated).
- 15 quantitative values (median 1.1, range 0.35-2.2micromol/l),
- 12 gave reference ranges (upper limit of normal: median 0.11, range 0.05-0.8micromol/l).

22 respondents did not suggest malonic aciduria

- 11 supplied spectra of butylated sample in which the peak at m/z 360 was clearly visible.
- 3 supplied MRM acquisition data which did not include malonyl carnitine.
- 4 described the sample as normal

ERNDIM QA scheme
Malonic acidemia

9c 49/60

9c 6.5y old male, large head circumference, learning difficulties.

49/60 malonic aciduria (malonyl CoA decarboxylase deficiency).

- ^C3DC, m/z 248 underivatised, m/z 360 butylated).
- 36 quantitative values (median 0.90μmol/l, range 0.29-2.09)
- 31 gave reference ranges (upper limit of normal: median 0.14μmol/l, range 0.00-0.60)

11 normal acylcarnitine profile. (8 butylation, 3 underivatised)

- 1 quantitative malonic: 0.66μmol/l, not flagged as abnormal
- 4/11 provided scans where a peak at the appropriate m/z was obvious.
- 1 gave a quantitative value for C3DC of 0.01μmol/l, based on m/z of 358.5.

Proportion missing diagnosis 2004: 52% 2007: 18%.
Increased awareness: QA scheme effect?

MRM acquisitions: blind to other acylcarnitines- importance of scan and knowledge base

Problems with quantitation and reference ranges

ERNDIM QA Scheme Glutaric Aciduria Type 1

2a 11month old male, collapse during intercurrent illness, large head with frontal bossing

GlutarylCoA dehydrogenase deficiency (type 1 glutaric aciduria) – enzyme confirmed

0/32 ^C5-dicarboxyl-carnitine

Blood spot/plasma acylcarnitines consistently normal

Urine organic acids - only once was 3OH-glutarate suspicious

5c 3y old male, intercurrent infection, altered consciousness, hypoglycaemia

52/53 ^C5-dicarboxyl-carnitine and suggested a diagnosis of GA1

15a 4y old, movement disorder subsequent to acute illness

39/44 ^C5-dicarboxyl-carnitine and suggested a diagnosis of GA1

20c 3 year old female, epilepsy, basal ganglia changes on MRI

50/52 ^C5-dicarboxyl-carnitine, 7/52 ^C5DC/C8 48/52 suggested a diagnosis of GA1

2016e 23y old male, movement disorder following intercurrent illness in childhood

56/56 ^C5-dicarboxyl-carnitine, 11/56 ^C5DC/C8. 56/56 suggested a diagnosis of GA1

A normal acylcarnitine result does not exclude type 1 glutaric aciduria

High awareness of GA1, unlikely to be missed if metabolite elevated

Increasing use of ratios

No apparent difficulty for underivatised analysis

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ERNDIM QA Scheme Glutaric Aciduria Type 1

Year Sample	correct	Median	Range	n	URL Median	URL Range	URL n
2005 5c	52/53	1.2	0.39-3.10	40	0.15	0.03-0.5	34
2010 15a	39/44	0.38	0.13-1.02	34	0.15	0.03-0.57	25
2012 20c	48/52	0.77	0.3-2.6	39	0.14	0.02-0.4	25
2016 e	56/56	4.38	1.47-12.1	46	0.17	0.03-0.51	34

Problems with quantitation and reference ranges

Some correlation between concentration & diagnostic accuracy

ERNDIM QA Scheme
VLCADD
2003 2c 16/32, 2005 6a 47/52, 2007 9a 59/59, **2012 19b 20/49** 2014 23c 57/58, 2015.04 53/56

2c 2month old female, Reye-like illness

16/32 respondents: increased tetradecenoylcarnitine (C14:1) & suggested VLCADD

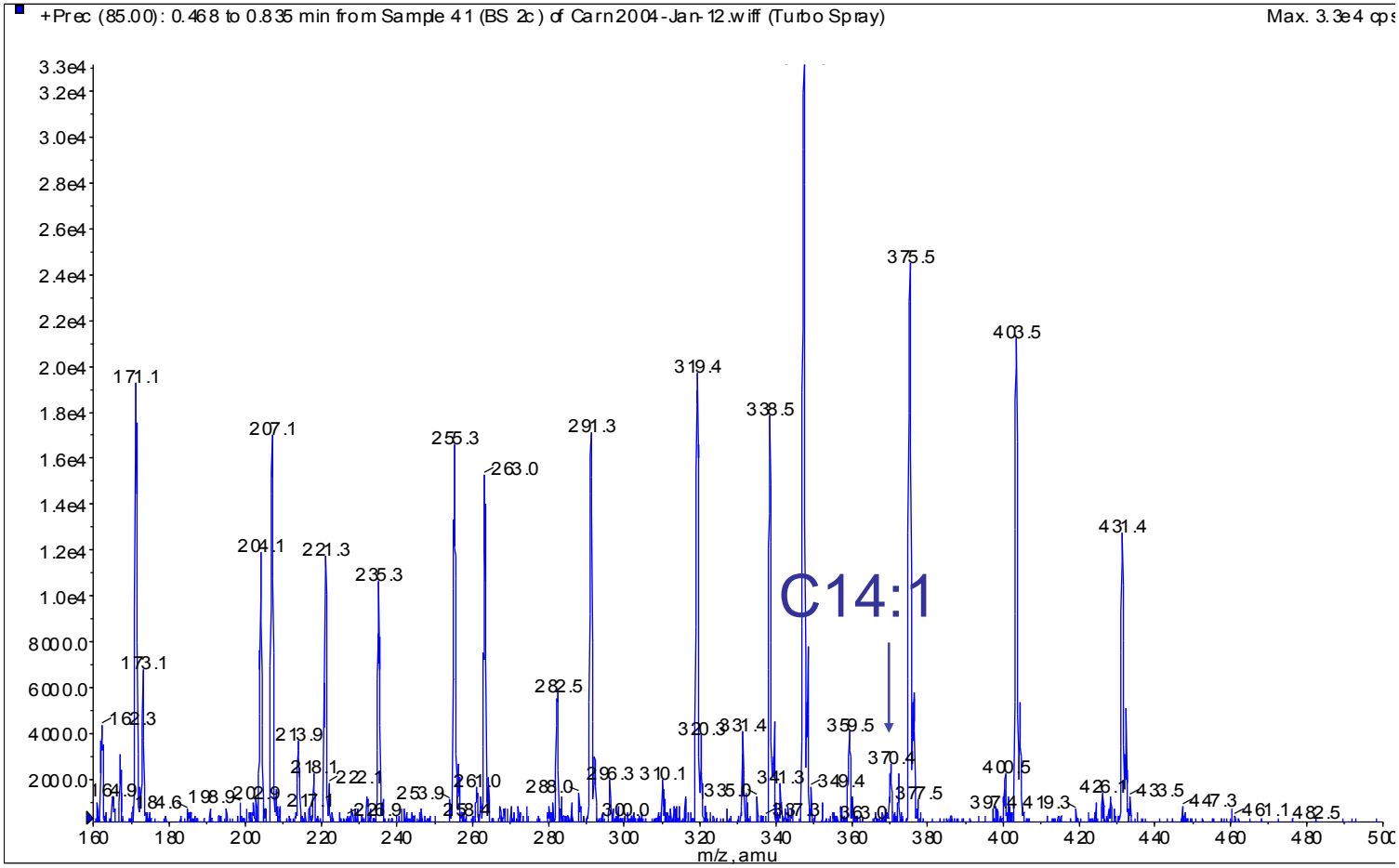
12/16 provided quantitation (median 0.35µmol/l, 0.19-0.55), laboratory normal ranges, upper limit of normal: median 0.22, 0.07-0.55

3/32 suggested CTD because of low free carnitine

16 did not suggest VLCADD or comment on C14:1 but 5/16 supplied scans in which peaks at m/z 370 (direct) or m/z 426 (butylated) were apparent

7/16 provided quantitation (median 0.27µmol/l, 0.09-0.38) – within their laboratory normal ranges, upper limit of normal: median 0.80, 0.54-1.07

ERNDIM QA scheme
VLCADD Sample 2c



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ERNDIM QA Scheme
VLCADD
2003 2c 16/32, 2005 6a 47/52, 2007 9a 57/59,
2012 19b 20/49 2014 23c 57/58, 2015.04 53/56

19b 24y old female. Cardiomyopathy & hypoglycaemic episode in childhood, currently well

15 quantitative results for C14:1: median 0.26μmol/l, range 0.18-0.32
15 median upper limit 0.18μmol/l, range 0.15-0.41
6 quoted C14:1/C16 ratios, median 0.65, range 0.38-0.86
6 median upper reference limit 0.2, range 0.07-0.4

35 quantitative results for C0: median C0 6.3μmol/l, range 2.8-15.0. 27 suggested CTD
22 median lower limit 12.2μmol/l, range 4.8-22.3

ERNDIM QA Scheme VLCADD

Year Sample	correct	Median C14:1	Range	n	URL Median	URL Range	URL n
2003 2c	16/32	0.35/ 0.27	0.19-0.55/ 0.09-0.38	12/ 7	0.22/ 0.80	0.07-0.55/ 0.54-1.07	12/ 7
2005 6a	47/52	0.79	0.47-1.78	34	0.23	0.07-1.3	31
2007 9a	59/59	0.83	0.31-25	49	0.24	0.02-0.89	42
2012 19b	20/49	0.26	0.18-0.32	15	0.18	0.15-0.41	15
2014 23 c	57/58	0.90	0.50-1.8	52	0.20	0.10-0.70	35
2015.04	53/56	0.80	0.35-1.39	48	0.21	0.06-0.75	34

ERNDIM QA Scheme VLCADD

Year Sample	correct	Median C0	Range C0	N C0	C14:1/C16	C14:1/C2	Other Ratio
2003 2c	16/32	-	-	-	-	-	-
2005 6a	47/52	5.4	2.5-14.3	32	-	-	-
2007 9a	59/59	-	-	-	-	-	-
2012 19b	20/49	6.2	2.8-15	36	6	-	-
2014 23 c	57/58	7	4.8-13	42	20	6	4
2015.04	53/56	6.2	0.56-10.5	7	20	6	8

ERNDIM QA Scheme
VLCADD

2003 2c 16/32, 2005 6a 47/52, 2007 9a 57/59,
2012 19b 20/49 2014 23c 57/58, 2015.04 53/56

Patients may not show gross elevation of diagnostic acylcarnitines when carnitine depleted or not decompensated

Carnitine supplementation not recommended in long chain disorders

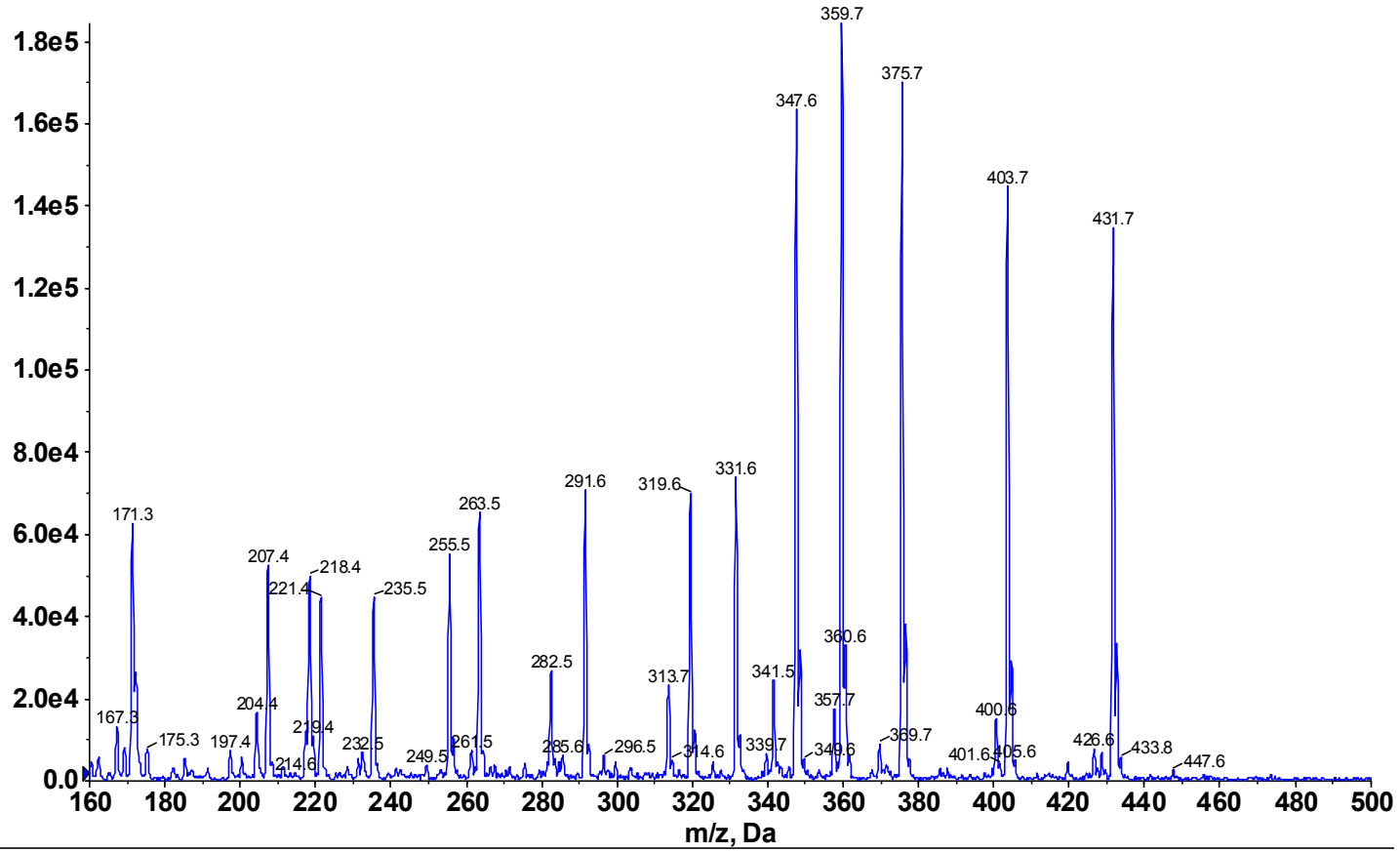
C14:1 non-specific marker of mitochondrial stress

Problems with quantitation and reference ranges

Ratios may provide better discrimination in carnitine depleted patients

ERNDIM QA Scheme

Sample 13a 8 day old male, hyperammonaemia, coma underivatised



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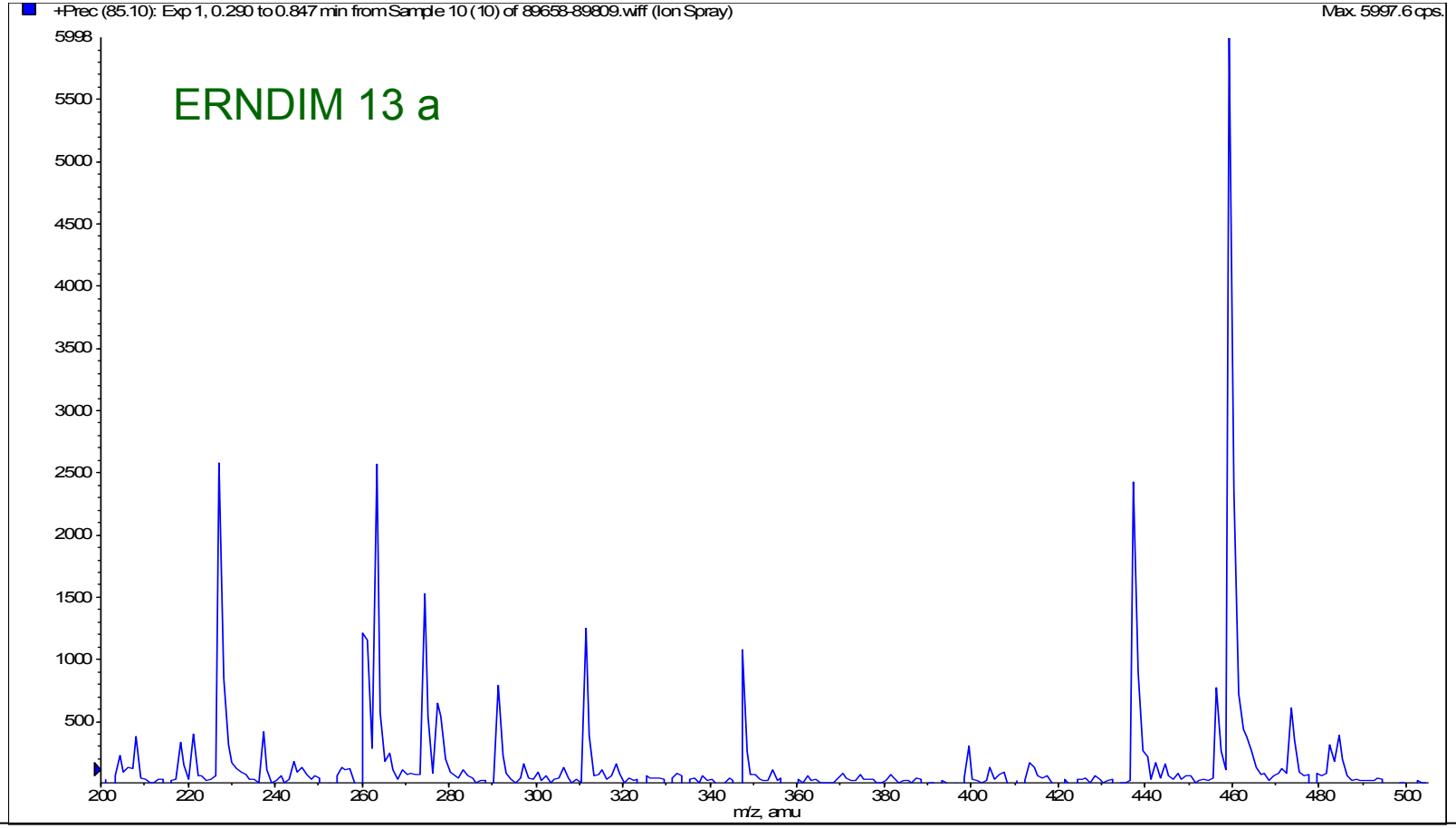
ERNDIM QA Scheme

Sample 13a 8 day old male, hyperammonaemia, coma butylated



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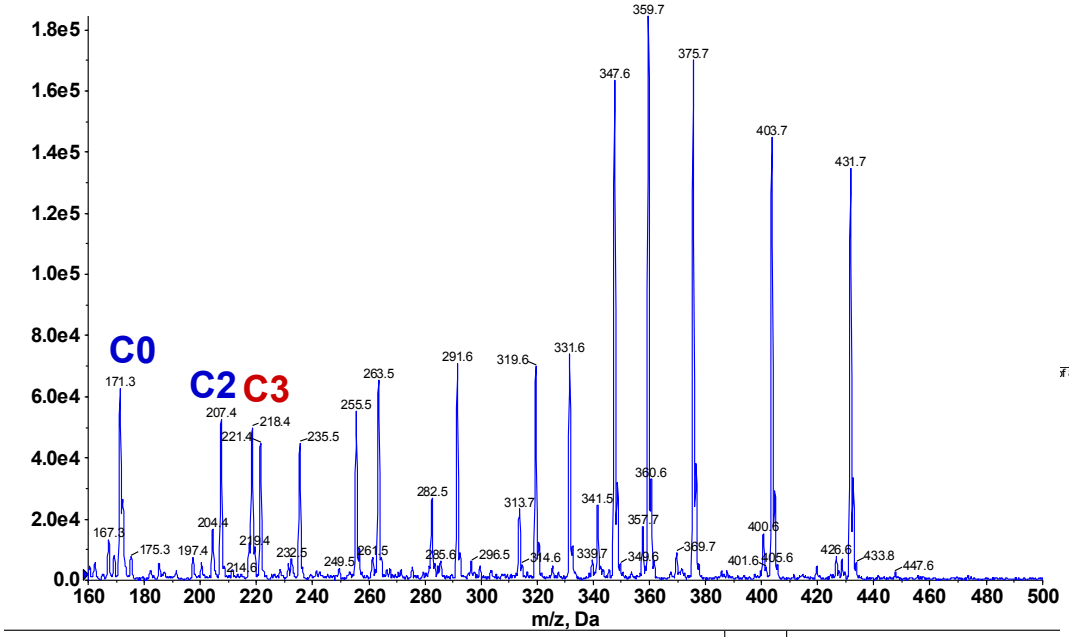
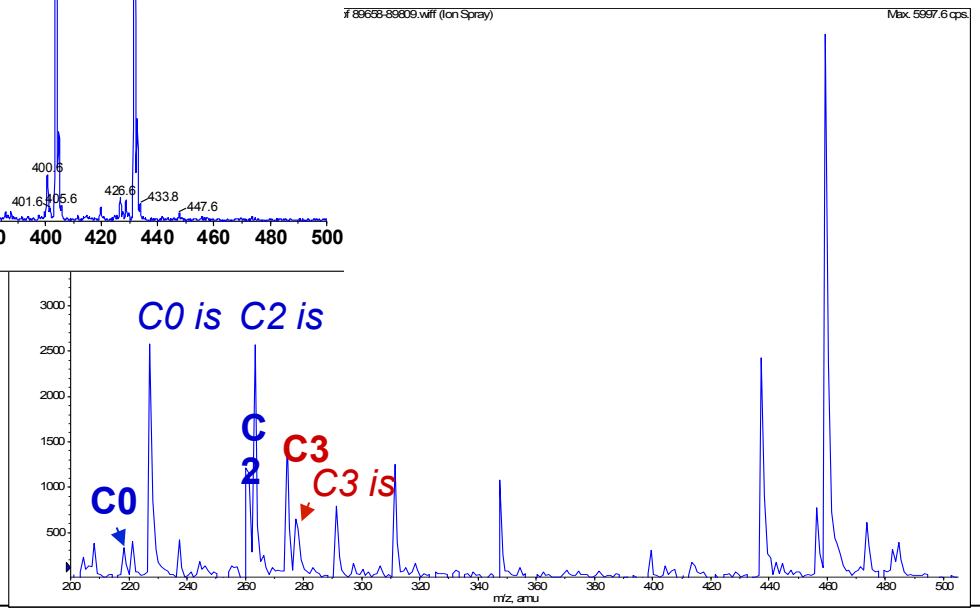
ERNDIM QA Scheme

Sample 13a 8 day old male, hyperammonaemia, coma





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ERNDIM QA Scheme
Propionic acidaemia Sample 13a

13a 55/73

8 day old male, hyperammonaemia, coma

44 C3 carnitine outside their age appropriate reference limit

13 C3 abnormal by ratios (C3/C2, C3/C0, C3/C16).

C3 concentration: median 3.4μmol/l, range 2.20-9.39, interquartile range 3.00-3.98 n=49. Upper limit: median 3.14μmol/l, range 1.10-10.70, IQ range 1.80-4.93 n=32

Free carnitine concentration: median 4.2μmol/l, range 2.0-15.0, IQ range 3.36-5.45 n=58. lower limit: median 9.62μmol/l, range 4.5-25.0, IQ range 7.00-14.00

16 carnitine transporter, 1 fatox, 1 urea cycle defect, 1 no diagnosis

Need for vigilance when carnitine is depleted

Huge variation in quantitative values & reference ranges

Overdependence on quantitative values in some laboratories

3/73 laboratories measured MMA & methylcitrate on the dbs

Critical error?

Carnitine palmitoyl transferase 2 deficiency
2013 21b 19/51 2014 24c 36/52 2016.D 15/56

21b 17 year old male, recent muscle pain & raised creatine kinase, currently well
24c 25 year old female, recent presentation with rhabdomyolysis, currently well
2016.D 20y old male, hypoglycaemia in childhood.

Year Sample	correct	Median C2	LRL Median C2	Median (C16+C18:1) /C2	URL (C16+C18:1) /C2
2013 21b	19/51	5.2	5.0	0.8	0.3
2014 24 c	36/52	3.6	7.8	0.78	0.3
2016.D	15/56	3.07	6.84	0.77	0.23

Carnitine palmitoyl transferase 2 deficiency
2013 21b 19/51 2014 24c 36/52 2016.D 15/56

21b 17 year old male, recent muscle pain & raised creatine kinase, currently well
24c 25 year old female, recent presentation with rhabdomyolysis, currently well
2016.D 20y old male, hypoglycaemia in childhood.

Very poor performance in detecting this disorder

Plasma offers lower background for long chains than DBS

Performance better on 24c:

?slightly lower C2, ?clinical description, ?Sample year before

Routine calculation of (C16+C18:1)/C2?

Carnitine palmitoyl transferase 2 deficiency
2016.D 15/56

2016.D 20y old male, hypoglycaemia in childhood.

Effect of butylation on reference ranges

Email dialogue with participant

9 respondents gave a quantitative value for (C16+C18:1)/C2 ratio and their upper reference limit: self selecting group; all included CPT2 in their differential

1 did not say whether they were using a derivatised method and gave a value of 0.74, with a URL of 0.26, they considered the profile most likely normal

5 used derivatised methods & gave a mean value of 0.57 (0.35-0.91), URL 0.20 (0.18-0.23). 1 also gave (C16+C18:1+C18:2)/C2: value 2.9 URL 0.87

3 used underivatised methods & gave a mean of 0.91 (0.77-1.10) and a URL of 0.44 (0.34-0.65)

Discussion point

Effect of butylation on reference range

Free carnitine higher

Acylcarnitines lower

Harmonisation of quantitation

Summary of ERNDIM QA results

Normal

- Most labs report normal scans as normal

MCADD, IVA, Carnitine transporter, CPT1, LCHAD, MADD

- No difficulty for most labs, some confusion between MADD & MCADD

3-methylcrotonyl carboxylase, HMGCoA lyase, beta ketothiolase, holocarboxylase synthase

- Most labs identified key acylcarnitines & suggested appropriate follow-up tests

GA1

- Some patients do not generate metabolites
- When C5DC present well detected
  - No difference between butylation & underivatised

Summary of ERNDIM QA results

Methylmalonic & Propionic

- All labs pick up grossly raised C3, C4DC less easy
  - Increasing use of second line tests on original blood spot: MMA, methylcitrate, 3OH-propionate
- Difficulties with reference range for C3: diagnoses missed when C3 not grossly elevated
  - Epimerase
  - Carnitine depleted acute propionyl CoA carboxylase
- Overdependence on quantitative values in some laboratories.
  - Underuse of ratios for flagging potential diagnoses?

Carnitine Depletion

Look beyond carnitine uptake disorder

High index of suspicion for secondary carnitine depletion

Look for the disproportionately raised acylcarnitine!

- Sick neonate
  - Gentamycin/AKI: reduced renal tubular reabsorption of free carnitine
- Well older child or adult
- Do not depend on reference ranges
- Ratios?

Quantitative analysis

Reported values vary over wide range

CDC quantitative DBS scheme (spiked normal donor blood) shows similar variation

Requires local normal ranges

Harmonisation is slow

some convergence 2003-2016 but not much!

Quantitative analysis

Issues with software designed for screening (Neolynx & Chemoview)

- Set up for scan data & isotope ratio

Stable isotope ratio quantitation can be misleading

- Traceability & accuracy of stable isotope labelled compounds
- All compounds not available labelled
- Manufacturing problems (PE derivatised kit 2009)
- Linearity of instrument
- Mass calibration drift
- Mass dependent variation on scans
- Between MRM variation in ionisation
  - Waters/Micromass product warning

Quantitative analysis

Use calibrators!

- Only proven route to accuracy, traceability & conformity
- Lack of DBS traceable calibration material
- Liquid calibrators
- Lack of defined reference material for all clinically relevant acylcarnitines

ERNDIM Quantitative acylcarnitines in serum

Operating as a separate full EQA scheme for first time in 2017; some acylcarnitines were previously included in the Special Assays in serum

Lyophilised, spiked human serum

Analytes (2017):

Free carnitine (C0)
Acetylcarnitine (C2)
Propionylcarnitine (C3)
Butyrylcarnitine (C4)
Tiglylcarnitine (C5:1)
Isovalerylcarnitine (iC5)
(2R)-3-Hydroxyisovaleroyl Carnitine (3-OH-iC5)
Hexanoylcarnitine (C6)
Octanoylcarnitine (C8)
Decanoylcarnitine (C10)
Cis-5-Tetradecenoylcarnitine (cis-5-C14:1)
Tetradecanoylcarnitine (C14)
Palmitoylcarnitine (C16)
3-Hydroxyhexadecanoyl-L-carnitine (3-OH-C16)
Oleoylcarnitine (cis-9-C18:1)
Stearoylcarnitine (C18)
3-Hydroxystearoylcarnitine (3-OH-C18)
Malonylcarnitine (C3DC)
Methylmalonylcarnitine (C4DC)
Glutarylcarnitine (C5DC)

Accuracy Precision Harmonisation

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QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Evelina London
Children's Hospital

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Samples

Current requirement for 1 circulation:

2.5-3.0 ml liquid lithium heparin anti-coagulated whole blood

50+ dried blood spots

Diagnosis, clinical details & statement of informed consent

DPT scheme offers 20% discount on scheme year after donation of suitable sample

Please donate samples!

erndim@mft.nhs.uk will put you in touch with a scheme organiser

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Discussion Points

Precursor Ion Scan vs MRM
Flow injection vs Chromatography
Inject-inject time (how many scans across the peak)
“Flat top” TIC vs smooth peak

Butylation vs Underivatised
Effect of butylation on reference range
Free carnitine higher Acylcarnitines lower

Harmonisation of quantitation
Carnitine depletion
Ratios vs reference ranges