Experience from the ERNDIM Acylcarnitine Scheme

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Acylcarnitine analysis
ERNDIM 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

Acylcarnitine measurement in blood spots ERNDIM QA scheme

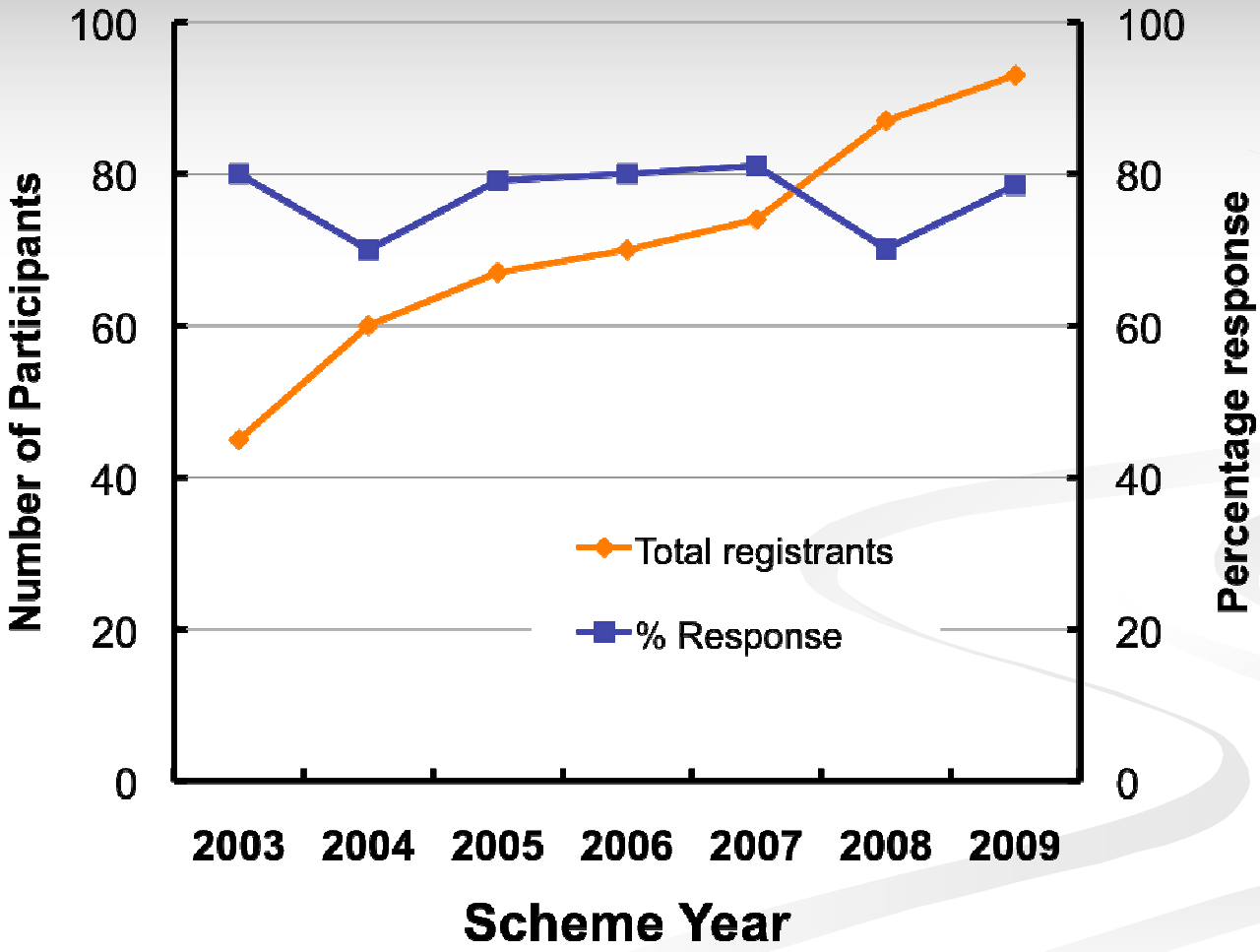
- Grew from 45 to 93 registered participants
- 2010 split between 2 centres (Heidelberg & London), 50+ participants in each centre

Acylcarnitine measurement in blood spots ERNDIM QA scheme (London)

Scheme Year	Dispatch Month
2003	April, January 2004
2004	July, November
2005	May, November
2006	June, January 2007
2007	April, November
2008	April, December
2009	August * 2
2010	August, October

Apologies for the variability & delays, particularly the reports for 2009!

Acylcarnitine measurement in blood spots ERNDIM QA scheme



Acylcarnitine measurement in blood spots ERNDIM QA scheme

Patients

Presenting to the the metabolic service at Guy's Hospital (now Evelina Childrens Hospital, St Thomas's)

Diagnoses confirmed by enzymology or DNA analysis

Routine blood sampling – written informed consent (patient/parent) obtained for use of excess blood for quality control purposes – authorised by the chairman of the Guy's Hospital Ethical Committee

Scheme participants encouraged to submit samples

4 donors so far

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Samples

Lithium heparin anti-coagulated whole blood
40µl aliquots spotted onto Schleicher & Schuell 903 paper
Dried for 24h at RT before packing and despatch
Any delay stored at –80^{o}C

Current requirement for 1 circulation:
approximately 3.5 ml liquid whole blood
60+ dried blood spots

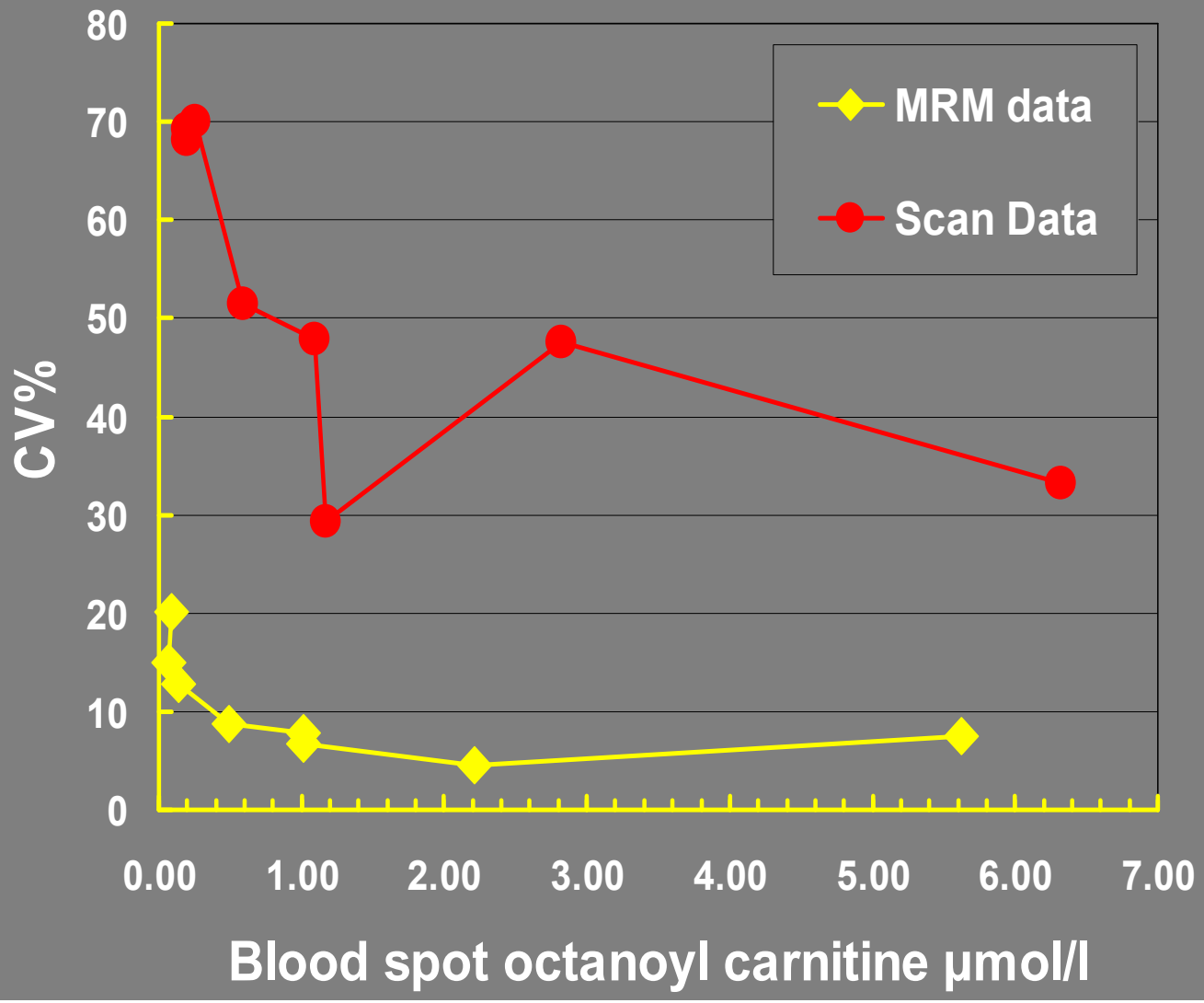
Acylcarnitine measurement in blood spots ERNDIM QA scheme

- Acylcarnitine analysis
- Methodological aspects
- Problems and Pitfalls

Acylcarnitine analysis

- Electrospray MSMS
  - Precursor ion scan, m/z 85
  - MRM acquisitions for specific acylcarnitines & quantitation
- Blood spots/plasma/serum
- Rapid analysis
  - Flow injection analysis
  - Simultaneous acquisitions for aminoacids etc
- Range of disorders
  - Organic acidaemias, fatty acid oxidation disorders
  - Particularly useful as window into mitochondrial fat oxidation
    - LCHADD, VLCADD

Octanoyl carnitine within run precision profile: MRM v Scan



Acylcarnitine measurement in blood spots ERNDIM QA scheme

Methodological aspects

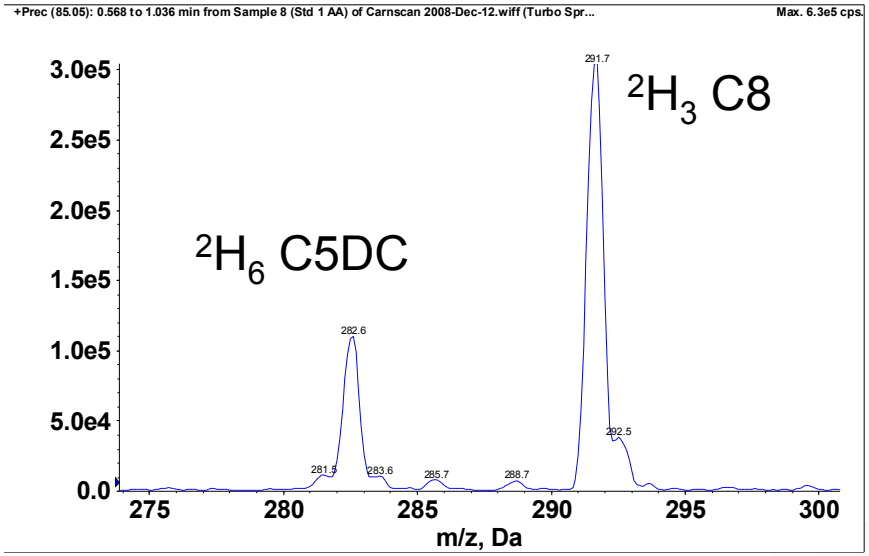
Direct assay (underivatised) v Butylation

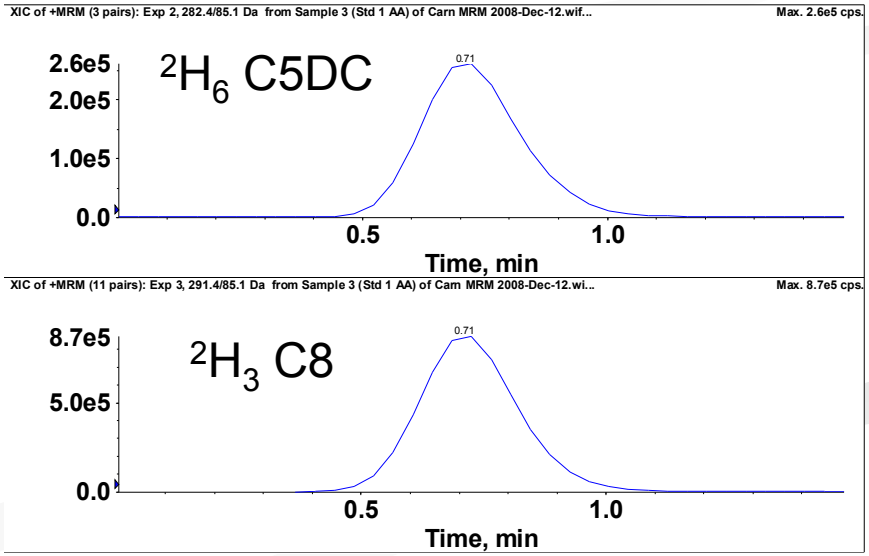
Disadvantages of underivatised	Advantages of underivatised
Different Mass difference -56 or -112 Sensitivity - dicarboxylic acylcarnitines Different isobaric acylcarnitines: e.g. C4DC & C5OH m/z 374.2 & 318.3 butylated: both m/z 262.3 underivatised	No sample transfers No corrosive reagents Less preparative equipment Less steps to QC No hydrolysis Rapid blood spot/plasma analysis

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Methodological aspects
Sensitivity

Dicarboxylics on direct assay
Scan vs MRM





Acylcarnitine measurement in blood spots ERNDIM QA scheme

Methodological aspects

Butylation v direct assay

Questionnaire on 1st circulation 2003

34/35 – butylation 1/35 – direct

Currently 30% underivatised

No obvious diagnostic advantage or disadvantage of either system has been evident from the scheme

Acylcarnitine measurement in blood spots
CDC QA scheme: annual report 2009

Butylation v direct assay

147 labs, 28 underivatised

PE Neogram derivatised kit: 2fold +ve bias for C5DC & C3DC, -ve bias for C10

No method showed bias or outliers for C3, C4, C5, C5OH, C6, C8, C10:1, C14, C14:1, C16, C18

No obvious diagnostic advantage or disadvantage of either system has been evident from the CDC scheme

Samples circulated 2003-2009

Diagnosis	No of samples	Primary acylcarnitine reported	% Correct diagnosis
Normal	9		88.2
Normal CRF	1		63.2
MCADD	7	C8, C10:1, C6	**98.9**
MMA (mutase)	3	C3, C4DC	95.5
MMA (Epimerase)	1	C3, C4DC	**49.2**
MMA (Cobalamin B)	1	C3, C4DC	92.6
PA	3	C3	87.1 (97.6, 94.6, **75.3**)
VLCADD	3	C14:1	83.9 (94.6, 96.6, **50.0**)
GA-1	2	C5DC	**(0.0, 98.1)**
IVA	2	C5	99.1
LCHAD	2	C18OH, C16OH, C16:1OH	89.6
Malonic	2	C3DC	**(18.0, 52.0)**
CTD	2	low C0	79.6
MADD	1	C5, C6, C8, C10. C12	61.4
CPT-1	1	C0, C16	94.7
3-MCC	1	C5OH	84.7
HMGCoA Lyase	1	C5OH, C6DC	79.1

ERNDIM QA Scheme
MCADD
Samples 2b, 3a, 5a, 7b, 10c, 12a, 14a

2b 32/32, 3a 39/41, 5a 53/53, 7b 56/56, 10c 60/60, 12a 61/61, 14a 65/67

3a 3y old female, seizures and developmental delay

- 39/41 respondents noted a high excretion of octanoylcarnitine & suggested MCADD
- 24 noted a low or low normal free carnitine
- 32 labs quantitative C_8 – median 1.00µmol/l (0.51-2.26)
- 39 labs suggested medium chain acylCoA dehydrogenase (MCADD)
- 1 normal and 1 carnitine transporter defect.

14a 61y old female, muscle pain

- 66/67respondents reported raised octanoyl carnitine,
- 65 suggested MCADD as the probable diagnosis.
- 57 reported quantitative values (median1.05, range 0.25-1.71micromol/l).
- 2 respondents did not suggest a specific disorder

Clinical details change reporting perspective

Variation in quantitation: Appropriate reference ranges?

ERNDIM QA Scheme
Glutaric Aciduria Type 1
Samples 2a, 5c

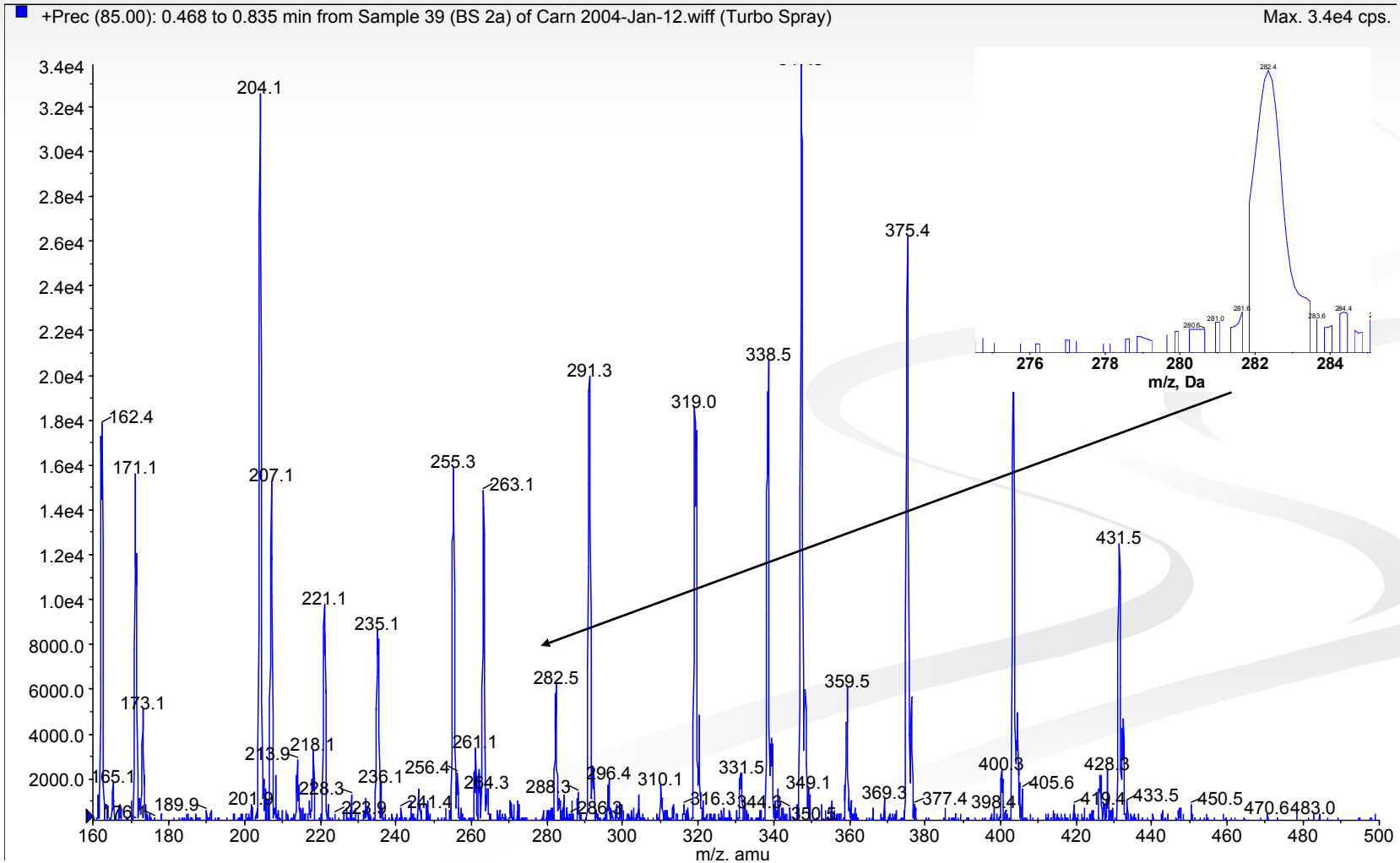
2a 0/32, 5c 52/53

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

- GlutarylCoA dehydrogenase deficiency (type 1 glutaric aciduria) – enzyme confirmed
- Blood spot/plasma acylcarnitines consistently normal
- Urine organic acids - only once was 3OH-glutarate suspicious
- 32/32 labs reported an essentially normal profile
- 8 respondents suggested further investigation to exclude type 1 glutaric aciduria

A normal acylcarnitine result does not exclude type 1 glutaric aciduria

Acylcarnitine analysis
ERNDIM QA scheme
Sample 2a



ERNDIM QA Scheme
Glutaric Aciduria Type 1
Samples 2a, 5c

2a 0/32, 5c 52/53

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

52/53 ^ C5-dicarboxyl-carnitine and suggested a diagnosis of glutaryl-CoA-dehydrogenase deficiency.

40 quantitative results (median 1.2, range 0.39-3.1micromol/l)

34 quoted an upper limit of normal (median 0.15, range 0.03-0.5micromol/l).

42 suggested followup tests, mainly urine organic acids & enzyme assay

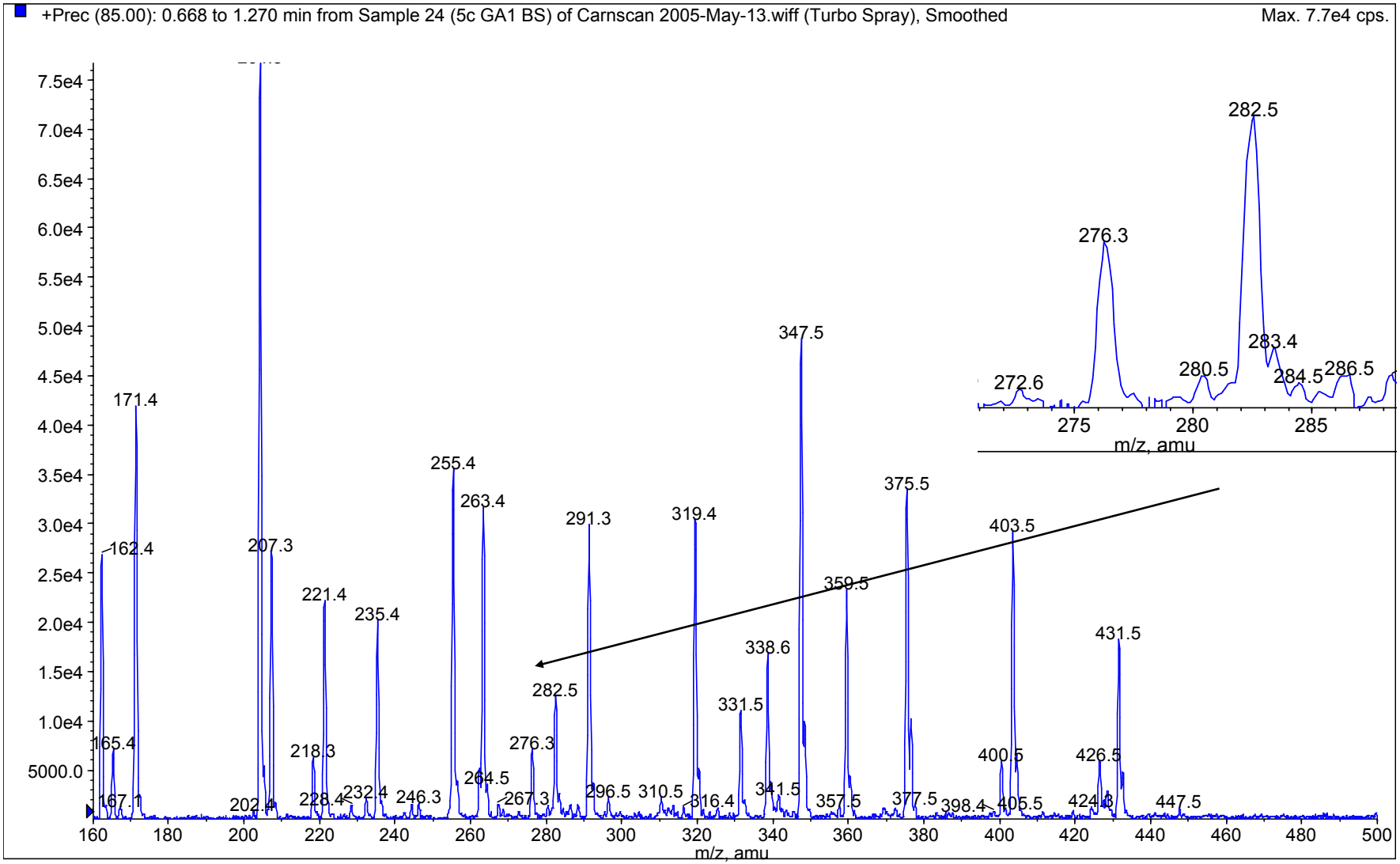
17 respondents made suggestions for clinical management

1 respondent reported the sample as normal.

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

No apparent difficulty for underivatised analysis

Acylcarnitine analysis
ERNDIM QA scheme
Sample 5c



ERNDIM QA scheme
Malonic acidaemia
Samples 4b, 9c

4b 20/42, 9c 49/60

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 acidaemia
Samples 4b, 9c

4b 20/42, 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

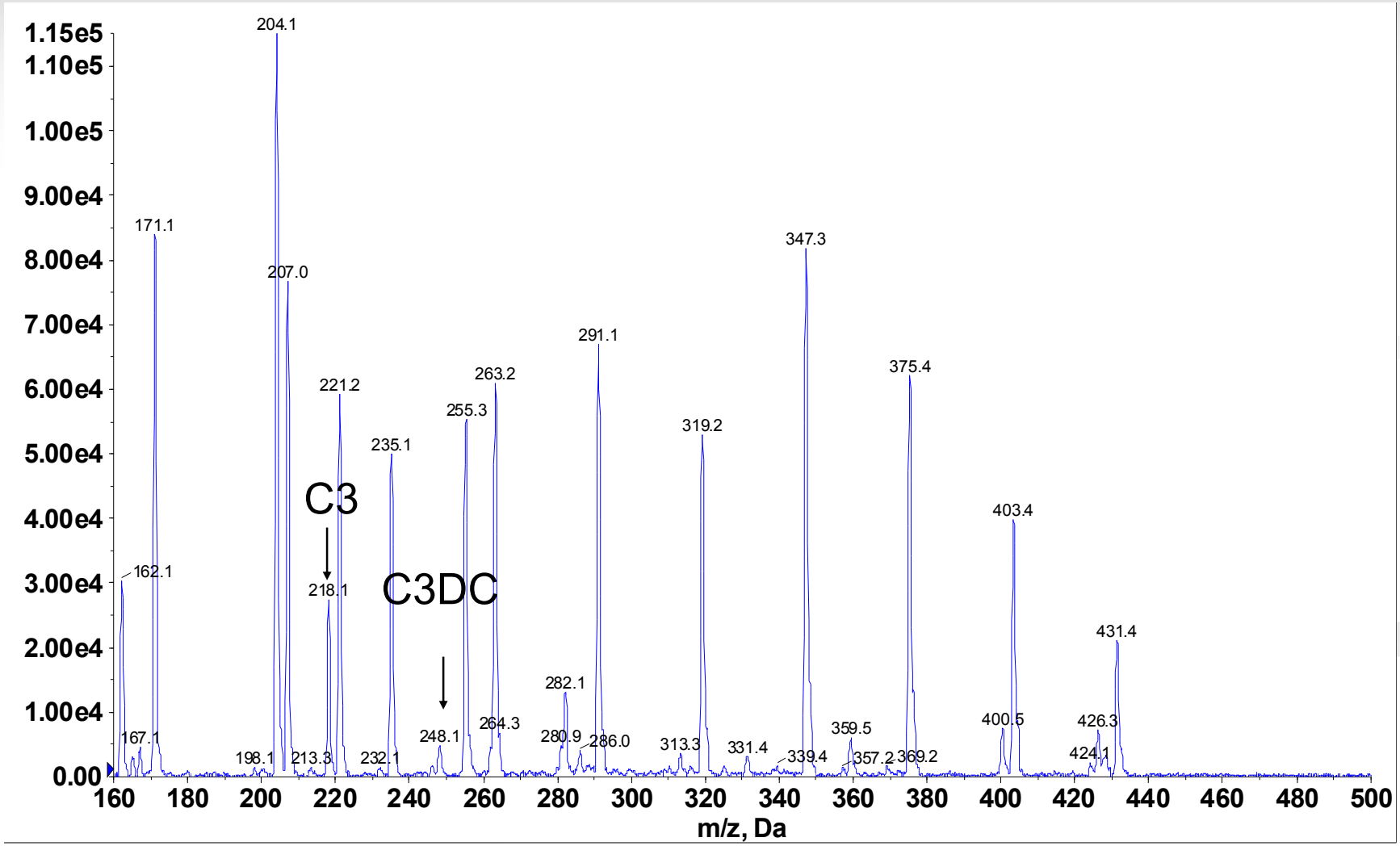
4/11 provided scans where a peak at the appropriate m/z was obvious.

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

Acylcarnitine analysis
ERNDIM QA scheme
Sample 9c



ERNDIM QA Scheme
VLCADD
Samples 2c, 6a, 9a

2c 16/32, 6a 47/52, 9a 57/59

2c 2month old female, Reye-like illness

16/32 respondents reported increased tetradecenoylcarnitine ($C_{14:1}$) & suggested VLCADD as the diagnosis

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

16 did not suggest VLCADD or comment on $C_{14:1}$ but 5/16 supplied scans in which peaks at m/z 370 (direct) or m/z 426 (butylated) were apparent

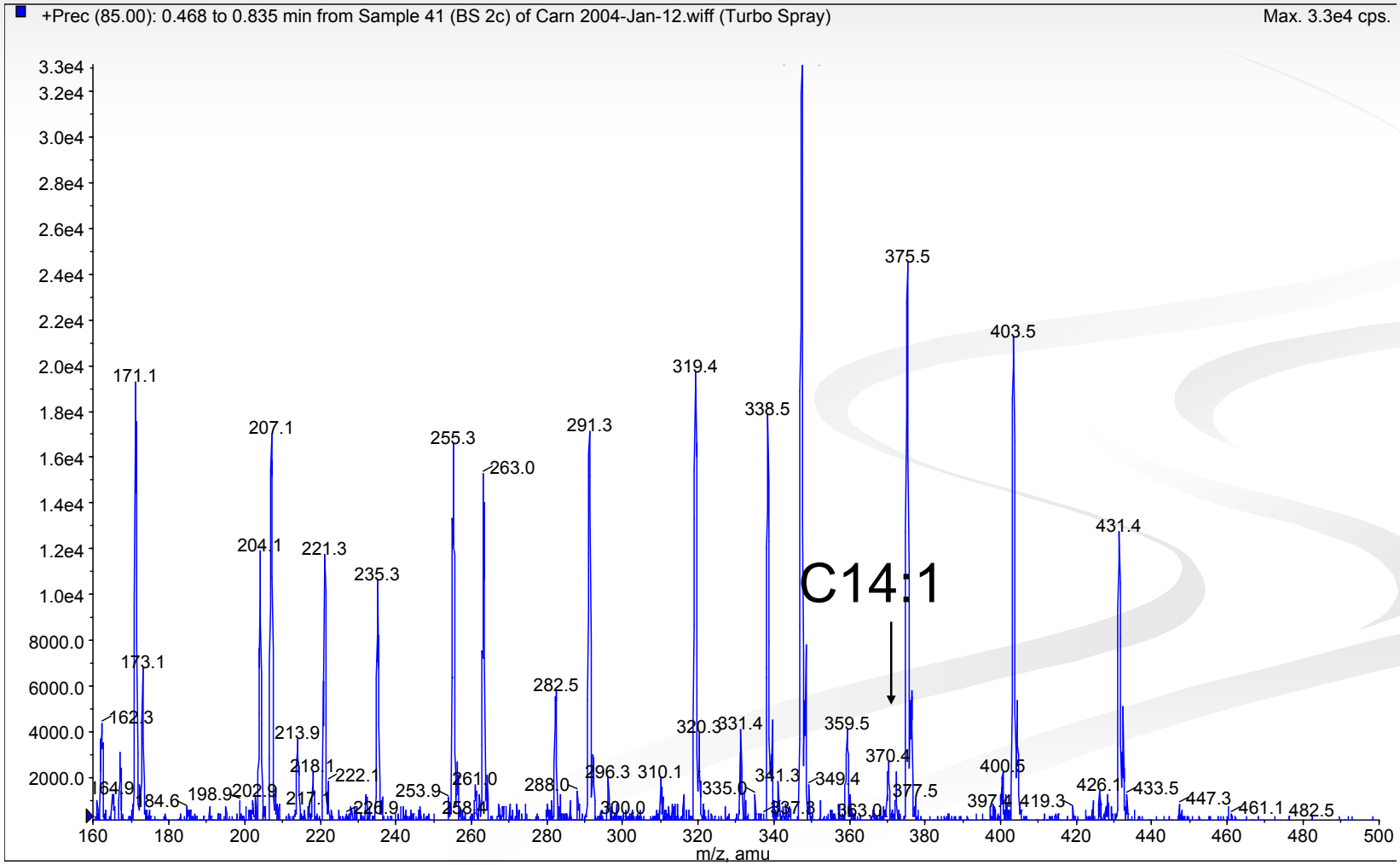
7/16 provided quantitation (median 0.27µmol/l, 0.09-0.38) – within the laboratory normal ranges, upper limit of normal: median 0.80, 0.54-1.07

Patient in recovery phase may not show gross elevation of C14:1

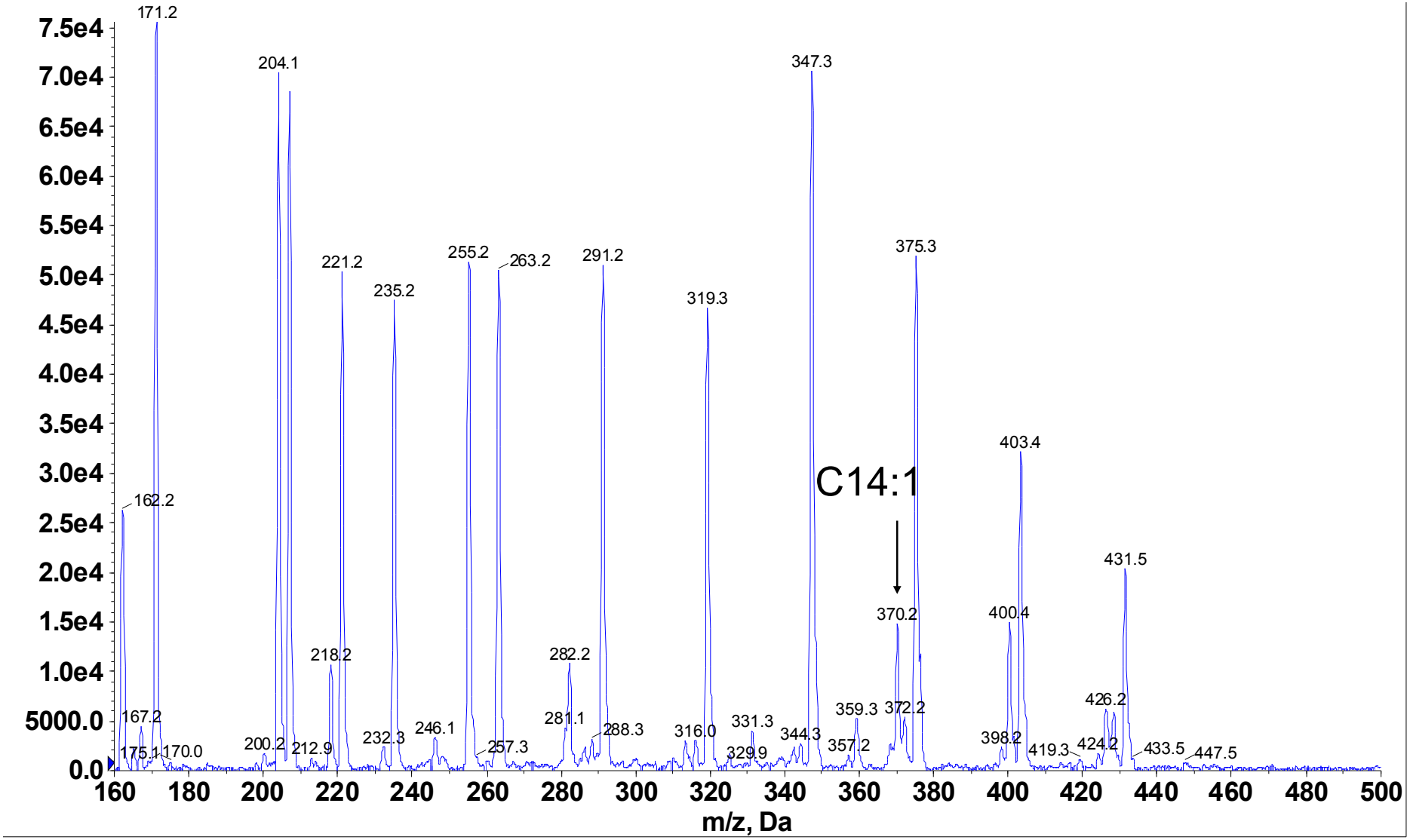
C14:1 non-specific marker of mitochondrial stress

Variation in normal ranges-false positives vs false negatives

ERNDIM QA scheme
VLCADD
Sample 2c



ERNDIM QA scheme
VLCADD
Sample 9a



ERNDIM QA Scheme
Methylmalonic acidaemia
Samples 1b, 6b, 14c (Mutase deficiency), 8c Cobalamin b

1b 34/36 6b 52/52 14c 62/67 8c 50/54

8c 11y old , clinical history of vomiting, lethargy, metabolic acidosis, hyperammonaemia

4 respondents suggested multiple carboxylase deficiency but suggested follow-up tests which would clarify diagnosis

ERNDIM QA Scheme
Methylmalonic acidaemia
Samples 1b, 6b, 14c (Mutase deficiency) , 8c Cobalamin b

1b 34/36 6b 52/52 14c 62/67 8c 50/54

Sample 14c was from the same adult patient as sample 1b

1b 44 year old male, epilepsy as child

both respondents not suggesting MMA saw no abnormality

14c 48y old male. Renal failure. Mild movement disorder.

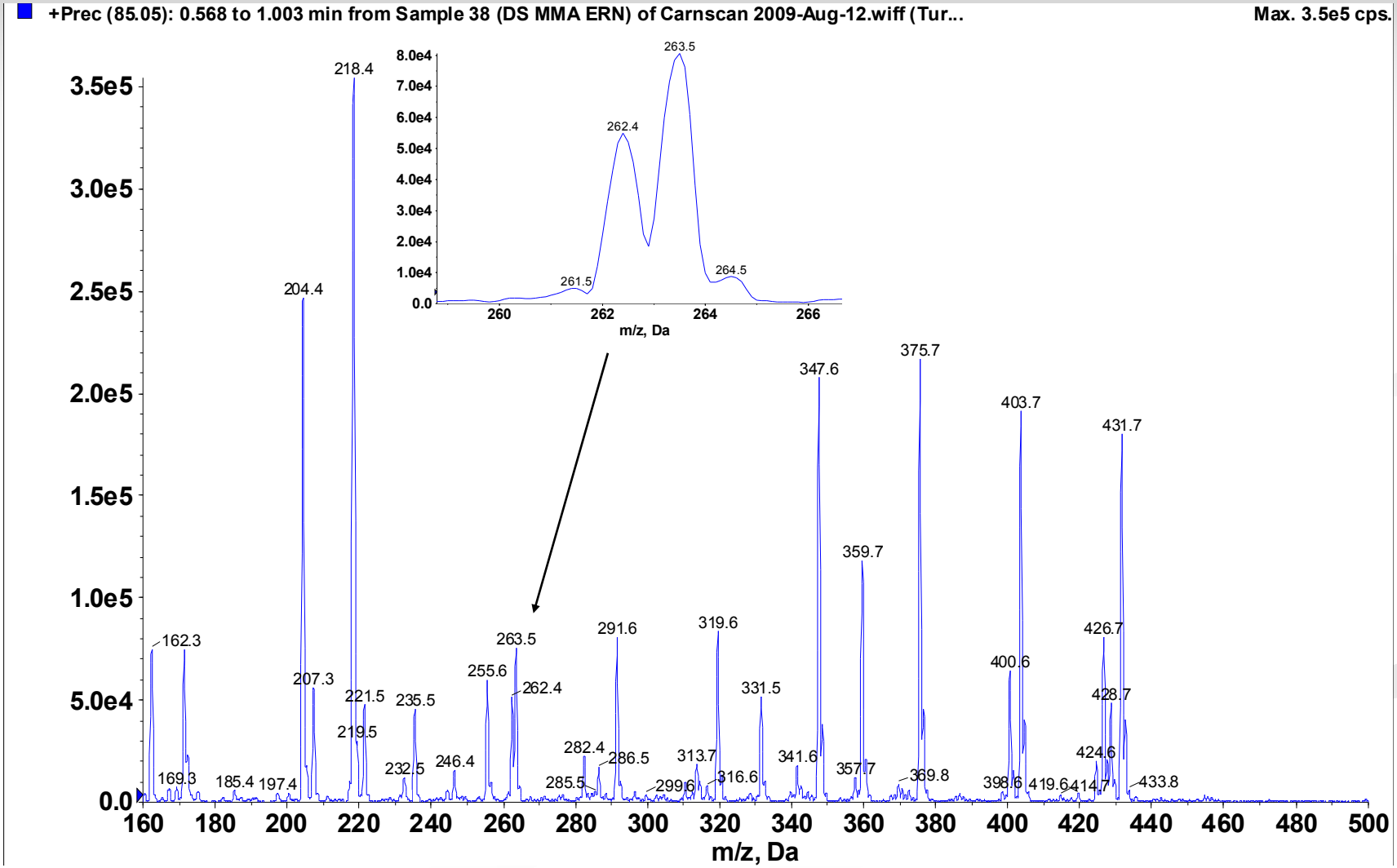
1 only suggested propionic acidaemia, 2 multiple carboxylase deficiency

1 rhabdomyolysis, 1 suspected supplementation with propionyl carnitine

Signorelli, S. S., P. Fatuzzo, et al. (2006). Propionyl- L -Carnitine Therapy: Effects on Endothelin-1 and Homocysteine Levels in Patients with Peripheral Arterial Disease and End-Stage Renal Disease." Kidney and Blood Pressure Research **29**(2): 100-107).

Clinical details change reporting perspective
Educational value of scheme (at least to organisers)

ERNDIM QA Scheme
Methylmalonic acidaemia
Sample 14c



ERNDIM QA Scheme
Methylmalonic acidaemia (Epimerase)
Sample 12b

12b 30/61

19y old female. history of hypotonia from the neonatal period. Two episodes of metabolic decompensation associated with intercurrent infection. Consanguinous family.

30/61 found increased C3 carnitine or C3/C2 ratio suggestive of a defect in propionate metabolism.

C3: median 2.8