Erndim Amino Acid Workshop

Friday 22nd October

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QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

Quantitative Amino Acids

- *Well established scheme*
- *Approx 290 labs participate*
- *8 samples distributed annually, lyophilised serum*
- *32 amino acids can be reported*
- *Annual report summarises key performance parameters*
  - *Accuracy*
  - *Recovery*
  - *Imprecision (intra-lab and inter-lab)*
  - *Linearity*

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QAA Method Groups

Methodology	**2001**	**2007**	**2015**	**2016**	**2017**	**2019**	**2020**	**2021**
LC-MS/MS	0%	3%	10%	12%	23%	25%	25%	28%
RP-HPLC	13%	14%	14%	14%	14%	13%	13%	12%
IEC	86%	82%	71%	67%	54%	54%	53%	52%
LC-MS					6%	3%	4%	4%
Other	2%	1%	5%	7%	3%	5%	5%	4%
Total number participants	*134*	*178*	*243*	*241*	*265*	*271*	*273*	*270*

Analyte	Precision (CV% duplicates)	Recovery (%added analyte)	Interlab CV	n
2-aminobutyric acid	7.9%	100%	13.1%	213
Alanine	4.4%	93%	8.30%	286
Alloisoleucine	6.4%	92%	16.9%	215
Arginine	5.8%	97%	10.5%	285
Arginino succinic acid	20.9%	70%	47.4%	139
Asparagine	11.4%	98%	23.5%	257
Aspartic acid	6.2%	68%	19.1%	270
Citrulline	5.5%	95%	14.0%	284
Cystine	11.1%	72%	15.0%	257
Glutamic acid	6.1%	82%	9.12%	283
Glutamine	6.4%	89%	11.0%	275
Glycine	4.3%	95%	7.76%	285
Histidine	5.7%	91%	9.53%	283
Homocitrulline	8.1%	90%	15.1%	123
Hydroxyproline	10.5%	98%	14.7%	240
Isoleucine	4.7%	94%	8.75%	290
Leucine	4.8%	92%	10.3%	290
Lysine	4.6%	95%	8.87%	286
Methionine	6.6%	94%	16.9%	283
Ornithine	4.2%	91%	8.85%	288
Phenylalanine	4.6%	92%	9.01%	293
Proline	6.4%	95%	11.4%	272
Sarcosine	12.4%	98%	20.5%	155
Serine	4.2%	86%	7.91%	284
Sulphocysteine	13.3%	78%	25.4%	88
Taurine	4.9%	97%	9.50%	266
Threonine	4.5%	96%	7.87%	284
Thryptophan	8.3%	90%	16.8%	220
Tyrosine	4.5%	93%	8.86%	293
Valine	4.4%	96%	7.54%	290

2021 Performance Data QAA scheme

Accuracy?

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- *Results reported relative to ALTM*
- *Do not reflect absolute accuracy*
- *Recovery relative to spiked values is summarised in annual report*
- *No matrix matched certified reference material exists*
- *Many labs use SigmaTraceCert or Sigma mixed amino acids to calibrate against (aqueous)*
- *Inter-laboratory variation of most amino acids is reasonable*
- *Accuracy less important for diagnosis, more important for monitoring*

Horwitz equation

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- Horwitz equation is independent of method, sample matrix or analyte
- Based on % purity of the analyte in the sample
- Calculates the Predicted Relative Standard Deviation of reproducibility (PRSD) e.g. the predicted between lab variation for a given analyte
- The RSDr, the repeatability CV, can then be calculated as 0.67 * PRSD
- The RSDr is a guide to what the within batch CV in a given lab should be

- The HorRat is the ratio of the actual RSD (from experimental data) to the PRSD
- The HorRat can then be used to provide an independent assessment of our performance
- HorRat > 1.3 indicates unacceptable variation

Bench Marking AA Performance

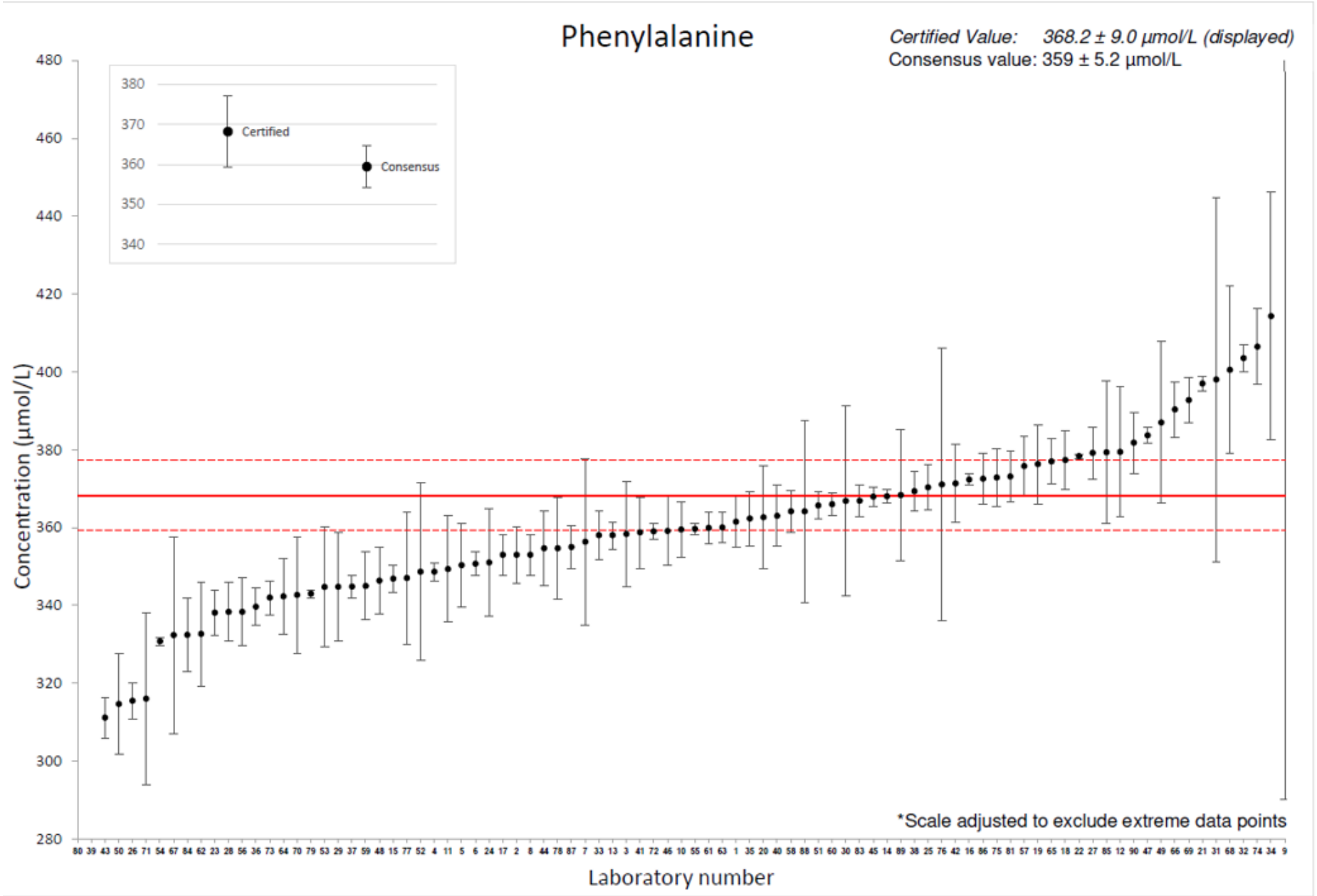
ERNDIM 2020 samples			Inter laboratory variation				Intra laboratory variation	
Analyte	Mean concentration 2020 samples (umol/L)	Mean concentration 2020 samples (ppm)	Predicted inter lab variation PRSDR (%)	Maximum Acceptable PRSD (%)	ERNDIM Inter Lab CV (%)	Hor Rat	RSDR (%)	ERNDIM Mean Intra Lab CV (%)
Alanine	449	40.0	4.8	9.7	8.3%	0.8	6.5	4.4%
Alloisoleucine	46	6.0	4.5	9.0	16.9%	1.9	6.0	6.4%
Arginine	187	32.5	3.3	6.6	10.5%	1.7	4.4	5.8%
Arginino succinic acid	15	4.2	3.0	6.0	47.4%	7.8	4.0	20.9%
Citrulline	605	105.9	3.0	6.1	14.0%	2.3	4.1	5.5%
Glutamine	721	105.3	3.4	6.9	11.0%	1.6	4.6	6.4%
Glycine	449	33.7	2.0	4.0	7.8%	2.0	2.7	4.3%
Isoleucine	300	39.4	1.8	3.6	8.8%	2.5	2.4	4.7%
Leucine	593	77.8	1.7	3.4	10.3%	3.0	2.3	4.8%
Lysine	214	31.2	1.6	3.2	8.9%	2.8	2.2	4.6%
Methionine	186	27.8	1.6	3.1	16.9%	5.4	2.1	6.6%
Ornithine	373	49.2	1.5	3.1	8.9%	2.9	2.0	4.2%
Phenylalanine	453	74.8	1.5	3.0	9.0%	3.0	2.0	4.6%
Proline	474	54.5	1.5	2.9	11.4%	3.8	2.0	6.4%
Serine	353	37.1	1.4	2.9	7.9%	2.8	1.9	4.2%
Taurine	253	31.6	1.4	2.8	9.5%	3.5	1.9	4.9%
Threonine	225	0.0	1.4	2.8	7.9%	2.9	1.9	4.5%
Tyrosine	342	62.0	1.4	2.8	8.9%	3.3	1.8	4.5%
Valine	313	36.7	1.4	2.7	7.5%	2.9	1.8	4.4%

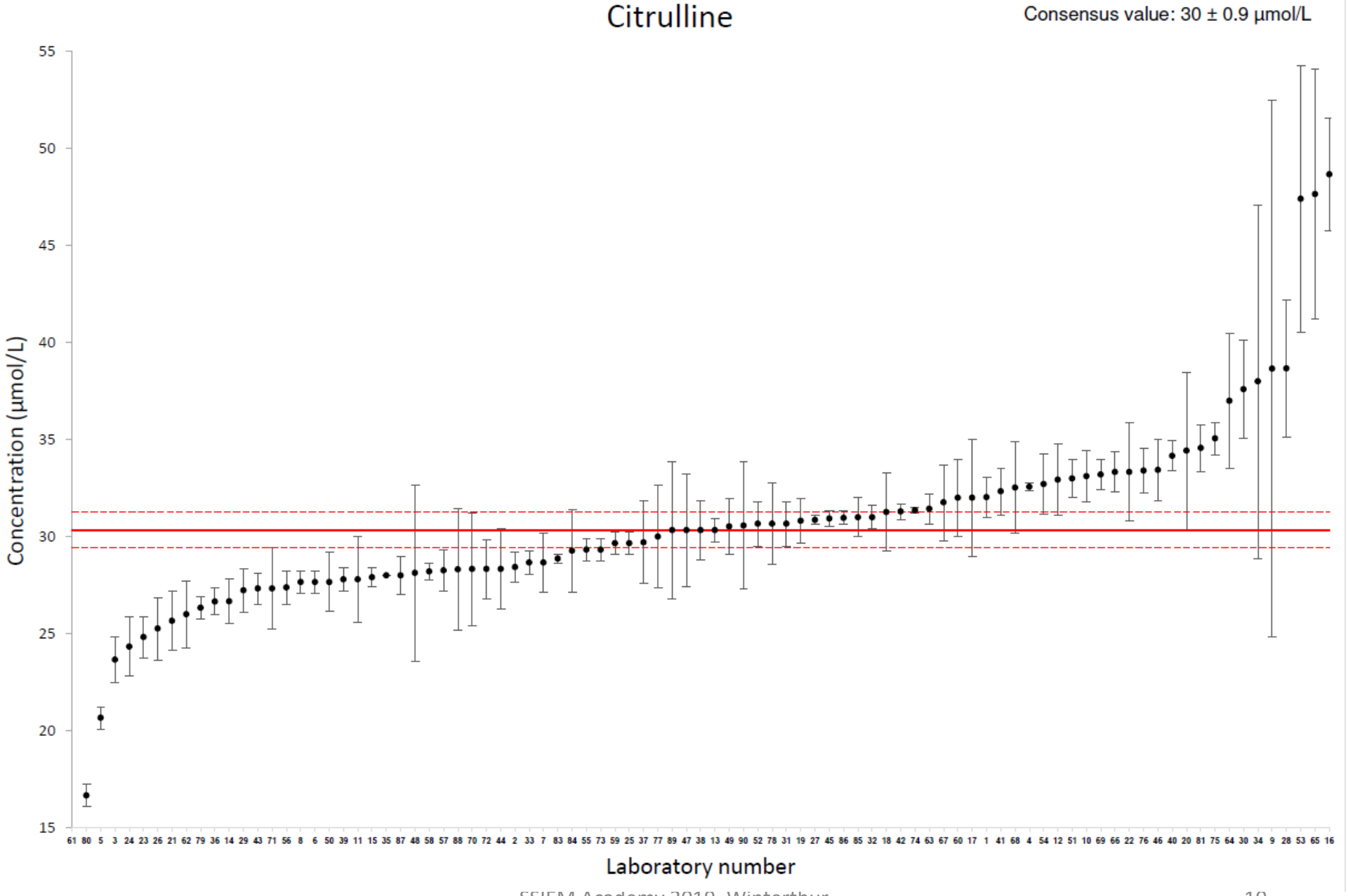
Collaboration with NML 2021

- *Frozen pooled human plasma prepared by NML*
- *Plasma phenylalanine and leucine concentration determined*
- *Concentrations traceable to the SI system of units (mass fraction)*
- *Stability and homogeneity characterised*
- *Sample distributed to national measurement labs across the world*
- *NML and Erndim collaborated to undertake inter-laboratory comparison exercise - 88 Erndim labs participated*

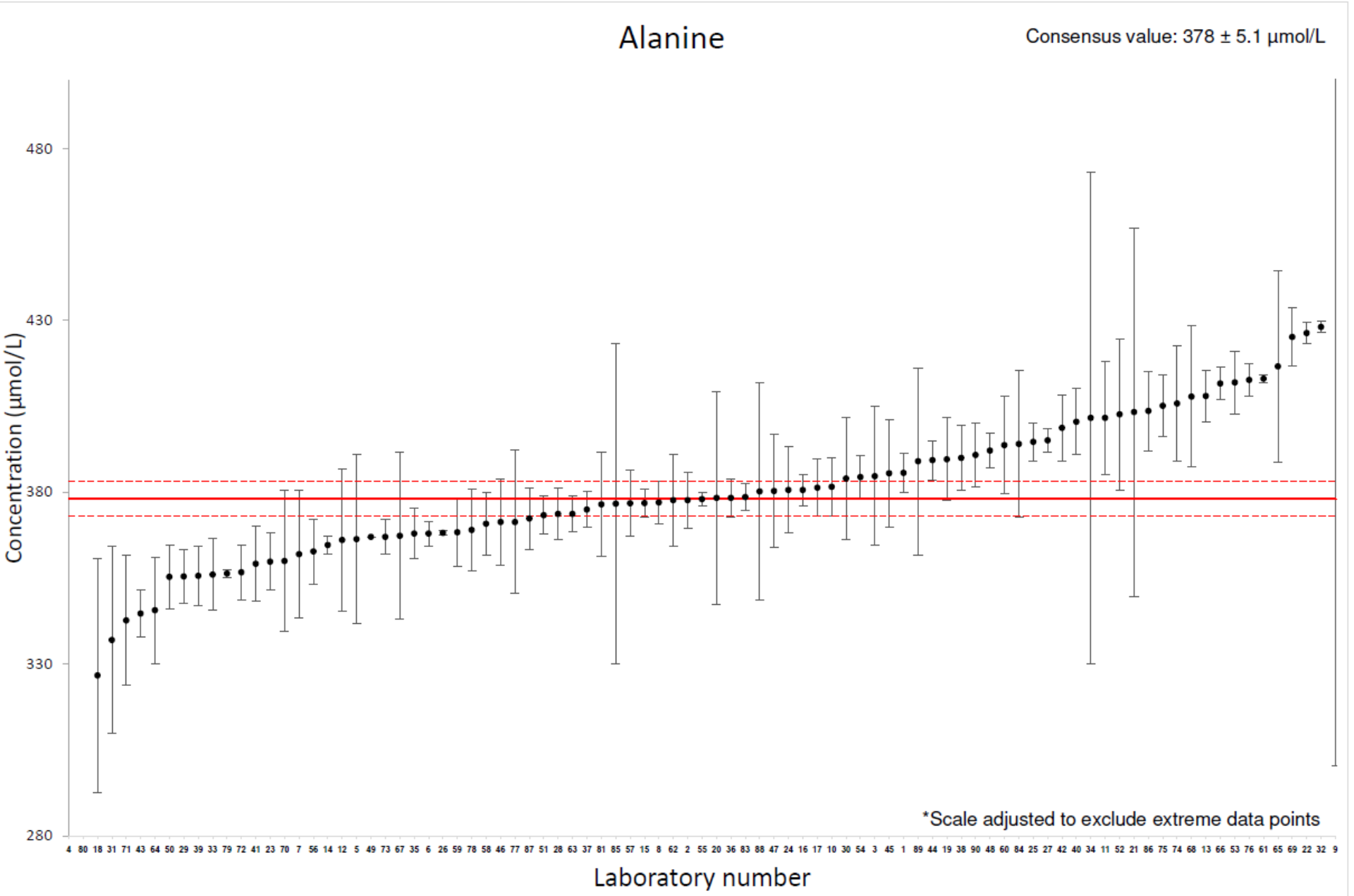
Aims of study

1. Compare method performance to an absolute value
2. Determine true inter-laboratory variation and bias for measurement of phe & leu
3. Understand instrument/calibration variation and bias


Phenylalanine
Certified Value: 368.2 ± 9.0 µmol/L (displayed)
Consensus value: 359 ± 5.2 µmol/L
Certified
Consensus
Concentration (µmol/L)
Laboratory number
*Scale adjusted to exclude extreme data points


Citrulline
Consensus value: 30 ± 0.9 µmol/L
Concentration (µmol/L)
55
50
45
40
35
30
25
20
15
61 80 5 3 24 23 26 21 62 79 36 14 29 43 71 56 8 6 50 39 11 15 35 87 48 58 57 88 70 72 44 2 33 7 83 84 55 73 59 25 37 77 89 47 38 13 49 90 52 78 31 19 27 45 86 85 32 18 42 74 63 67 60 17 1 41 68 4 54 12 51 10 69 66 22 76 46 40 20 81 75 64 30 34 9 28 53 65 16
Laboratory number

Analyte	Total results submitted (Number of not detected)	Consensus value (µmol/L ± U (*k*=2))	Average %CV	Inter-laboratory %CV
1-methylhistidine	63 (1)	-	3.5	-
3-methylhistidine	63 (10)	-	9.1	-
Alanine	89	378 ± 5.1	2.4	5.0
Anserine	47 (40)	-	-	-
Arginine	89 (1)	59 ± 1.6	7.1	10.0
Carnosine	50 (47)	-	-	-
Citrulline	89 (1)	30 ± 0.9	4.0	11.4
Glutamate	88	104 ± 2.6	3.8	9.3
Glutamine	89	450 ± 10.8	2.9	9.1
Glycine	89	258 ± 3.9	2.6	5.8
Histidine	89	80 ± 1.9	3.1	8.7
Isoleucine	89	81 ± 1.6	3.2	7.4
Leucine* *151.0 ± 3.2 µmol/L*	89	149 ± 2.7	2.3	6.9
Lysine	89	175 ± 3.0	2.3	6.5
Methionine	89	27 ± 0.6	3.3	8.5
Ornithine	89 (1)	109 ± 2.4	3.4	8.3
Phenylalanine* *368.2 ± 9.0 µmol/L*	89	359 ± 5.2	2.0	5.4
Proline	88 (1)	229 ± 4.3	3.6	7.0
Serine	89	107 ± 2.1	3.2	7.3
Taurine	84 (1)	49 ± 0.8	3.6	6.2
Threonine	89 (1)	147 ± 2.1	2.7	5.4
Tryptophan	74 (3)	53 ± 2.9	4.2	18.6
Tyrosine	89	66 ± 1.1	2.4	6.3
Valine	89	236 ± 3.8	2.8	6.0


Alanine
Consensus value: 378 ± 5.1 µmol/L
Concentration (µmol/L)
480
430
380
330
280
*Scale adjusted to exclude extreme data points
4 80 18 31 71 43 64 50 29 39 33 79 72 41 23 70 7 56 14 12 5 49 73 67 35 6 26 59 78 58 46 77 87 51 28 63 37 81 85 57 15 8 62 2 55 20 36 83 88 47 24 16 17 10 30 54 3 45 1 89 44 19 38 90 48 60 84 25 27 42 40 34 11 52 21 86 75 74 68 13 66 53 76 61 65 69 22 32 9
Laboratory number

Cognitive Amino Acid Scheme

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- *Pilot scheme, started in 2017*
- *Approx 50 labs participate (limited to one return per lab)*
- *Six cases distributed annually (3 cases, twice a year)*
- *18 cases distributed to date*
- *Participants receive clinical details and a set of amino acid results*
- *Participants score based on*
  - *abnormalities identified in profile*
  - *suggested diagnosis*
  - *recommendations for further testing*
- *Scientific Advisor: Sabine Scholl-Burgi plus 3 additional advisors*

Case 1
Brian

Past medical history/family history

- *Female infant*
- *Consanguineous parents*
- *Born at 38 weeks gestation, emergency C section*
- *Maternal type 2 diabetes*
- *Treated during her pregnancy with insulin and metformin*

- *Presented day 1 of life*
- *Hypoglycaemia*

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Presentation

- *? sepsis*
- *Admitted to NICU (age 2 hrs)*
- *Blood glucose 0.7 mmol/L*
- *Required dextrose at 20% to maintain normoglycameia*

- *Hypoglycaemia screen requested*

- *Ammonia - normal*

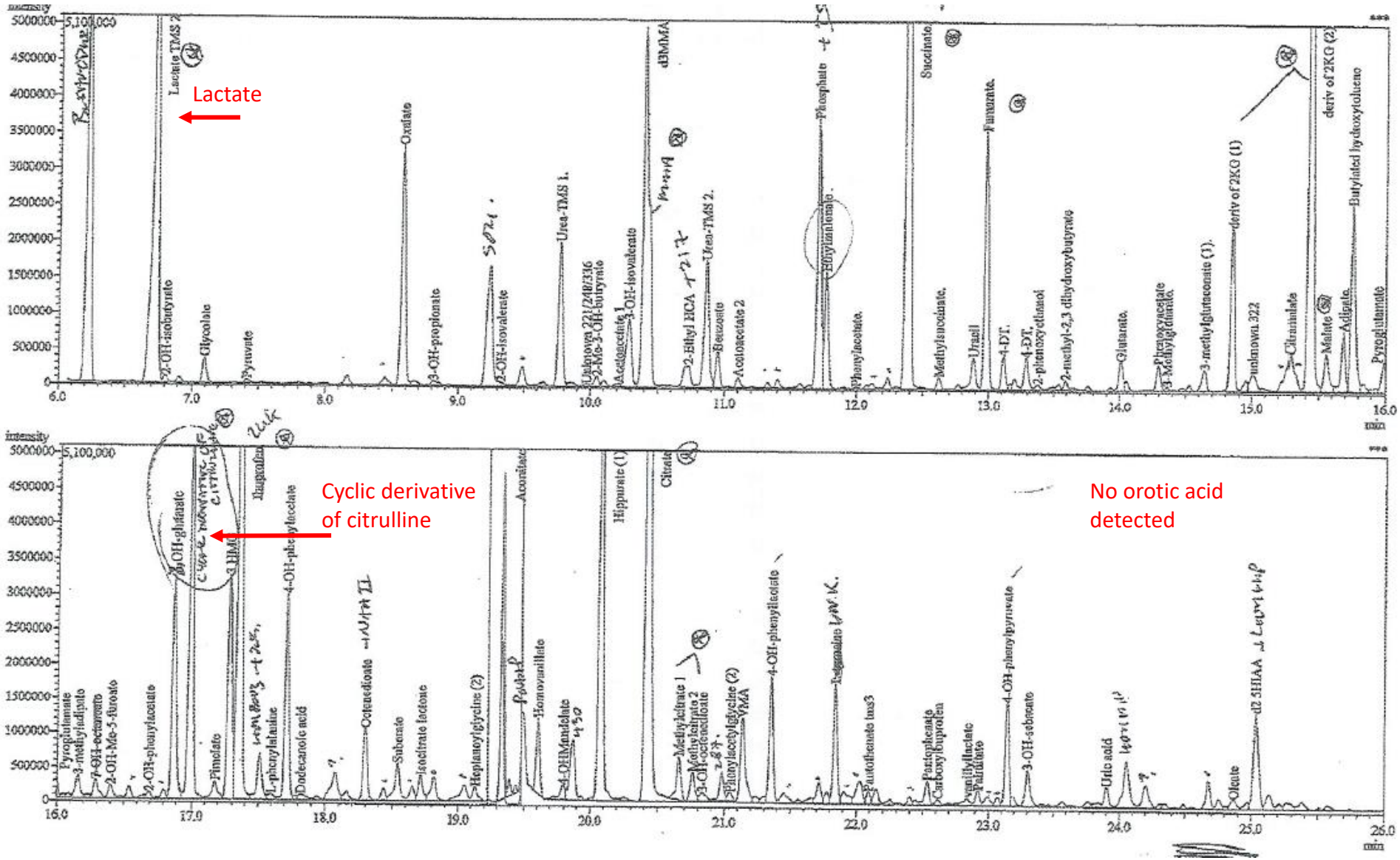
Case 1

Plasma Amino Acids

Amino acid	µmol/L	Reference range		
Taurine	130	19		173
Aspartate				
Threonine	**279**	32		239
Serine	177	52		231
Asparagine				
Glutamate	81	21	-	174
Glutamine	719	307	-	768
Proline	261	45		452
Glycine	**486**	81	-	303
Alanine	551	112		686
Citrulline	**294**	8	-	57
a-aminobutyrate				
Valine	126	95		566
Cystine				
Methionine	37	10		53
Isoleucine	38	15		159
Leucine	55	5		264
Tyrosine	90	26		154
Phenylalanine	68	34		110
Ornithine	38	20		144
Lysine	250	61		337
Histidine	97	40		143
Arginine	**23**	26	-	180

Case 1

Organic Acids



Diagnosis: Citrullinaemia type 1

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Confirmatory testing

Original report issue[redacted]

Gender: Female
Hospital:
Hospital Number:
Referring Physician: [redacted]
Referral for: Citrullinaemia
Investigation: Citrulline incorporation
Specimen: Skin biopsy
Specimen Number:
Date Received: [redacted]
Date Taken:
Notes: ? Citrullinaemia type 1 but mild.

Reason for referral: Term female infant with biochemical parameters suggestive of citrullinaemia type I but with normal ammonia t

Incorporation of label f
normal and one affected

RESULTS (patom/6h/m

[redacted]

Unaffect

Affected

All samples assayed at t

COMMENT

The patient values are
affected control.

[redacted]sults are con
(Results must
acid analysis).

RESULTS (patom/6h/mg protein):

[redacted]

Unaffected controls	1	64	72	61
	2	4955	6320	6290
		3459	3403	2744
Affected Control		10	21	12

All samples assayed at the same time.

NOTE: DNA sequencing of the *ASS1* gene is currently underway to confirm this diagnosis: report to follow.

Functional studies in fibroblasts consistent with citrullinaemia type 1

RESULTS

Gene	Ref Seq	Disorder	Detected Sequence Variants	Pathogenic Potential[2]
ARG1	NM_000045.3	Argininaemia	-	-
ASL	NM_000048.3	Argininosuccinic aciduria	-	-
ASS1	**NM_000050.4**	**Citrullinaemia**	**Homozygous c.950T>C p.(Phe317Ser)**	**Likely pathogenic**
CPS1	NM_001122633.2	Carbamyl phosphate synthetase deficiency	-	-
NAGS	NM_153006.2	N-acetylglutamate synthase deficiency	-	-
OAT	NM_000274.3	Ornithine aminotransferase deficiency	-	-
OTC	NM_000531.5	OTC deficiency	-	-
SLC25A13	NM_001160210.1	Citrin deficiency	-	-
SLC25A15	NM_014252.3	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	-	-

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Case 1 – Brian

Confirmatory testing

Original report issue [redacted]

Gender: Female
Hospital:
Hospital Number: [redacted]
Referring Physician:
Referral for: Citrullinaemia
Investigation: Citrulline incorporation
Specimen: Skin biopsy
Specimen Number: [redacted]
Date Received:
Date Taken:
Notes: ? Citrullinaemia type 1 but mild.

Reason for referral: Term female infant with biochemical parameters suggestive of citrullinaemia type I but with normal ammonia test results.

Incorporation of label from ^{14}C-citrulline into protein was measured in triplicate in cultured fibroblasts. Two normal and one affected fibroblast cultures were assayed in parallel.

RESULTS (patom/6h/mg protein):

[redacted] 64 72 61

Functional studies in fibroblasts consistent with citrullinaemia type 1

RESULTS

Gene	Ref Seq	Disorder	Detected Sequence Variants	Pathogenic Potential[2]
ARG1	NM_000045.3	Argininaemia	-	-
ASL	NM_000048.3	Argininosuccinic aciduria	-	-
ASS1	**NM_000050.4**	**Citrullinaemia**	**Homozygous c.950T>C p.(Phe317Ser)**	**Likely pathogenic**
CPS1	NM_001122633.2	Carbamyl phosphate synthetase deficiency	-	-
NAGS	NM_153006.2	N-acetylglutamate synthase deficiency	-	-
OAT	NM_000274.3	Ornithine aminotransferase deficiency	-	-
OTC	NM_000531.5	OTC deficiency	-	-
SLC25A13	NM_001160210.1	Citrin deficiency	-	-
SLC25A15	NM_014252.3	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	-	-

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Citrullinemia type 1

- *Autosomal recessive*
- *Argininosuccinate synthetase deficiency*
- *SLC25A3 gene encoding aspartate-glutamate transporter*
- *Classical citrullinemia – typical plasma citrulline in range 2000-50000 umol/L. Accumulation of glutamine and ammonia, depletion of arginine, increased orotate*

- *Mild variant*
- *Biochemical phenotype, asymptomatic*

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Mild citrullinemia in Caucasians is an allelic variant of argininosuccinate synthetase deficiency (citrullinemia type 1)

Johannes Häberle,* Silke Pauli, Eva Schmidt, Barbara Schulze-Eilfing, Christoph Berning, and Hans Georg Koch

Universitätsklinikum Münster, Klinik und Poliklinik für Kinderheilkunde, Albert-Schweitzer-Strasse 33, D-48149 Münster, Germany

Received 26 June 2003; received in revised form 15 August 2003; accepted 15 August 2003

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Management

- *Arginine 75mg/Kg/day*
- *Mild protein restricted diet*
- *Emergency regimen*

Proficiency scoring 2021.01

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Mild variant citrullinaemia type 1.

The results provided were from an asymptomatic girl who exhibited hypoglycaemia at age of 1 day. Repeat plasma amino acids taken on 14th day of life showed citrulline 2605, arginine 22, ornithine 490, glycine 667, glutamine 584 and glutamate 300 µmol/L. Ammonia = 54 µmol/L.

Current treatment comprises low protein intake plus essential amino acid mixture, sodium benzoate and arginine.

The diagnosis was confirmed by mutation analysis showing a homozygosity for likely pathogenic, novel variant (c.950T>C) in *ASS1*-gene.

Correct findings / abnormalities

Increases of plasma citrulline (1 point), threonine and (glycine) (0.5 points) and mention of low concentration of arginine (0.5 points) were considered necessary for full points. Maximum 2 points.

Correct Diagnosis

The diagnosis of mild variant citrullinaemia type 1 was scored with 2 points. One point was scored for mention of urea cycle disorder or citrin deficiency. Maximum 2 points.

Further tests

Genetic testing (*ASS1*-gene) and functional studies (including repeat amino acids) were scored with 1 point each.

Comments on overall performance

Overall proficiency was relatively poor with a score of 68%. The diagnosis and further testing recommendations scored lower than identification of the abnormalities.

Case 2
Brian

Past medical history/family history

- *Female*
- *28 years old*
- *Diagnosed with alveolar proteinosis age 16yrs*
- *Short stature, treated with GH in teens*

Presentation

- *Gastrointestinal symptoms*
- *Liver histology showed glycogen deposits*
- *? GSD*

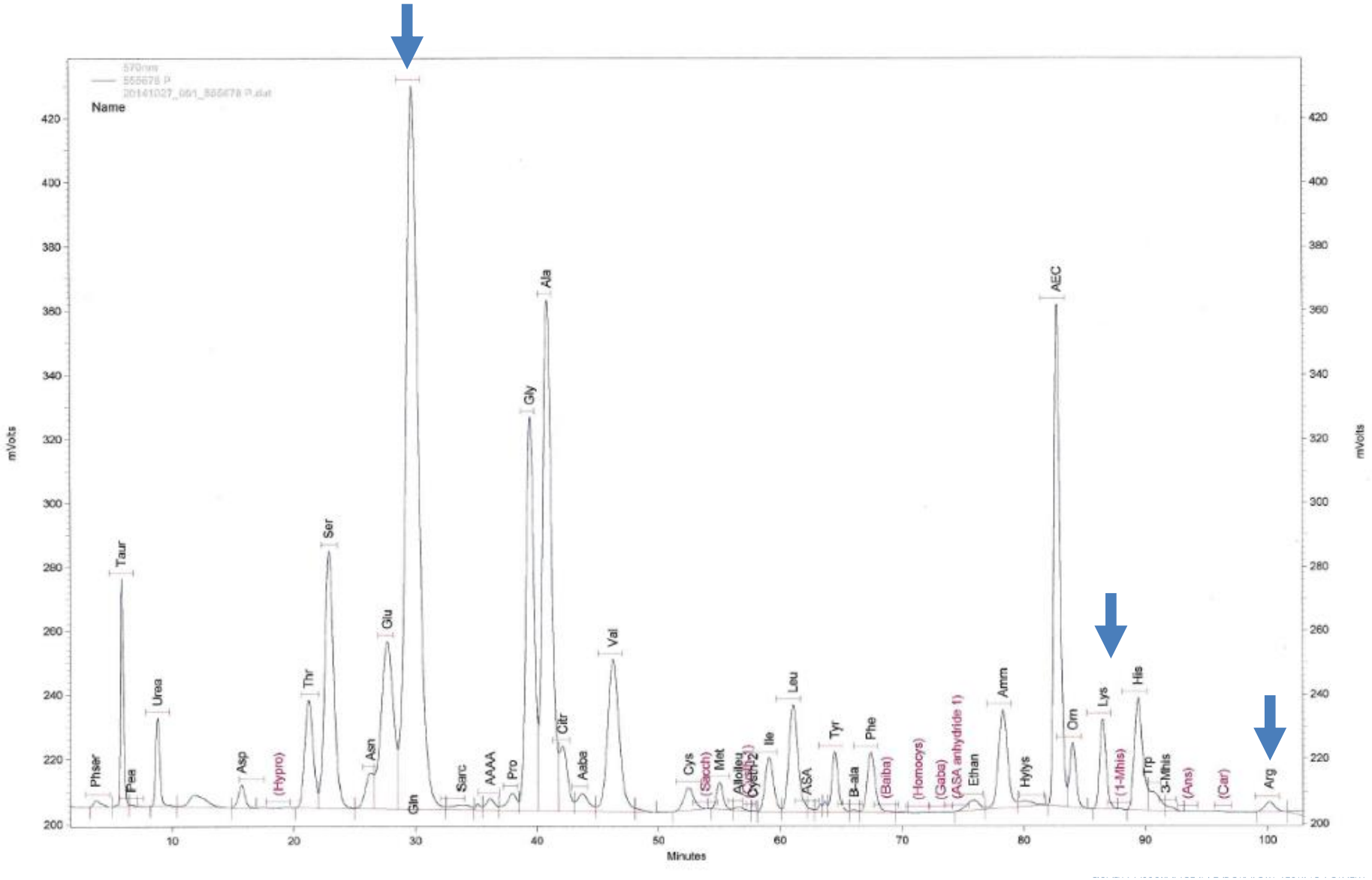
Case 2 – Brian

Plasma Amino Acids

Plasma Amino acids	Value (umol/L)	Reference interval	
Taurine	66	19-173	
Threonine	76	38 - 239	
Serine	181	51 - 231	
Glutamate	160	21 - 174	
Glutamine	**788**	307 - 768	
Proline	197	45 - 452	
Glycine	242	81 - 303	
Alanine	384	112 - 686	
Citrulline	39	8 - 57	
Valine	151	96 - 566	
Methionine	13	10 - 53	
Isoleucine	39	26 - 159	
Leucine	81	50 - 264	
Tyrosine	31	26 - 154	
Phenylalanine	39	34 - 110	
Ornithine	28	20 - 144	
Lysine	**44**	61 - 337	
Histidine	65	40 - 143	
Arginine	**8**	26 - 180	

Case 2 – Brian

Plasma Amino Acids



Urine Amino Acids

Urine organic Acids

No obvious organic disorder
Orotic acid – not detected

Urine Amino acids	Value (umol/L)	Reference interval
Ornithine	6	4 - 15
Lysine	394	7 - 58
Cystine	41	4 - 15
Arginine	52	3 - 11

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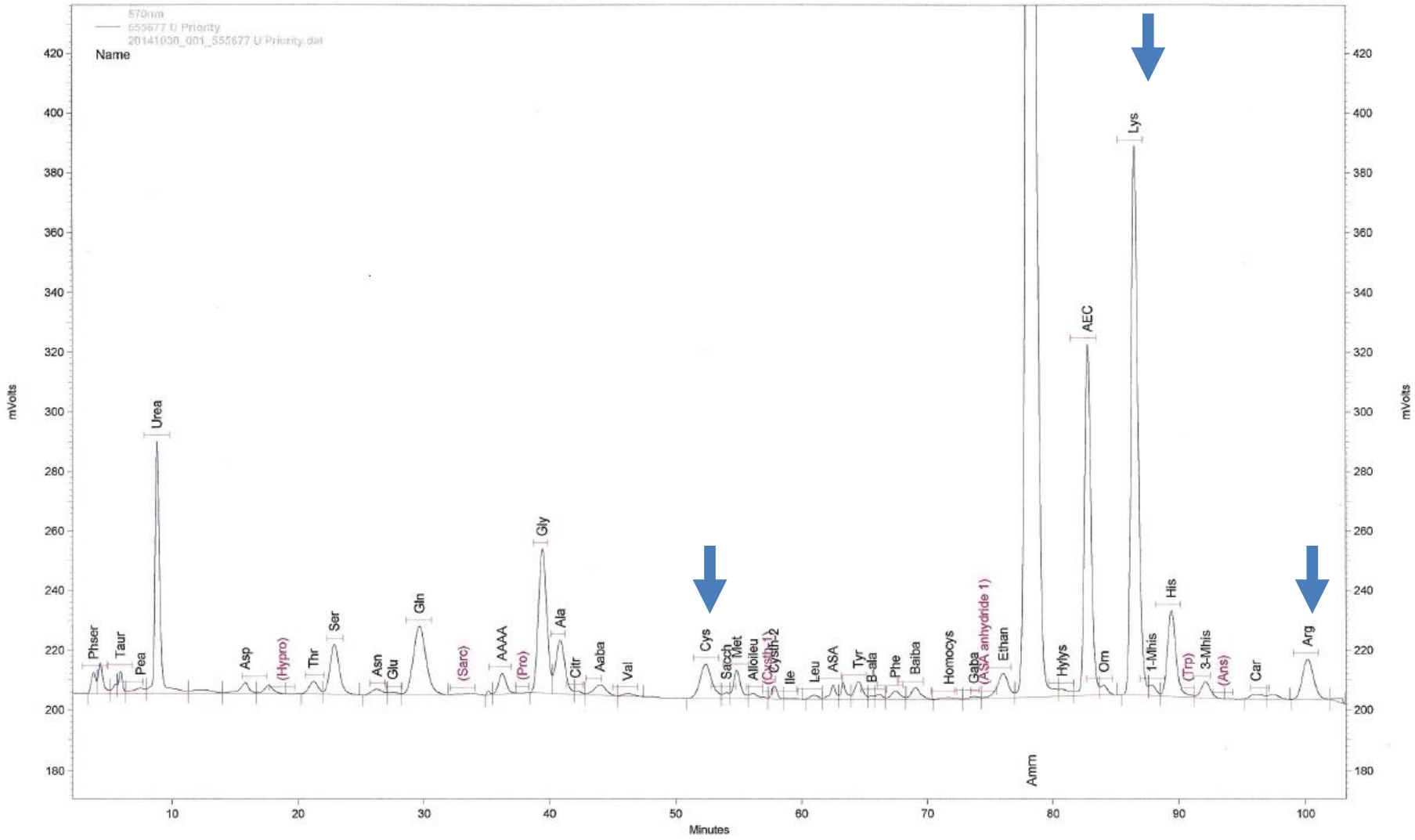
Case 2 – Brian

Urine Amino Acids

Urine organic



Diagnosis: Lysinuric protein intolerance

Confirmatory testing

- *Plasma ammonia*
- *Mutation analysis*
- *SLC7A7 gene*
- *LDH/ferritin*

LPI

- *Inherited aminoaciduria caused by defective cationic amino acid transport*
- *Mutations in the SLC7A7 gene*
- *Initially described in Finland*
- *Symptoms typically present after weaning*
- *Refusal of feeds, vomiting, FTT*
- *Hepatosplenomegaly, haematological and neurological involvement*
- *Hyerammonemic coma*

- *Major complications – pulmonary alveolar proteinosis and renal disease*
- *Variable clinical presentation*
- *Delayed / misdiagnosis common*

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Management

- *Protein restricted diet*
- *Citrulline supplementation*
- *Medical control of hyperammonaemia*
- *Lysine and carnitine supplements*

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Proficiency scoring 2017.02

Sample details:
These results were from a patient with LPI (lysinuric protein intolerance). Plasma amino acids with selected urinary levels were provided.

Correct findings / abnormalities:

There was slightly elevated plasma glutamine with low levels of arginine and lysine together with elevated lysine, cystine and arginine in urine. This was considered correct. Mention of some, if not all of these abnormalities scored two points.

Correct Diagnosis and further tests:
LPI was correct (1 point).
Further testing should include plasma ammonia, urinary orotic acid and confirmation by mutation analysis of the SLC7A7 gene (1 point). LDH/ferritin measurement was correctly recommended by ten labs.

Comments on overall performance:
Excellent with 99% overall proficiency.

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Case 3
Mary Anne

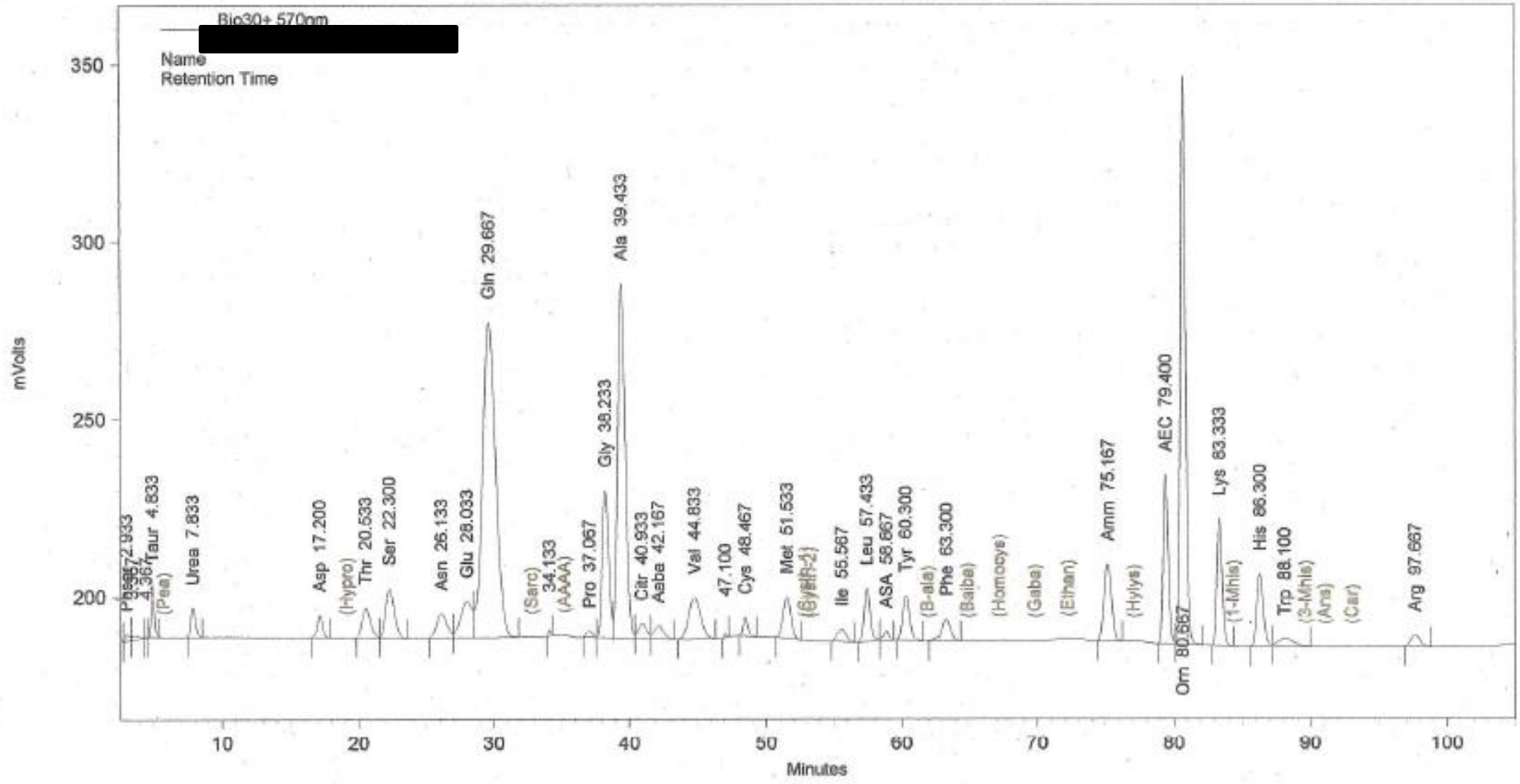
Case 3 - 1y old

- Tongue bleed for 4 days, required suturing
- Referred to haematology team
- Anaemic, prolonged PT and PTT
- Mild transaminitis
- Low clotting factors VII, V and X
- Treated with transfusion, tranexamic acid, FFP
- Repeat bloods showed abnormal LFTs and clotting

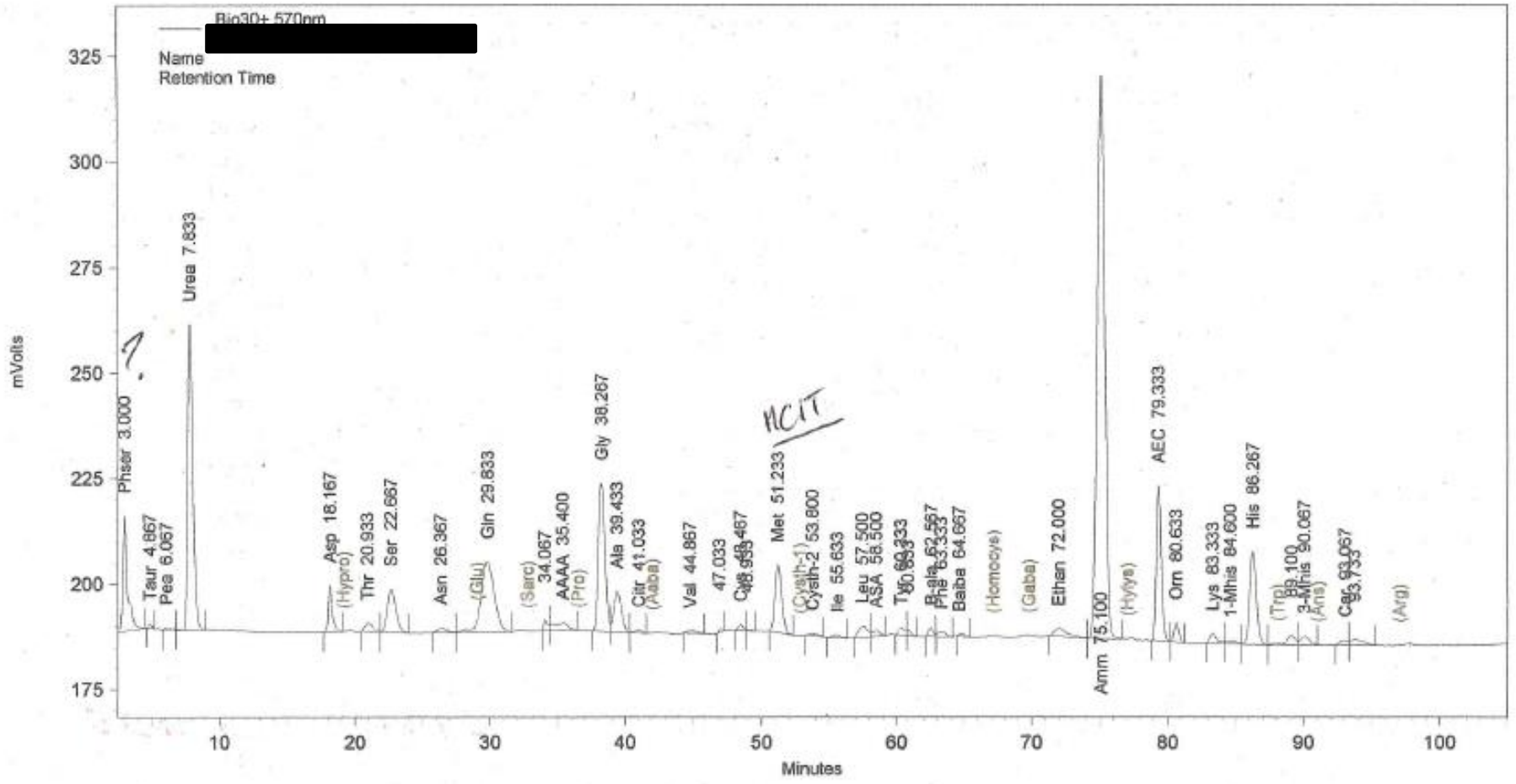
- Admitted under liver team
  - Ast 208 IU/L (0-41)
  - Alt 861 umol/L (0-41)
  - Urea 2.0 mmol/L (2.5-6.5)
  - Ammonia 207umol/L (<75)
  - PT 60 sec (9-13)
  - PTT 57 sec (24-32)
  - Plasma quantitative amino acids
  - Urine amino acids - grossly raised homocitrulline
  - Urine organic acids - increased orotate and uracil
- Referred to IMD team
  - Commenced Ravicti, citrulline, low protein diet
  - HHH syndrome confirmed by DNA analysis (SLC25A15 gene)
  - LFTs and clotting have now normalised

- HHH may present with liver failure and severe coagulation abmormalities

Plasma amino acids



Urine amino acids



Comment on the amino acid results

- Increased glutamine
- Increased ornithine
- Increased alanine & proline (lactate 2.4 mmol/L)
- Increased methionine
  - May be due to interference from homocitrulline on Biochrom analyser or due to liver failure?
  - I have reviewed amino acids on our HHH patients and this is the only one that has an increased ‘methionine’

Plasma Amino Acids

	µmol/L	reference range		
Glutamine	1023	356	-	768
Alanine	727	305	-	700
Glycine	219	119	-	525
Proline	331	95	-	332
Valine	139	83	-	384
Threonine	62	115	-	385
Lysine	159	36	-	171
Serine	103	63	-	245
Glutamic Acid	105	53	-	175
Leucine	75	26	-	134
Taurine	54	58	-	225
Histidine	105	32	-	125
Ornithine	605	40	-	102
Arginine	26	21	-	103
Tyrosine	71	17	-	105
Phenylalanine	45	33	-	129
Isoleucine	32	37	-	95
Cystine	17	29	-	127
Methionine	77	5	-	38
2-amino n-butyric acid	33	9	-	42
Aspartic Acid	26	7	-	37
Citrulline	32	5	-	24

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Plasma Amino Acids

	µmol/L	reference range		
Glutamine	1023	356	-	768
Alanine	727	305	-	700
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Glutamic Acid	105	53	-	175
Leucine	75	26	-	134
Taurine	54	58	-	225
Histidine	105	32	-	125
Ornithine	605	40	-	102
Arginine	26	21	-	103
Tyrosine	71	17	-	105
Phenylalanine	45	33	-	129
Isoleucine	32	37	-	95
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Diagnosis: HHH syndrome

HHH syndrome

- *Autosomal recessive*

- *Neonatal onset (8%) – hyerammonemia within 24 – 48hrs of feeding*

- *Infantile, childhood and adult onset (92%)*
- *May present with:*
  - *chronic effects - developmental delay, ataxia, spasticity, learning disabilities, cognitive deficits, and/or seizures*
  - *acute encephalopathy secondary to hyperammonemic crisis*
  - *Chronic liver dysfunction*

Management

- *Control hyperammonemia*
- *Protein restricted diet*
- *Citrulline supplementation*

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Proficiency scoring 2021.02

HHH Syndrome (hyperammonaemic, hyperornithinaemia, homocitrullinaemia)

Sample details
The results were from a 1.5 year *old boy presenting initially with mouth bleed. The patient has HHH syndrome due to a mutation in *SLC25A15*-gene.

Correct findings / abnormalities
Increased plasma glutamine (0.5), alanine (0.5), ornithine (0.5), citrulline (0.5) and methionine (0.5) were the main findings scored with maximum of two points. Mention of the slightly decreased concentration of threonine, cysteine and isoleucine were not scored for this case.

Correct Diagnosis
The diagnosis of HHH syndrome scored two points.

Further tests
Maximum 2 points were given. Measurement of ammonia, homocitrulline and/or functional testing scored one point, molecular genetic studies of *SLC25A15*-gene was scored with 1 point.

Comments on overall performance
Performance was good with 89% overall proficiency.

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Case 4
Sabine

Medical history/family history

- *1. child, 2. pregnancy*
- *Normal pregnancy, normal birth*

- *Newborn screening positive, with highly elevated methionine*

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Amino acids

Laboratory results	
homocysteine	40.8 µmol/L

amino acids in plasma				
	µmol/L	reference range*		
Taurine	105	10	-	167
Aspartate	6	0	-	31
Threonine	187	46	-	222
Serine	213	92	-	178
Asparagine	99	38	-	121
Glutamate	52	8	-	179
Glutamine	480	402	-	776
Proline	282	97	-	254
Glycine	188	154	-	338
Alanine	319	142	-	421
Citrulline	14	8	-	36
Valine	196	79	-	217
Cystine	51	6	-	43
Methionine	1087	9	-	44
Isoleucine	76	12	-	77
Leucine	132	46	-	147
Tyrosine	114	13	-	91
Phenylalanine	48	25	-	74
Ornithine	96	41	-	129
Lysine	241	69	-	200
Histidine	93	37	-	83
Arginine	107	7	-	128

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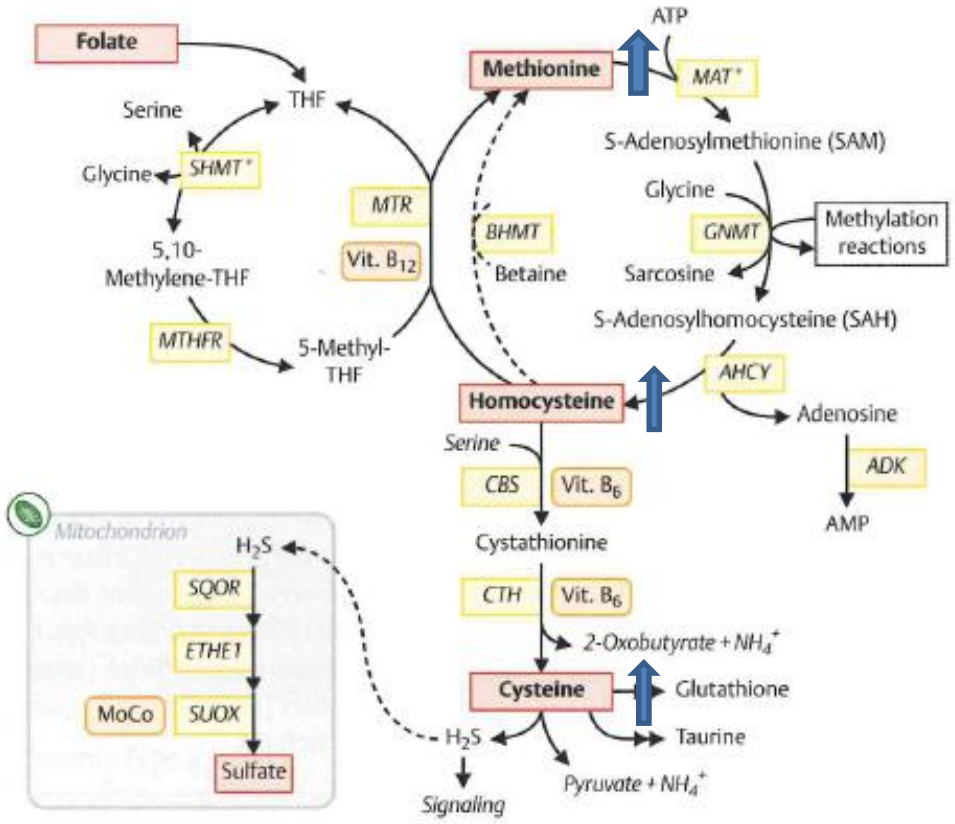
Metabolic pathway



From Zschocke and Hoffmann, Vademecum metabolicum, 2021, p. 88

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- ***Abnormalities:*** *"Increased homocysteine, extremely large increase in methionine, normal phenylalanine and insignificant increase in tyrosine, small increase in cystine and insignificant increases in other amino acids."*
  *(**Scoring:** elevated met 1 point, elevated hcy 1 point, cys not decreased 1 point, maximum 2 points)*
- ***Interpretation:*** *"MAT1/III deficiency. Other conditions associated with increased methionine are symptomatic. Methionine of this magnitude in CBS deficiency would be on betaine treatment and treated from birth."*
  *(**Scoring:** MATI/III deficiency 2 points, CBS deficiency 1 point, maximum 2 points)*
- ***Recommendation:*** *"Plasma S-adenosylmethionine (where available). DNA mutation analysis of relevant genes i.e. MAT1/III, (GNMT, SAHH, ADK, CBS)."*
  *(**Scoring:** SAM/SAH 1 point, molecular genetic studies (MATI/III gene) 2 points, CBS-gene 1 point, maximum 2 points)*

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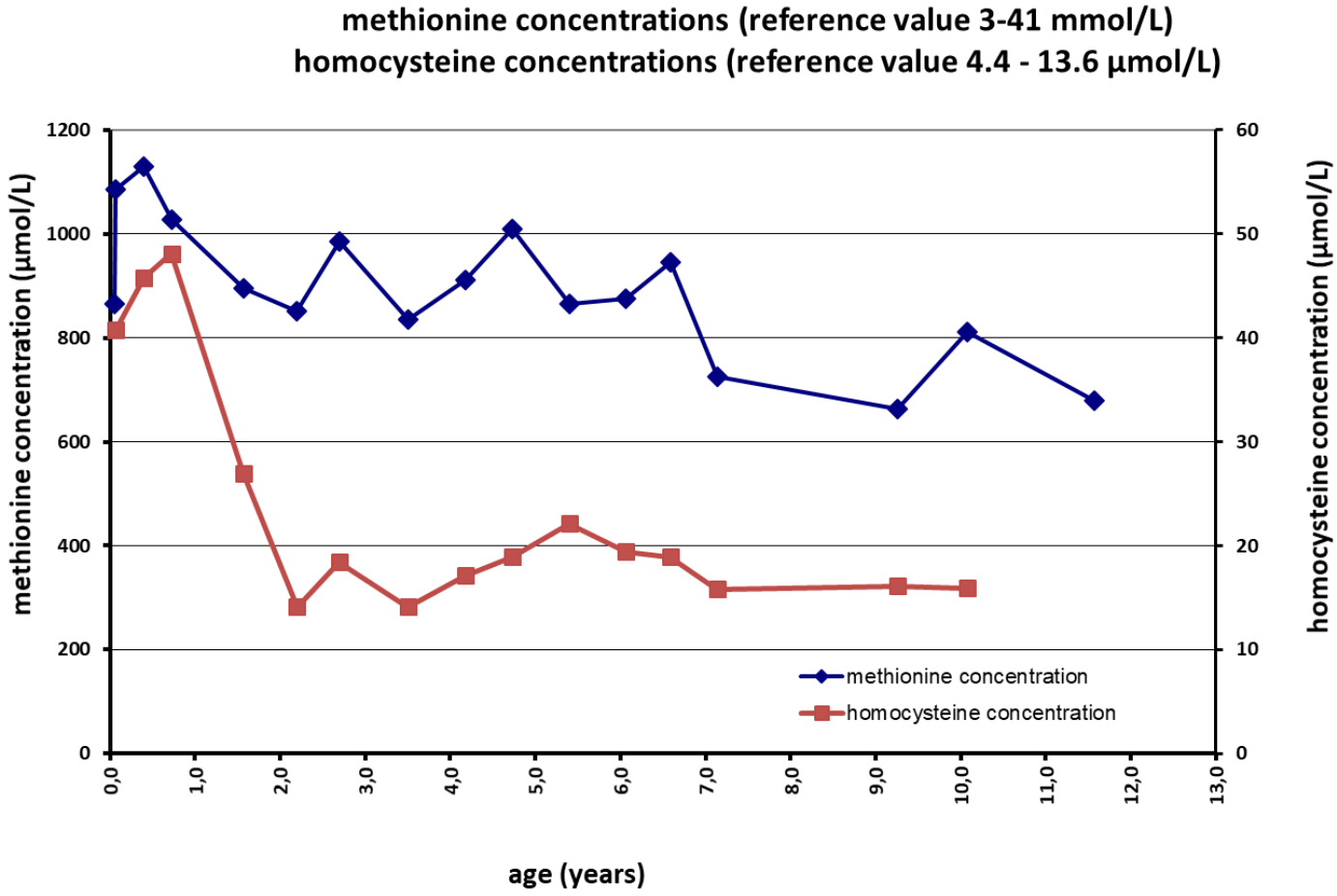
Outcome



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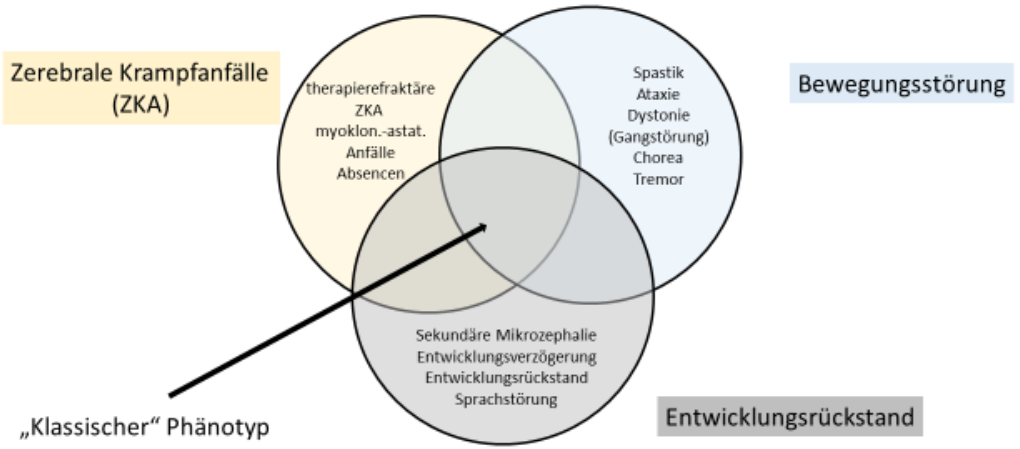
Case 5
Sabine

Clinical Signs & Symptoms

- *periodic ataxia*
- *movement disorder*
- *muscular hypotonia (since age 3 years)*
- *(family history unremarkable)*



CSF glucose	↓	30 mg/dL
Plasma glucose		76 mg/dL
Ratio	↓	0.39

Schönlaub A et al, Neuropädiatrie 2021, in press

Glut1-DS on ketogenic diet
ketosis (ß-hydroxybutyric acid 3.447 mmol/L)
Heterocygous for mutation (c.1A>G in intron 1) in *SLC2A1*- gene

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Amino acids

amino acids in plasma				
	µmol/L	reference range*		
Taurine	91	20	-	120
Aspartate	6	1	-	17
Threonine	192	40	-	204
Serine	166	70	-	194
Asparagine	51	13	-	83
Glutamate	40	14	-	78
Glutamine	484	333	-	809
Proline	184	40	-	332
Glycine	248	107	-	343
Alanine	239	120	-	600
Citrulline	23	8	-	47
Valine	514	132	-	480
Cystine	14	23	-	68
Methionine	30	3	-	43
Alloisoleucine	1			
Isoleucine	160	6	-	122
Leucine	274	30	-	246
Tyrosine	77	19	-	119
Phenylalanine	65	26	-	98
Ornithine	108	20	-	136
Lysine	191	66	-	270
Histidine	97	47	-	135
Arginine	75	12	-	112

Ratio BCAA/alanine helpful?

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- ***Abnormalities:*** *"Slightly increased branched chain amino acids. Valine > Leucine."*
  *(**Scoring:** elevated ile, leu, val each 1 point, maximum 2 points)*
- ***Interpretation:*** *"Results suggest ketosis, but may be dietary. Is patient on ketogenic diet (e.g. GLUT1 deficiency)? Is patient on protein restriction + aa supplementation (e.g. GA-1)? Has MSUD been excluded?"*
  *(**Scoring:** ketosis due to ketogenic diet in a patient with Glut1-DS, 2 points, (mild) MSUD 1 point, maximum 2 points)*
- ***Recommendation:*** *"Check for alloisoleucine to exclude MSUD. CSF glucose and plasma acylcarnitines if GLUT1 & GA-1 have not previously been excluded. Contact requestor for dietary information."*
  *(**Scoring:** clinical investigation, medical history 1 point, amino acids (with alloisoleucine) 1 point, ketone bodies in plasma 1 point, organic acids in urine 1 point, maximum 2 points)*

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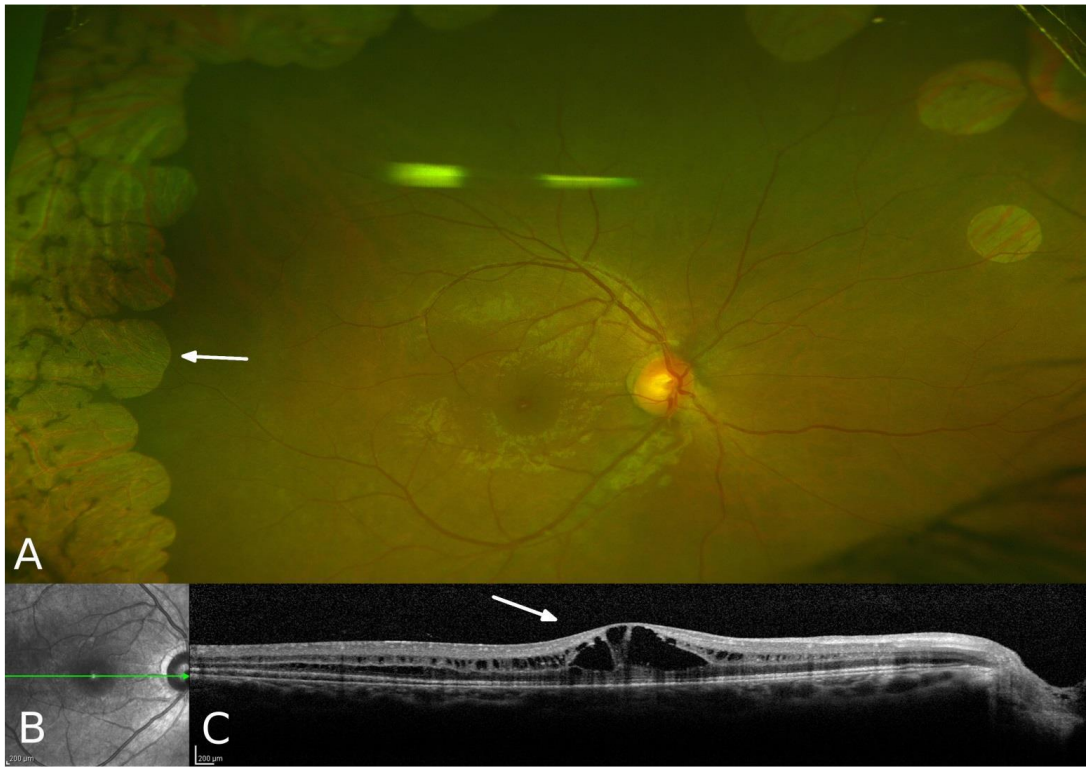
Case 6
Sabine

Clinical Signs & Symptoms

- *Myopia*
- *Short stature (delay of bone age)*



Fundus: *chorioretinal changes*

Optical coherence tomography: *bilateral cystoid edema*

Michel M et al, Klin Pediat 2015

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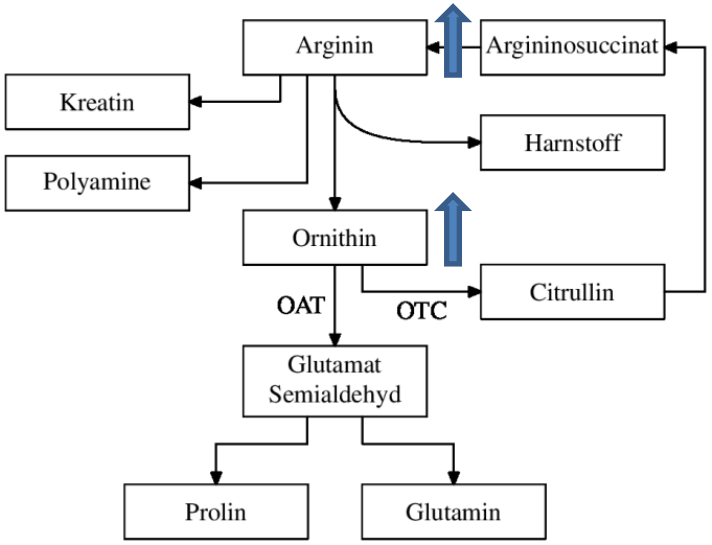
Amino acids

amino acids in plasma				
	µmol/L	reference range*		
Taurine	48	20	-	90
Aspartate	4	3	-	15
Threonine	112	102	-	246
Serine	138	92	-	196
Asparagine	39	34	-	94
Glutamate	25	17	-	69
Glutamine	525	457	-	857
Proline	279	58	-	324
Glycine	199	166	-	330
Alanine	240	242	-	594
Citrulline	31	19	-	52
Valine	208	155	-	343
Cystine	36	36	-	58
Methionine	21	13	-	41
Isoleucine	56	34	-	106
Leucine	104	86	-	206
Tyrosine	87	35	-	107
Phenylalanine	60	34	-	86
Ornithine	878	47	-	195
Lysine	89	116	-	276
Histidine	81	68	-	108
Arginine	92	1	-	81



Michel M et al, Klin Pediat 2015

ornithine aminotransferase deficiency (OAT deficiency)
Homozygous for mutation (c.498C>A in exon 4) in *OAT*- gene

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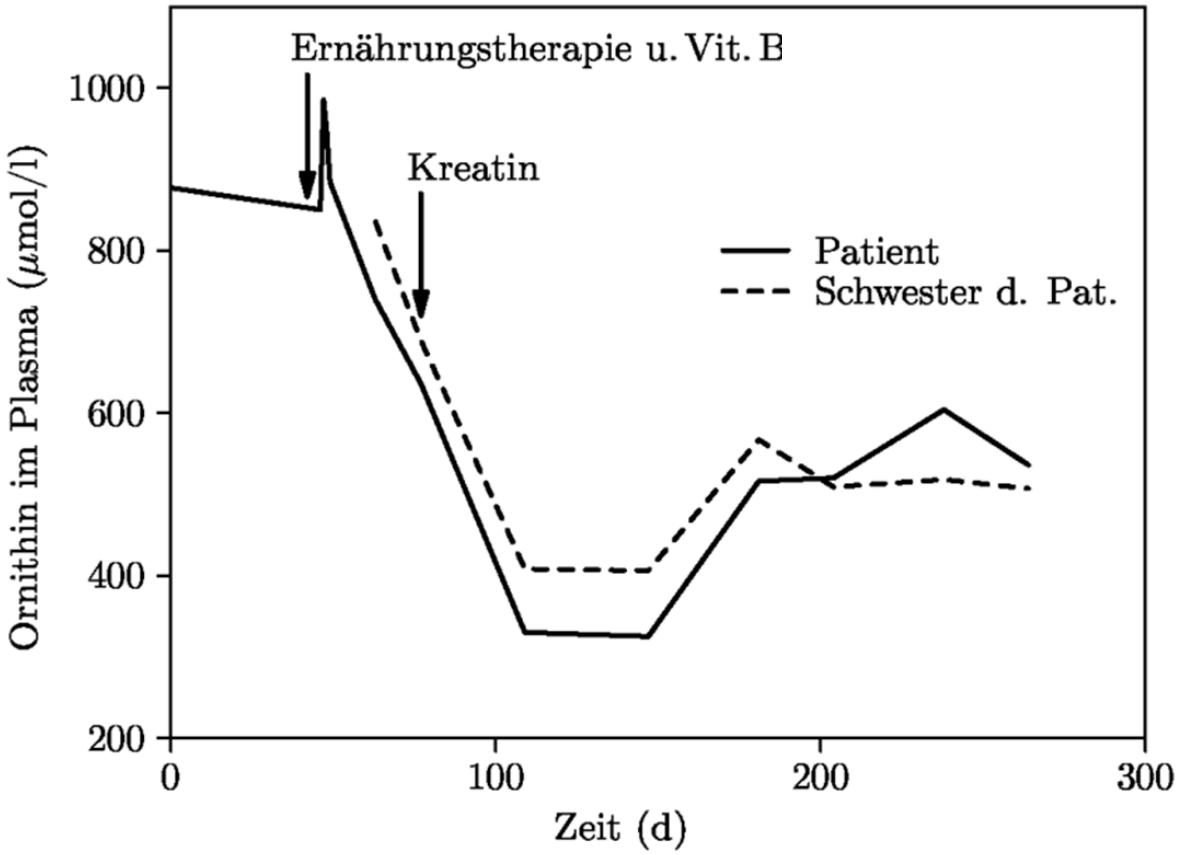
Therapy



- *Protein defined nutrition*
- *Creatine*
- *Amino acids mixture*
- *Vitamin B6*

Michel M et al, Klin Pediat 2015

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- ***Abnormalities:*** *"Grossly elevated ornithine and slightly elevated arginine. Slightly low alanine and lysine are probably unspecific."*
  *(**Scoring:** elevated orn 2 points, slightly elevated arginine, maximum 2 points)*
- ***Interpretation:*** *"Hyperornithinemia with gyrate atrophy (OMIM 258870). Differential diagnosis: HHH syndrome (238970), but this does not fit with phenotype.."*
  *(**Scoring:** OAT deficiency 2 points, HHH syndrome 1 point, maximum 2 points)*
- ***Recommendation:*** *"Repeat the amino acids in plasma. Go for genetics (OAT gene).Order ophthalmological examination (myopia and cataract). Start with arginine-restricted diet/low-protein diet, check the bones!"*
  *(**Scoring:** orotic acid, ammonia, homocitrulline (=amino acids), creatine each 1 point, molecular genetic studies (gene) 1 point, ophthalmologic examination; maximum 2 points)*

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ERNDIM Planning

			changes in plasma			2021	2020	2019	2018	2017
			elevated	decreased						
Participation						Pilot	Pilot	Pilot	Pilot	Pilot
primary amino acid changes (= diagnostic)	urea cycle disorders	OTC deficiency	gln	cit, arg				84%	63%	
		ASS deficiency	gln, cit	arg		1				
		ARG deficiency	gln, arg						88%	
		HHH syndrome	gln, orn			2				
		Citrin deficiency	cit, thr, met, tyr						75%	
	disorders of aromatic AA	tyrosinemia type III	tyr						79%	
	disorders of sulfuric AA metabolism	MAT1A mutation	met			4				
		homocystinuria	met, hcy	cys						96%
		NKG	gly		CSF					98%
	other disorders of AA metabolism	gyratatrophia	orn			6				
		LPI	(cit)	(arg, lys, orn)	urine					98%
		hyperlysinemia	lys					100%		
secondary amino acid changes	preanalytical condition	arginine infusion	orn, arg			3				
		antibiotic therapy	hcy							
	organic aidurias	propionic acidemia	gly, ala	bcaa	urine				76%	
		PDH deficiency						86%		
	other disorders of metabolism, other contions	GSD I	ala						75%	
		ketogenic diet	bcaa			5				

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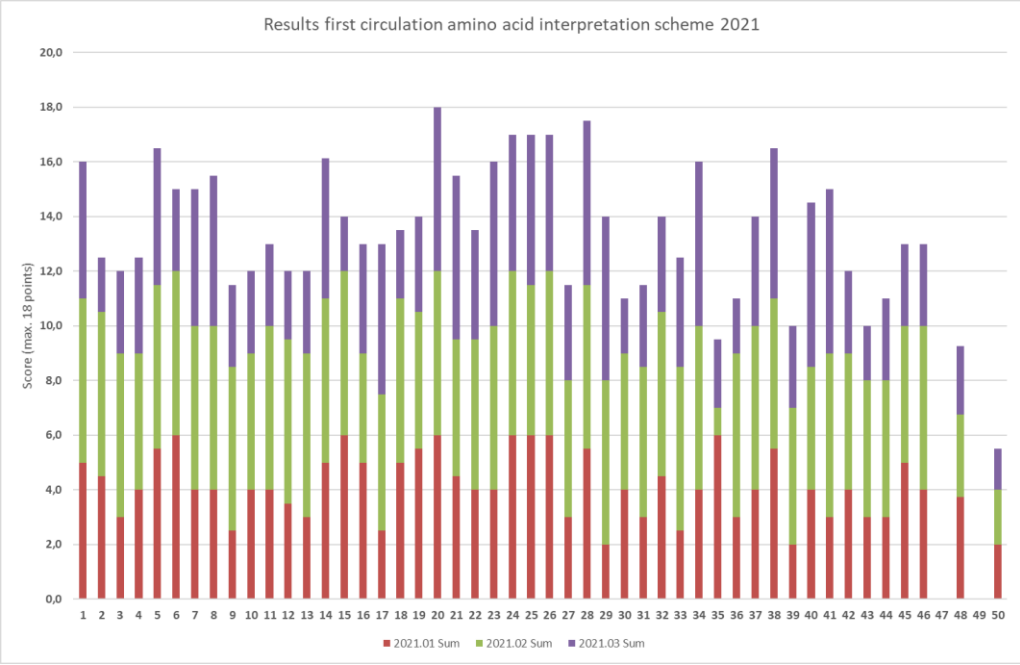
ERNDIM Scoring

Part. No.	Case 6 abnormalities	1	2	3	4	2021.06 abnormalities	Case 6 diagnosis	1	2	3	4	2021.06 diagnosis	Case 6 further testing recommendations	1	2	3	4	2021.06 recommendations	2021.06 Sum	Sum 4-6	2021.04 Sum	2021.05 Sum	2021.06 Sum
	elevated orn 2 points, sightly elevated arginine, maximum 2 points						OAT deficiency 2 points, HHH syndrome 1 point, maximum 2 points						orotic acid, ammonia, homocitrulline (=amino acids), creatine each 1 point, molecular genetic studies (gene) 1 point, ophthalmologic examination; maximum 2 points										
2	Highly elevated concentration of ornithine. Also slightly elevated concentration of arginine. and reduced concentration of lysine.	#	#	#	#	2,0	Hyperornithinemia due to ornithine aminotransferase-deficiency (gyrate atrophy) DD: Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome	#	#	#	#	2,0	Repeat the amino acids in plasma Go for genetics (OAT gene).Order ophthalmological examination (myopia and cataract). Start with aginine-restricted diet/ low-protein diet, Check the bones!	#	#	#	#	2,0	6,0	8,0	1,0	1,0	6,0



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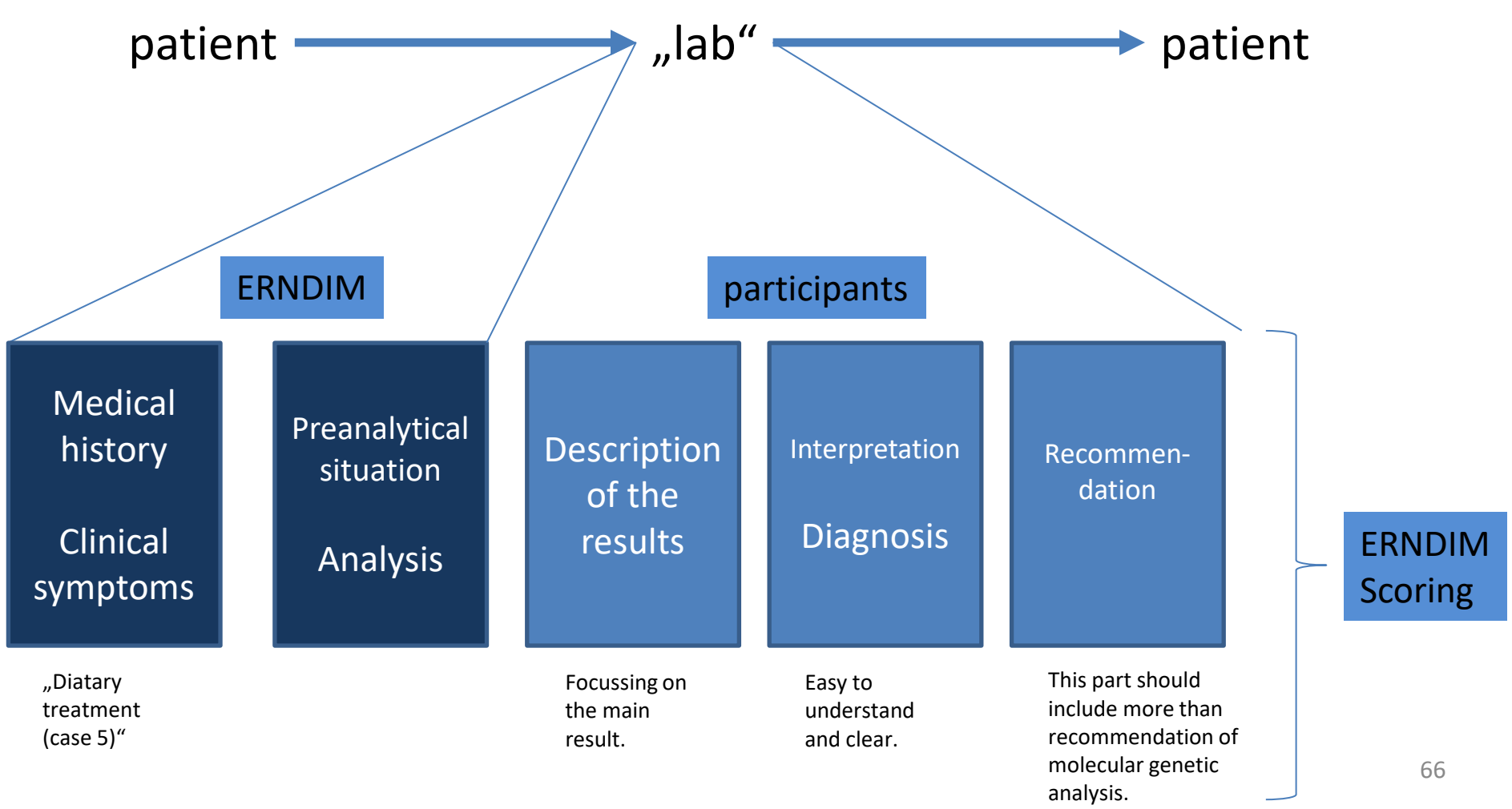
Take Home Messages



Feedback on Cognitive Amino Acid Scheme

What works well?

What could we do to improve the scheme?

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