ERNDIM DPT Center Basel

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Proficiency Testing Centre Basel
Annual Report 2007

1. Introduction

In 2007 proficiency testing in our centre was run as a regular ERNDIM scheme.

2. Geographical distribution of participants

Twenty one laboratories from 9 countries have participated in our Diagnostic Proficiency Testing scheme in 2007, for details see the table below:

Country	Number of participants
Austria	1
Canada	2
Estonia	1
Germany	6
Norway	1
Sweden	2
Switzerland	2
UK	1
USA	5
total	**21**

3. Logistics of the scheme

- Two surveys: **2007/1** – samples A, B and C / **2007/2** – samples D, E and F
- Origin of samples: Five urines were obtained from patients with known diagnoses and one sample was obtained from a patient with no metabolic disorder (samples were provided by the organizer). The common sample provided by our UK colleagues was distributed in all 5 DPT schemes. All samples were analyzed in our lab after heat-treatment, diagnostically relevant metabolites were detected in all six samples after 3-day incubation at RT mimicking possible changes during transport.
- Six heat-treated urines together with result protocols were shipped in one batch to the participants at ambient temperature using the TNT courier service. Twelve packages were received within 5 days, five within 6 - 8 days and the date of receipt was not indicated in four cases.
- The following protocol for heat inactivation was used: 1. Add thiomersal 100 mg/l of urine; 2. Heat urine to 56°C for one hour in water bath. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. The urinary samples have to be frozen until shipment.
- Tests required in 2007: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2007

Sample distribution	April 25, Wednesday
Start of analysis of Survey 2007/1	May 7, Monday
Survey 2007/1 – results submission	May 28, Monday
Survey 2007/1 – report	June 18, Monday
Start of analysis of Survey 2007/2	June 25, Monday
Survey 2007/2 – results submission	July 16, Monday
Survey 2007/2 – report	August 10, Friday
Annual meeting of participants	September 4, Tuesday
Annual report 2007	December

5. Receipt of samples and results

Date of receipt of samples (samples sent on April 25, 2007)

Date of receipt (reported by participants)	Number of participants
1 day	8
2 days	2
5 days	2
6 days	4
8 days	1
Date n.a.	4

Deadlines of results submission

	2007/1	2007/2
Deadline or before	18	19
1 day delay	-	2
3 days delay	1	-
4 days delay	1	-
10 days delay	1	-

6. Scoring system

Analytical performance, interpretative proficiency and recommendations for further investigations are evaluated. Due to the large variability in reporting results in various countries recommendations pertaining to treatment are not evaluated in proficiency testing, however, they are still reported and summarized by the scheme organizers.

A	Analytical performance	Correct results of the appropriate tests Partially correct or non-standard methods Unsatisfactory or misleading	2 1 0
I	Interpretative proficiency	Good (diagnosis was established) Helpful but incomplete Misleading/wrong diagnosis	2 1 0
R	Recommendations	Helpful Unsatisfactory or misleading	1 0

The **total score** is calculated as a sum of these three criteria. The maximum that can be achieved is 5 points per sample, i.e. 15 points per survey and 30 points per year. The scores were calculated only for laboratories submitting results.

7. Results of samples and evaluation of reporting

Sample A (MSUD)

Patient: The sample was obtained from a 23 year old boy with maple syrup urine disease (MSUD) who was receiving treatment and cared for in Basel. The diagnosis was based on examination of urine and plasma amino acids. The enzymatic or mutational analysis has not been performed but the plasma and urine amino acid profiles and clinical symptoms leave no doubt as to the diagnosis.

Analytical performance: 21 Laboratories reported amino acid analyses and 18 were able to correctly identify some abnormality. 21 also reported organic acid analysis and 20 reported abnormalities. 1 point was given for each analysis. The analytical performance of this sample was 90 %.

Interpretative proficiency: Diagnosis of maple syrup urine disease due to branched chain ketoacid dehydrogenase deficiency was considered correct. The proficiency score was 100%.

Recommendations: We consider follow up by measurement of plasma amino acids as essential and further confirmatory tests helpful.

Overall impression: Straigthforward sample with good performance.

Quantitative data:

- **Creatinine:**
  4.78; 5.07; 5.3; 5.7; 5.82; 5.9; 5.92; 5.99; 6.0; 6.03; 6.08; 6.2; 6.22; 6.31; 6.34; 6.4; 6.41; 6.5; 6.5; mmol/l, mean: 5.97, median: 6.03
- **Amino acids:**
  Leucine: 15.77, 21.3, 22.18, 22.3, 22.5, 24, 24, 25, 25.2, 26.1, 28, 30.9, 31, 32.6, 34.48, 39, 45, 67, mmol/mol creat. (Ref. 2- 11); Isoleucine: 4.3, 5, 5, 6, mmol/mol creat. (Ref. 0- 4); Allo-isoleucine: 3.04, 4, 4, 5.3, 5.7, mmol/mol creat.

Sample B (IVA)

Patient: This sample came from a female patient with isovaleric aciduria (IVA) who was treated in Basel. The diagnosis of isovaleryl CoA dehydrogenase deficiency was confirmed by the finding of reduced fixation of label from [^{14}C] isovalerate in fibroblasts and by mutation analysis (Dr. G. Vockley).

Analytical performance: The performance of organic acid analysis was considered essential for the diagnosis in this case. Proficiency score: 100%.

Interpretative proficiency: The diagnosis of Isovaleryl-CoA dehydrogenase deficiency is correct. Proficiency score: 100 %.

Recommendations: Confirmation of diagnosis by acylcarnitine measurement, enzyme assay or mutation analysis was considered helpful.

Overall impression: Straigthforward sample with excellent performance.

Quantitative data:

- **Creatinine:**
  2.74; 2.8; 2.9; 2.92; 2.99; 3.0; 3.03; 3.06; 3.1; 3.1; 3.17; 3.2; 3.24; 3.26; 3.32; 3.35; 3.4; 3.41; 3.53; mmol/l, mean: 3.13; median: 3.1.
- **Organic acids:**
  N-isovalerylglycine: 710, 1000, 1889, 1956, 2000, 2170, 2220, 2466, mmol/mol creat.

Sample C (OTC)

Patient: This sample was obtained from a 3.5 year old female who had first presented at the age of 9 months due to episodic vomiting, failure to thrive and intermittent hyperammonemia. Clinical and biochemical findings both before and during treatment indicate a diagnosis of OTC deficiency. Although mutation analysis did not reveal any mutant allele in the coding region a mutation in the promoter or intronic region of the OTC-gene has not been excluded.

Analytical performance Amino acids and organic acids or purine/pyrimidine analysis was considered essential. The finding of citrulline and arginine related to treatment but not argininosuccinic acid was considered correct. The finding of orotic acid is essential. 20 laboratories performed amino acid analysis, 12 with correct result. The reporting of ASA was considered incorrect. All laboratories correctly found orotic acid to be elevated. The analytical performance of this sample was 78%.

Interpretative proficiency: Several participants reported the presence of ASA but the amino acid profile seen in this patient is much different from that seen in ASA-lyase deficiency even under treatment. A diagnosis of a urea cycle defect received one point and one point was given for OTC-deficiency. A diagnosis of ASA-uria was considered incorrect. The interpretative proficiency for this sample was 69%.

Recommendations: Plasma amino acids ideally together with ammonia is the appropriate follow up with suggestions for confirmatory enzyme or DNA Studies.

Overall impression: This sample mimics the situation in a female subject with OTC deficiency out of crisis. It is reassuring that all labs found increased orotic acid. The false finding of ASA and interpretation thereof can be attributed to the presence of a small peak in the ASA position on amino acid column chromatography which appears to be due to administration of a macrolide (clarithromycin) at the time of urine collection. This peak was not seen in this patient in the absence of such treatment. Note the very different findings in a true case of ASA-uria. See appendix 1- 3.

Quantitative data:

- **Creatinine:**
  2.48; 2.8; 2.85; 2.9; 2.92; 3.0; 3.05; 3.1; 3.1; 3.13; 3.14; 3.2; 3.21; 3.22; 3.23; 3.28; 3.29; 3.34; 3.4; (mmol/l), mean 3.08, median 3.13
- **Amino acids:**
  Citrulline: 14.9, 27.8, 29, 30.9, 31, 31.3, 32, 32, 33.6, 36.6, 38, 38, 38, 40, 43, 44.7, 47.6, 51.5, mmol/mol creat. (Ref. 0- 6)
  Ornithine: 1.2, 3.8, 4.3, 6, 7.4, 7.7, 7.8, mmol/mol creat. (ref. 0- 7)
  Arginine: 12.8, 31.6, 39.8, 42, 42.5, 43, 43, 44, 45.4, 45.5, 48, 49, 50, 50, 54, 59.4, 65.9, mmol/mol creat. (Ref. 0- 9)
- **Organic Acids:**
  Orotic acid: 45, 99.4, 147, 190, 190, 213, 230, 231, 259, 276, 284, 289, 308.7, 1347, mmol/mol creat.

Sample D (MPS)

Patient: The sample was obtained from a female subject diagnosed with MPS Type I at the age of 4 years. The diagnosis was confirmed by determination of α-iduronidase activity in leucocytes (0.07 µmol/g/h, controls 10 – 30). The clinical progress points to the Scheie variant. The urine was collected at 30 years of age.

Analytical performance: 18 Laboratories reported increased total glycosaminoglycans by direct measurement and/or extraction and separation. Of these, 14 further differentiated the GAGs and identified specific abnormities suggesting the type of MPS disorder. 2 points were scored by these

laboratories. 1 point was given for increased GAG without any differentiation. Overall analytical performance was 76% which increased to 89% when laboratories not offering MPS analysis were excluded.

Interpretative proficiency: MPS type I probably Scheie was the correct conclusion.
A number of laboratories reported elevation of only dermatan sulphate whilst others found heparan sulphate to be increased as well. Due to the variability of MPS excretion in such disorders 2 points were given if MPS I was mentioned in the conclusion. The proficiency score was 76 % (89 % excluding labs not performing the analysis).

Recommendations: Confirmation of the defect by enzyme assay with or without mutation analysis is considered correct. Laboratories recommending appropriate further tests based on total GAG results or clinical grounds if no analysis was performed also received 1 point.

Overall impression: A straight forward sample with good overall proficiency of 91%, if labs not performing the test are excluded. These labs recommended the correct analysis based on the clinical picture.

Quantitative data:

- **Creatinine:**
  5.46; 6.63; 6.8; 6.8; 6.9; 7.08; 7.1; 7.12; 7.38; 7.43; 7.45; 7.45; 7.5; 7.6; 7.7; 7.79; 7.8; 7.9; 7.95; mmol/l, mean 7.25; median 7.43
- **Mucopolysaccharides:**
  GAG total: 11.6, 18.2, 19.2, 22, 24, 25, 27, 30, 31 31.2, 32, 32.5, 36.8, 37, g/mol creat.

Sample E (no disorder, Valproate)

Patient: This sample was obtained from a 14 year old female with no metabolic disorder but who was treated with valproate because of epilepsy.

Analytical performance: It was considered essential to analyse amino acids and organic acids. The finding of mildly elevated glycine or normal amino acids (1 point) were both accepted as correct due to the wide variation of glycine excretion although this needs to be debated. The finding of valproate metabolites but no organic acid disorder was judged as correct (1 point). Analytical performance was 86 %.

Interpretative proficiency: The conclusion of no metabolic disorder and identification of valproate treatment with or without the possibility of non-ketotic-hyperglycinaemia was considered correct. Emphasis on increased glycine or NKHG scored one point. The interpretative proficiency score for this sample was 88 %.

Recommendations: We consider the findings in this sample are satisfactorily explained by the treatment with valproate and that no further tests are needed, although this could be debated. Nevertheless one point was given for recommendations to specifically exclude NKHG but not for a more extensive list of follow up tests. This is reflected by the relatively low total score for recommendations of 81 %.

Overall impression: The overall score of 86 % was somewhat lower than expected and the lack of reporting of valproate derivatives in some cases, which help the overall interpretation, was surprising. This type of sample is commonly seen and should not yield too many follow up tests. See appendix 4, showing clear evidence of valproate derivatives.

Quantitative data:

- **Creatinine:**
  1.68; 2.3; 2.37; 2.48; 2.48; 2.5; 2.5; 2.6; 2.67; 2.68; 2.7; 2.75; 2.8; 2.83; 2.9; 2.9; 2.92; 2.92; 3.1; mmol/l, mean: 2.63, median: 2.68
- **Amino acids:**
  Glycine**:** 266; 278; 288; 311; 311; 329; 334; 349; 349; 371; 374; 469; mmol/mol creat.

Sample F (α-aminoacid semialdehyde synthase deficiency)

Patient: This sample was obtained from a 18 year old girl affected by α-aminoacid semialdehyde synthase deficiency. The diagnosis had been made on the basis of examination of urinary amino acids.

Analytical performance: All laboratories analyzed amino acids and 19 reported increased lysine with only seven reporting the presence of saccharopine in addition.
The finding of both saccharopine and an increased level of lysine in urine was considered good analytical performance. The presence of increased lysine only in the amino acid analysis was considered to be only partially correct and scored one point. The analytical performance of this sample was 62%.

Interpretative proficiency: The key was to find increased lysine without increased cystine or other dibasic amino acids with the finding of saccharopine pointing to the defect more precisely. The diagnosis of α-aminoacid semialdehyde synthase deficiency, hyperlysinemia I or II, saccharopinuria, hyperlysinemia or lysinuria was considered to be satisfactory. The interpretative proficiency score for this sample was only 64%.

Recommendations: appropriate follow up was judged to be plasma amino acid analysis and/or further confirmation of α-aminoacid semialdehyde synthase deficiency by enzymatic assay and/or mutation analysis although this is not considered to be essential. Proficiency for recommendations was 76%.

Overall impression: The rather low total proficiency score of 66% points to the need for a more careful interpretation of the amino acid chromatogram, especially to distinguish between cystine and saccharopine. See appendix 5 and 6. Although the lack of increased arginine in this sample makes a diagnosis of lysinuric protein intolerance unlikely, one point was given for this conclusion. One laboratory reported increased pipecolic acid and two increased guanidine acetic acid.

Quantitative data:

- **Creatinine:**
  0.7; 0.8; 0.84; 0.98; 1.0; 1.04; 1.06; 1.06; 1.07; 1.09; 1.1; 1.1; 1.14; 1.17; 1.17; 1.2; 1.2; 1.2; 1.44; mmol/l, mean 1.07, median: 1.09
- **Amino Acids:**
  Lysine: 188, 193, 200, 210, 216, 225, 232, 234, 244, 244, 265, 268, 274, 289, 289, 308, 402, 1374 (Ref. 62- 513), 1467 (Ref 153- 634), mmol/mol creat., 830 µmol/g creat.

8. Score of participants for individual samples

Survey 2007/1

Lab No.	Sample A MSUD				Sample B IVA				Sample C OTC			
	A	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**
1	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
2	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
3	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
4	1	2	0	**3**	2	2	1	**5**	1	0	0	**1**
5	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
6	2	2	0	**4**	2	2	1	**5**	1	0	0	**1**
7	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
8	2	2	1	**5**	2	2	1	**5**	2	1	1	**4**
9	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
10	1	2	1	**4**	2	2	1	**5**	1	2	1	**4**
11	2	2	1	**5**	2	2	1	**5**	2	1	1	**4**
12	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
13	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
14	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
15	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
16	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
17	1	2	1	**4**	2	2	1	**5**	1	0	1	**2**
18	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
20	2	2	0	**4**	2	2	1	**5**	2	2	1	**5**
21	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
22	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
	90%	100%	86%	**93%**	100%	100%	100%	**100%**	78%	69%	90%	**77%**

Survey 2007/2

Lab No.	Sample D MPS Type I				Sample E No disorder, Valproate				Sample F α-AA semialdehyde synthase deficiency			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
2	2	2	1	**5**	2	2	0	**4**	1	0	0	**1**
3	1	1	1	**3**	2	2	1	**5**	1	0	1	**2**
4	2	2	1	**5**	1	2	1	**4**	0	0	0	**0**
5	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	1	2	1	**4**	2	2	1	**5**
7	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
8	2	2	1	**5**	1	2	1	**4**	1	1	1	**3**
9	1	1	1	**3**	1	1	1	**3**	1	2	1	**4**
10	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
11	2	2	1	**5**	2	2	0	**4**	1	2	1	**4**
12	2	2	1	**5**	2	2	1	**5**	1	0	0	**1**
13	2	2	1	**5**	2	2	1	**5**	1	0	0	**1**
14	1	1	1	**3**	1	0	0	**1**	1	1	1	**3**
15	2	2	1	**5**	2	1	1	**4**	1	2	1	**4**
16	0	0	1	**1**	2	2	1	**5**	2	2	1	**5**
17	0	0	1	**1**	1	2	0	**3**	0	0	0	**0**
18	0	0	1	**1**	2	2	1	**5**	2	2	1	**5**
20	1	1	1	**3**	2	2	1	**5**	1	2	1	**4**
21	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
22	2	2	1	**5**	2	1	1	**4**	2	2	1	**5**
	76%	76%	100%	**81%**	86%	88%	81%	**86%**	62%	64%	76%	**66%**

excluding labs which did not perfom MPS analysis – No. 16, 17, 18

89%	89%	100%	**91**%

A - Analytical score, I – Interpretative score, R – Recommendations

Survey 2007 – Score summary

Sample	Diagnosis	Analytical [%]	Interpreta-tative [%]	Recommen-dations [%]	Total [%]
A	**MSUD**	90%	100%	86%	93%
B	**IVA**	100%	100%	100%	100%
C	**OTC**	78%	69%	90%	77%
D	**MPD Type I**	76%/ *89	76% /*89	100%	81%/ *91
E	**No disorder, Valproate**	86%	88%	81%	86%
F	**α-AA semialdehyde synthase deficiency**	62%	64%	76%	66%

* (excluding labs which did not perform MPS analysis)

9. Total score of participants for individual surveys and their performance in 2007

Lab no	Survey 2007/1 (points)	Survey 2007/2 (points)	Total points 2007
1	15	15	**30**
2	15	10	**25**
3	15	10	**25**
4	9	9	**18**
5	15	15	**30**
6	10	14	**24**
7	13	13	**26**
8	14	12	**26**
9	15	10	**25**
10	13	14	**27**
11	14	13	**27**
12	13	11	**24**
13	13	11	**24**
14	13	7	**20**
15	14	13	**27**
16	15	11	**26**
17	11	4	**15**
18	15	11	**26**
20	14	12	**26**
21	15	15	**30**
22	13	14	**27**

10. Assessment of performance

Steps have been taken within the Scientific Advisory Board of ERNDIM to set the level of good performance within a proficiency scheme. Letters of support to those laboratories with clear poor performance will be issued. See also remarks on the certificate below.

11. Annual meeting of the participants

The annual meeting of participants of the Proficiency Testing Centre Basel took place in Hamburg at the SSIEM Annual Symposium on September 4, 2007.

12. Changes planned for 2008

A system for submission and evaluation of results and reporting via internet is now being developed by B. Fowler and V. Kozich. It is hoped to be able to introduce this system on a pilot scale to allow testing by participants from the Basel and Prague centres. Participants will be notified of developments in due course.

13. Tentative schedule of DPT scheme and fee in 2008

Sample distribution	April 23, Wednesday
Start of analysis of Survey 2008/1	May 05, Monday
Survey 2008/1 - results submssion	May 26, Monday
Survey 2008/1 - report	June 16, Monday
Start of analysis of Survey 2008/2	June 23, Monday
Survey 200/2 – results submission	July 14, Monday
Survey 2008/2 - report	August 08, Friday
Annual meeting of participants	September 2, Tuesday
Annual report 2008	December

The next annual meeting of participants will take place on September 2, 2008 at the SSIEM Annual Symposium in Lisbon Portugal.

The Executive Board of ERNDIM determined the fee for 2008 in the amount of 284 €.

14. Certificate of participation in Proficiency Testing for 2007

The certificate of participation will be provided by ERNDIM to all participants who returned the results of both surveys. In addition we are introducing a new type of certificate which will now indicate whether satisfactory performance was achieved in the scheme. Please see the ERNDIM website for more details.

Basel, February 2008

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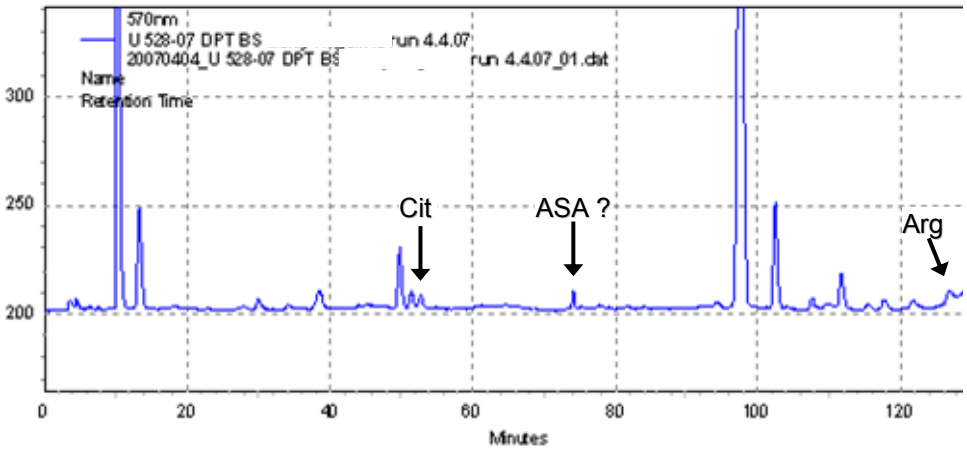
Marianne Zaugg

Piotr Litynski

15. Appendix

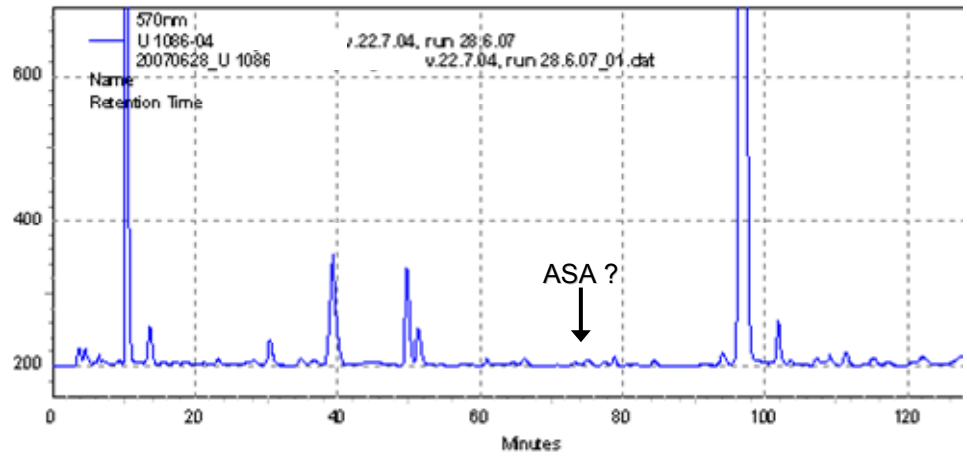
Appendix 1 Basel DPT 2007, Sample C: amino acids

Patient on a macrolide (clarithromycin)

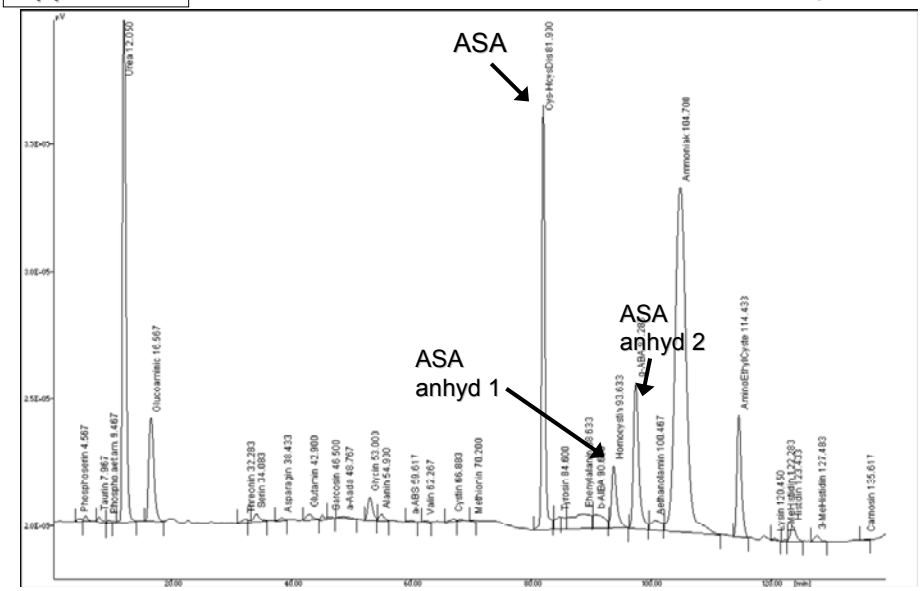


Appendix 2 Basel DPT 2007, Sample C: amino acids

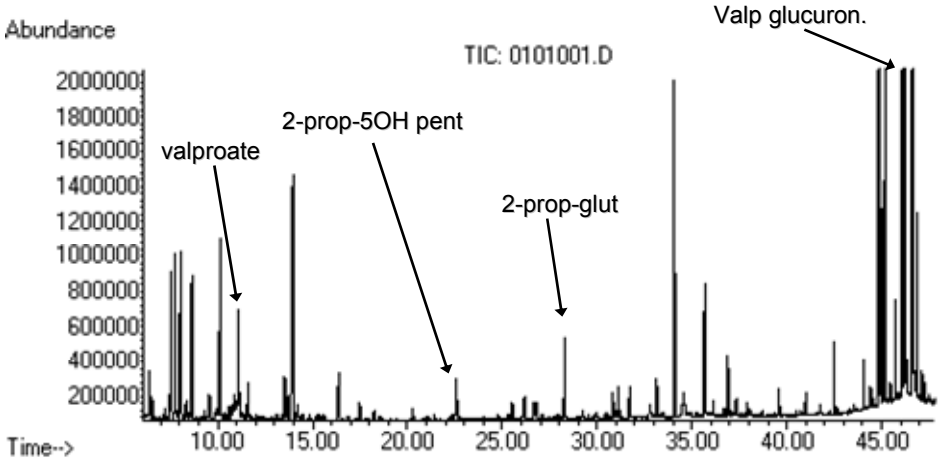
Patient no antibiotics



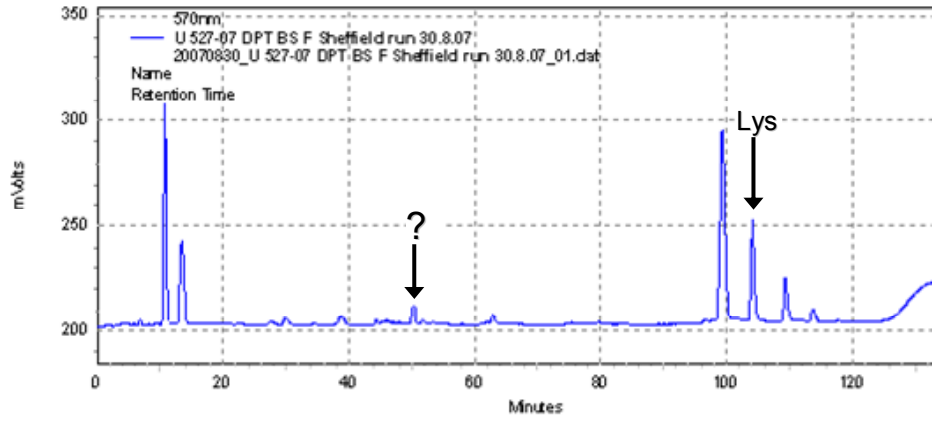
Appendix 3 Amino acids: Basel DPT 2007, Sample A



Appendix 4 Basel DPT 2007 Sample E: organic acids



Appendix 5 Amino acids: Basel DPT 2007 Sample F



Appendix 6 Amino acids: Basel DPT 2007 Sample F Expanded Scale