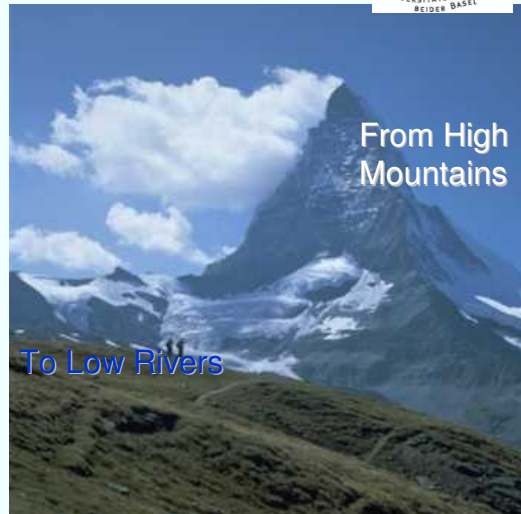


Strategies for Screening for Amino Acid Disorders Selective versus Population Screening

B. Fowler
University Childrens
Hospital
Basel, Switzerland



Strategies for Screening for Amino Acid Disorders Selective versus Population Screening



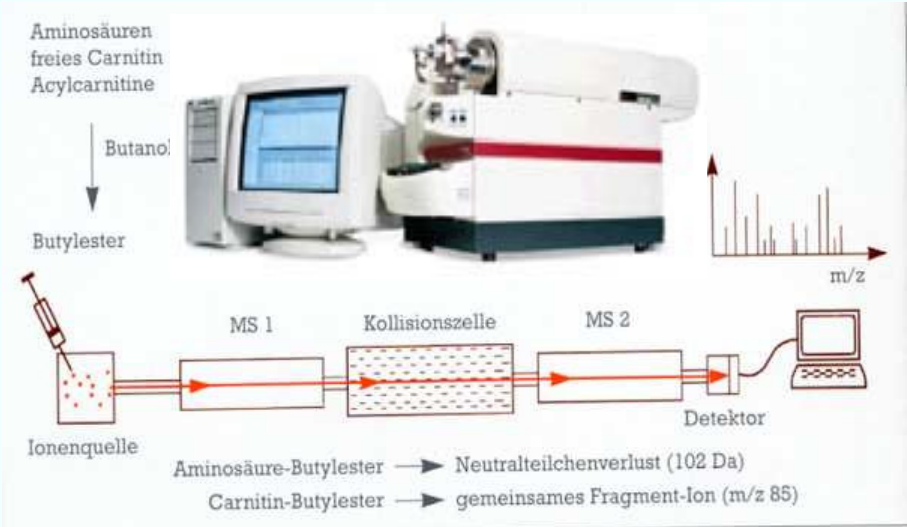
Strategies for Screening for Amino Acid Disorders Selective versus Population Screening

- **Potential and Limitations of Newborn Screening**
- Necessity for selective (symptomatic, high risk) screening
How to detect the great majority of disorders not detectable by NBS
- **Overview of diagnostic approaches**
- The role of the IEM diagnostic laboratory
- **Examples of specific cases: discussion of diagnostic approach**
- EQA = ERNDIM

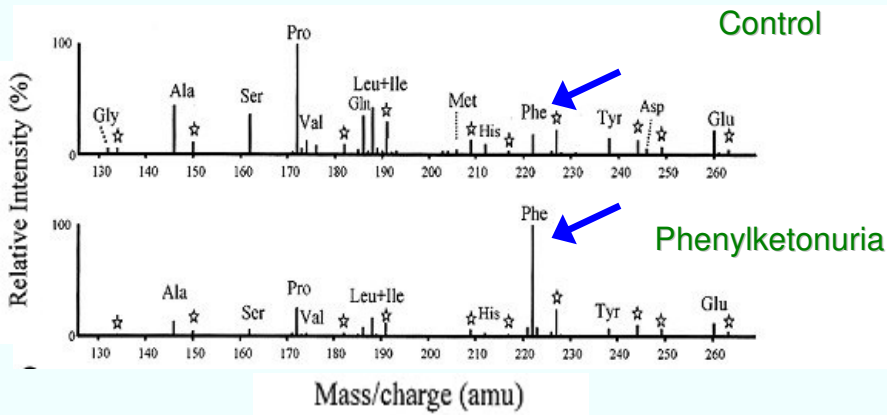
systematic

done or acting according to a fixed plan or system; methodical.

Tandem Mass Spectrometry



NB-Screening - Amino acids



IEMs currently investigated by Tandem MS

(Munich group)

Organic acid disorders

Isovaleric acidaemia	Glutaric aciduria type I
Methylmalonic acidaemia	Propionic acidaemia
Methylcrotonyl CoA carboxylase def.	β -Ketothiolase def.
3-Methylglutaconyl CoA hydratase def.	3-OH-3-Me-glutaryl CoA lyase def.
3-OH-Isobutyryl CoA Hydrolase def.	
Short Chain Acyl CoA DeH def.	Medium Chain AcylCoA DeH. def
Very Long Chain Acyl CoA DeH. def.	Multiple Acyl CoA DeH. def.
Long Chain 3-Hydroxy-Acyl CoA DeH. def.	
Carnitine Palmitoyl Transferase def. I	Carnitine Palmitoyl Transf. def. II
Carnitine Translocase def.	

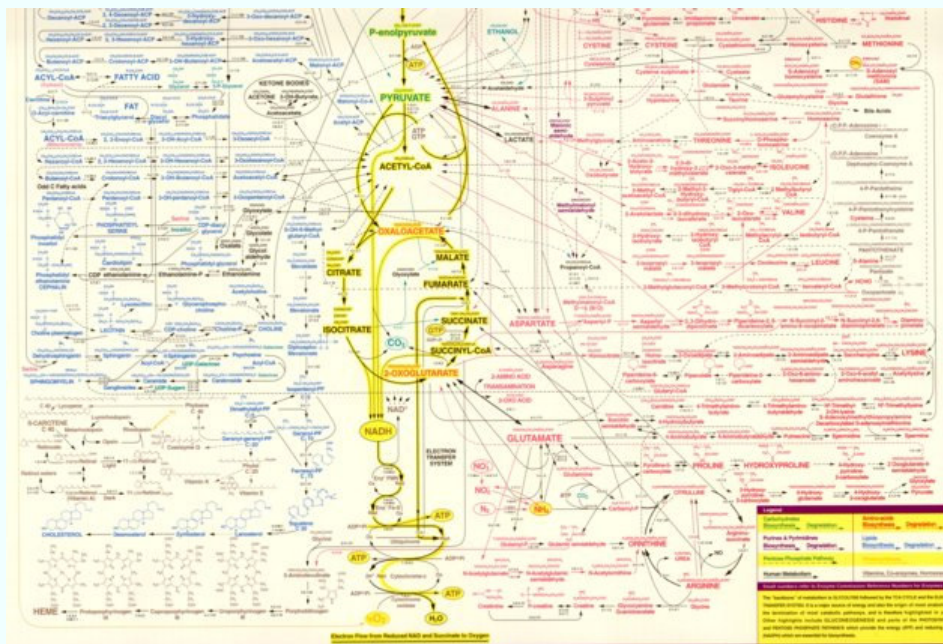
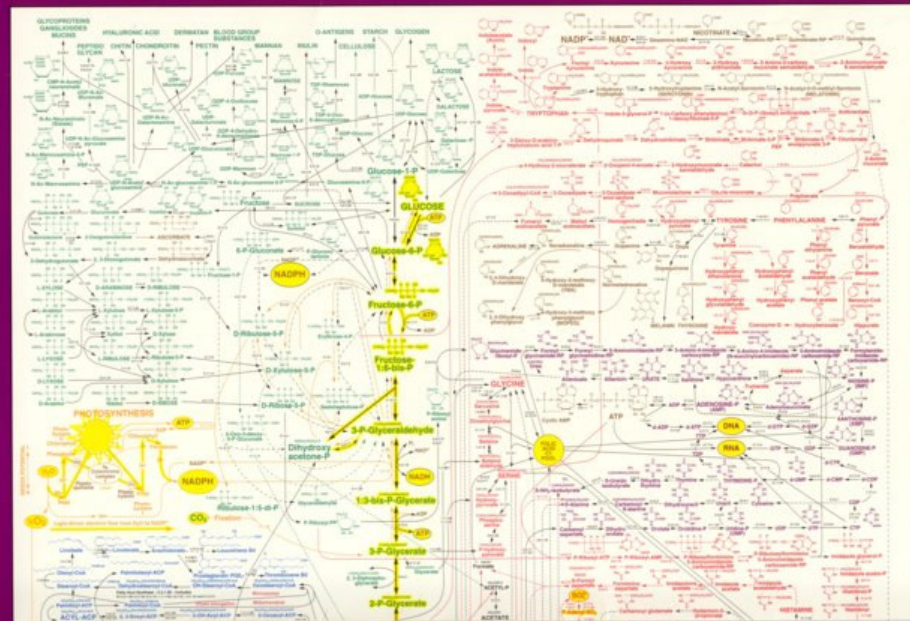
IEMs currently investigated by Tandem MS

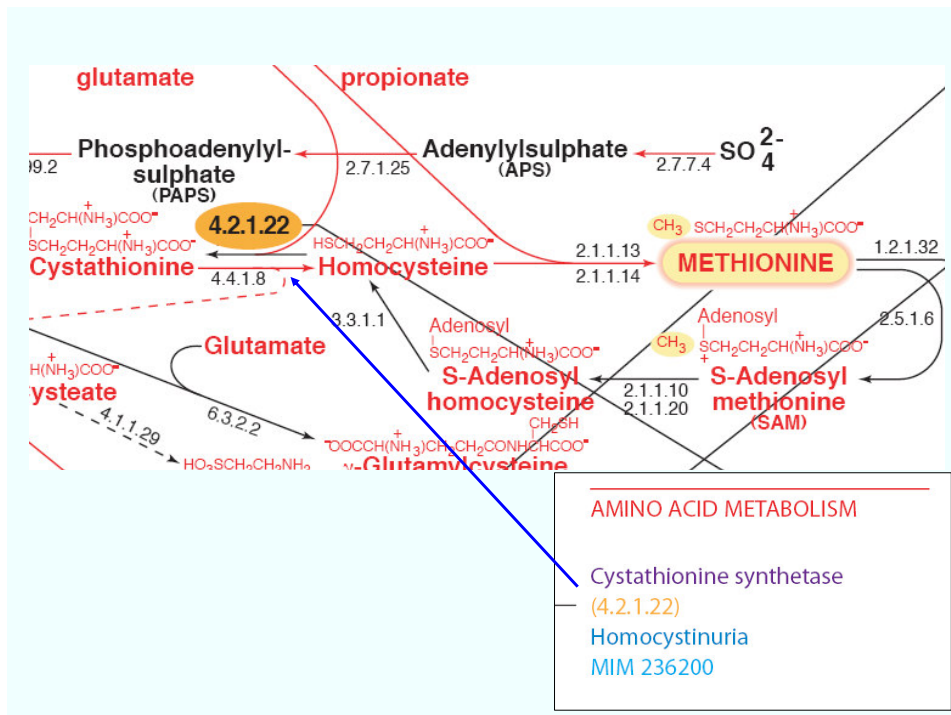
(Munich group)

Amino acid disorders

Phenylketonuria	Tetrahydrobiopterin deficiencies
Branched Chain Ketoacidaemia (MSUD)	Tyrosinaemia I, II
Non-Ketotic Hyperglycinaemia	Homocystinuria CBS def.
Methionine Adenosyl Transferase def. (Methionine Synthase def.)	(MethyleneTHF Reductase def.) (Cobalamin C/D defect)
Citrullinaemia	Argininosuccinic aciduria

Metabolic Pathways





Amino Acid Metabolism and Disorders

21 amino acids found in proteins

1. Monoamino monocarboxylic acids

- Aliphatic
- Aromatic - phenylalanine and tyrosine
- Heterocyclic - tryptophan and histidine

2. Monamino dicarboxylic acids

3. Diamino monocarboxylic acids

4. Sulphur-containing amino acids

5. Acids containing an imino group

Amino Acid Metabolism and Disorders

21 amino acids found in proteins

Non-protein amino acids

Formed by intermediary metabolism of amino acids

Ornithine
β-aminoisobutyric acid
taurine
homocystine

Disorders of metabolism of varying severity from benign to incompatible with life

Amino Acid Metabolism and Disorders

Invention of amino acid analyser in 1950s (Stein and Moore)
started investigation of inherited metabolic disorders

→ diagnosis, monitoring of treated patients

Steady improvements of reliability and sensitivity needed for minor changes in some disorders and CSF investigations

Amino acid analysis ca. 1970

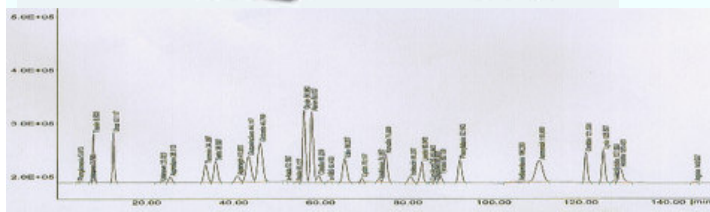


Run time 18
hours

Amino acid analysis 2000



Total Run time
150 minutes



Amino Aciduria in Amino Acid disorders

- Protein amino acids – low renal loss disorders → hyper-aminoacidaemias
e.g. phenylalanine
- Non-protein amino acids – higher renal loss disorders → hyperamino acidurias
e.g. argininosuccinic aciduria
- Transport defects
→ renal amino aciduria
e.g. cystinuria – cystine, lysine
hartnup disease – all neutral and acidic amino acid

Secondary metabolic changes in Amino acids

Amino acids associated with disturbances of

- nutritional state
- Organ function
 - liver
 - kidney
 - intestine
 - muscles
 - tumours

Amino acid disorders, M. Duran, Lab Guide Methods in IEM

1. N-Acetylglutamate synthase deficiency	plasma
2. S-Adenosylhomocysteine hydrolase deficiency	plasma
3. α -Amino adipic semialdehyde dehydrogenase deficiency	plasma urine
4. α -Amino adipic aciduria	plasma urine
5. Argininemia	plasma urine
6. Argininosuccinic aciduria	plasma urine
7. Aspartylglucosaminuria	urine
8. γ -Aminobutyric acid transaminase deficiency	urine, plasma, csf
9. β -Aminoisobutyric aciduria	urine
10. Δ -Aminolevulinic acid dehydratase deficiency	urine
11. Carbamyl phosphate synthase deficiency	urine
12. Carnosinemia	urine
13. Citrullinemia type 1	plasma
14. Citrullinemia type 2 (citrin)	plasma
15. Cystathioninuria	urine
16. Cystinosis	urine(renal Fanconi)
17. Cystinuria	urine
18. Dicarboxylic aminoaciduria	urine
19. Formiminoglutamic aciduria	urine

Amino acid disorders, M. Duran, Lab Guide Methods in IEM

20. γ -Glutamylcysteine synthase deficiency	urine
21. γ -Glutamyl transpeptidase deficiency	urine
22. Glutamine synthetase deficiency	plasma
23. Glycine N-methyltransferase deficiency	plasma
24. Hyperglycinemia	urine, plasma, csf
25. Hartnup disorder	urine
26. Histidinemia	urine, plasma
27. Homocarnosinosis	csf
28. HHH-syndrome	plasma
29. Hawkinsinuria	urine
30. Homocystinuria/CBS	plasma
31. Homocystinuria/MTHFR	plasma
32. Homocystinuria/Cbl E or G	plasma urine FIGLU \uparrow
33. Hydroxykynureninuria	urine
34. Hydroxylysineuria	urine, plasma
35. Hypophosphatasia	urine
36. Iminoglycinuria	urine
37. Iminopeptiduria (prolidase deficiency)	urine
38. Lowe syndrome	urine

Amino acid disorders, M. Duran, Lab Guide Methods in IEM

39. Hyperlysinemia	urine plasma
40. Lysinuric protein intolerance	urine plasma
41. Methionine adenosyltransferase deficiency	plasma
42. β -mercaptolactate-cysteine disulfiduria	urine
43. MSUD	plasma
44. Ornithine carbamoyltransferase deficiency	plasma
45. Ornithinemia	plasma
46. Hyperphenylalaninemas	plasma
47. 3-Phosphoglycerate dehydrogenase deficiency	plasma,csf
48. Phosphohydroxylysinuria	urine
49. Prolinemia type 1	plasma urine
50. Prolinemia type 2	plasma urine
51. Pyrroline-5-carboxylate synthase deficiency	urine
52. Saccharopinuria	plasma urine
53. Sarcosinemia	urine, plasma
54. Sulfite oxidase deficiency (+Mo-cofactor)	urine plasma
55. Tyrosinemia type 1	plasma urine
56. Tyrosinemia type 2	plasma urine
57. Tyrosinemia type 3	plasma urine

Laboratory Guide to the Methods in Biochemical Genetics

Blau, N.; Duran, M.; Gibson, K.M.
(Eds.)
Springer -Verlag Berlin Heidelberg,
2008
ISBN 978-3-540-76697-1



Acute Metabolic Disorders: major symptoms

- Acidosis / Alkalosis
- Hypoglycaemia
- Liver dysfunction: hepatomegaly
 liver enzyme synthesis disturbance
- Cardiomyopathy: arrhythmia, heart insufficiency
- Neurological symptoms
 lethargy, feeding problems, vomiting
 seizures, coma

⇒ Unspecific symptoms

Inherited Metabolic Disorders Clinical Presentation

A. early days / weeks of life

Newborn Child ⇒ deterioration after feeding or catabolism

Symptoms mostly unspecific !

⇒ feeding problems, vomiting
 breathing, neurological problems
 jaundice, cardiomyopathy, sleepy, coma,
 dysmorphic features

⇒ Unusual smell: - maple syrup (maggi): MSUD
 - sweaty odour: Isovaleric aciduria

Inherited Metabolic Disorders Clinical Presentation

B. Infancy

“healthy” child ⇒ Deterioration after diet change or catabolic state (e.g. infection)

⇒ unspecific symptoms !

e.g. growth delay dysmorphic features
neurological abnormalities hepatomegaly
cardiomyopathy

Typical example: [hereditary fructose intolerance](#)

⇒ change from breast milk to modified milk feeding
(contains sucrose):

vomiting, hypoglycaemia, hypophosphataemia,
fructosaemia, later poor growth, seizures, liver disorder

Inherited Metabolic Disorders Clinical Presentation

C. after first year of life

Chronic appearance of:

- Mental retardation
- Growth retardation
- Dysmorphic features
- Organomegaly
- Neurological features, seizures
- Etc. etc. etc. etc.

Inherited metabolic disorders Laboratory diagnosis

Diagnostic Parameters

- Ammonia → increased in Urea Cycle defects
- Blood gas analysis → metabolic acidosis (organic acid defects)
- Glucose → hypoglycaemia (energy metabolism defects)
- Lactate → increased in energy metabolism defects
- Uric acid → indication of glycogenoses, fructose intolerance
- Urine → Reducing substances (Clinitest), ketone bodies
odour (smell) !
- Routine-Laboratory parameters as also in other patients
(Blood film, electrolytes, Ca, Mg etc.)
- Guthrie-card → Acyl carnitines (Tandem-Mass Spectrometry)
→ **Amino acids**, organic acids

Methods for Diagnosis of Amino Acid Disorders

Spot Tests

Cyanide-Nitroprusside

Sulphur amino acids

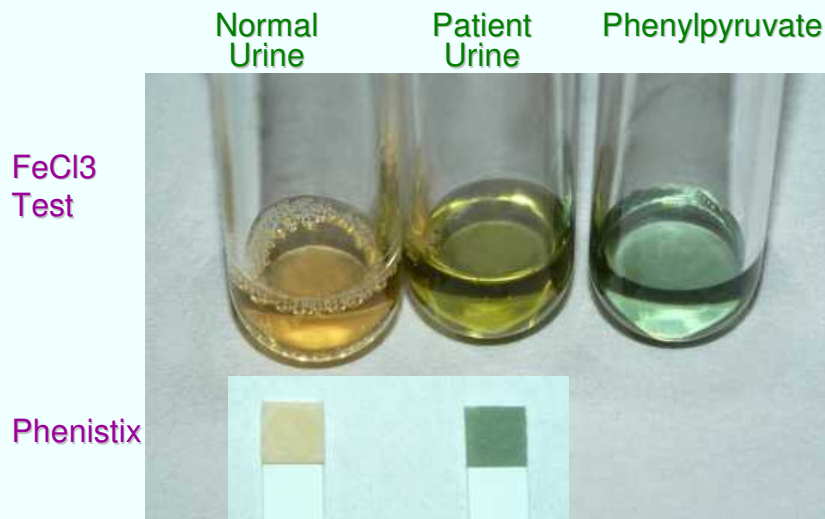
Ferric Chloride

Phenylalanine metabolites
e.g. phenylpyruvic acid

DiNitrophenylhydrazine

keto acids
branch chain amino acid
derivatives

Urine – Phenylalanine-metabolites:Spot-Test



MSUD: Spot-Test

Dinitrophenylhydrazine (DNPH)-Test

Normal Urine

Maple Syrup Urine
Disease Patient Urine

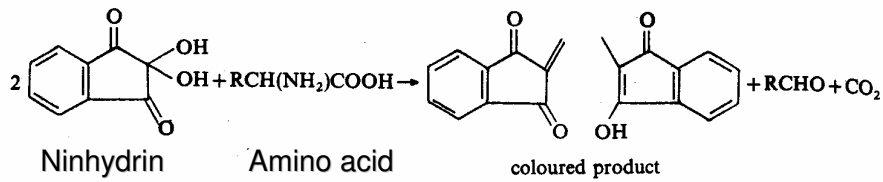


Methods for Diagnosis of Amino Acid Disorders

Semi quantitative (Urine)

One-, two-dimensional paper chromatography
thin layer-chromatography
Electrophoresis,

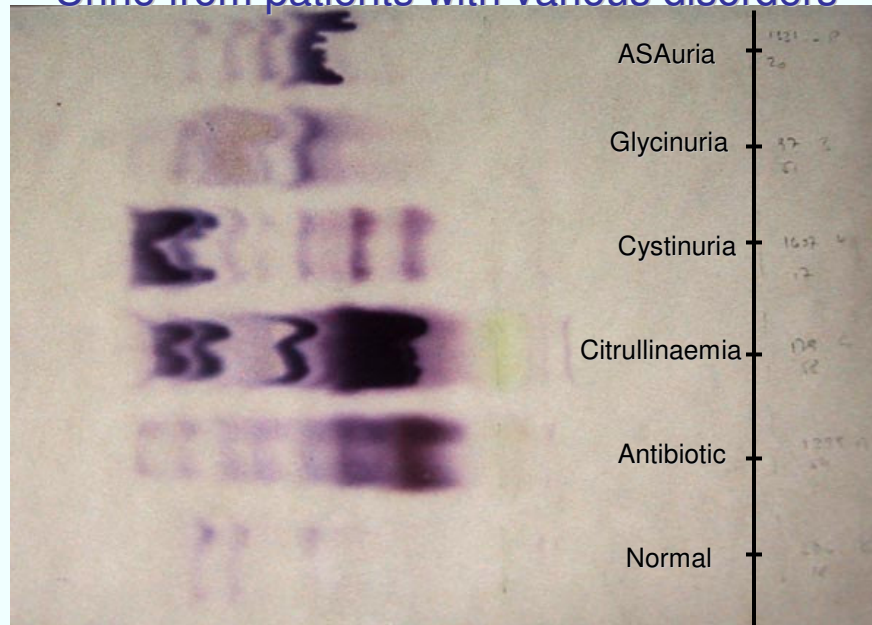
Ninhydrin detection



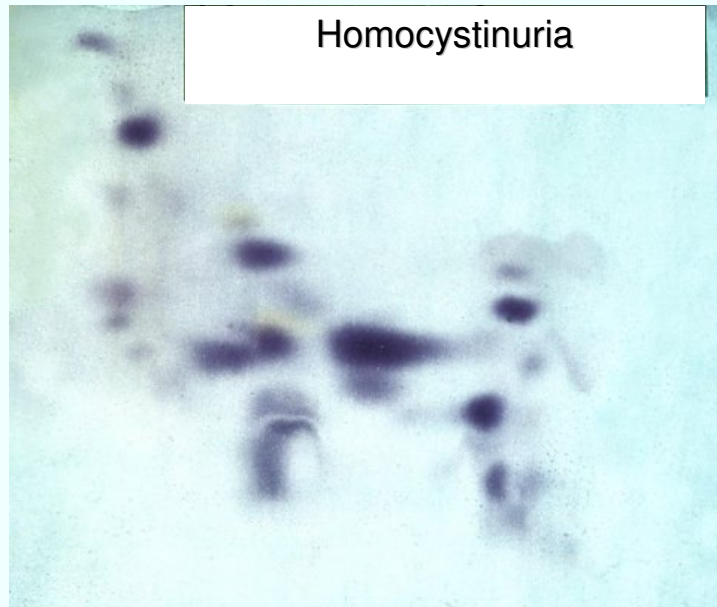
Aminoacids- High-voltage electrophoresis apparatus



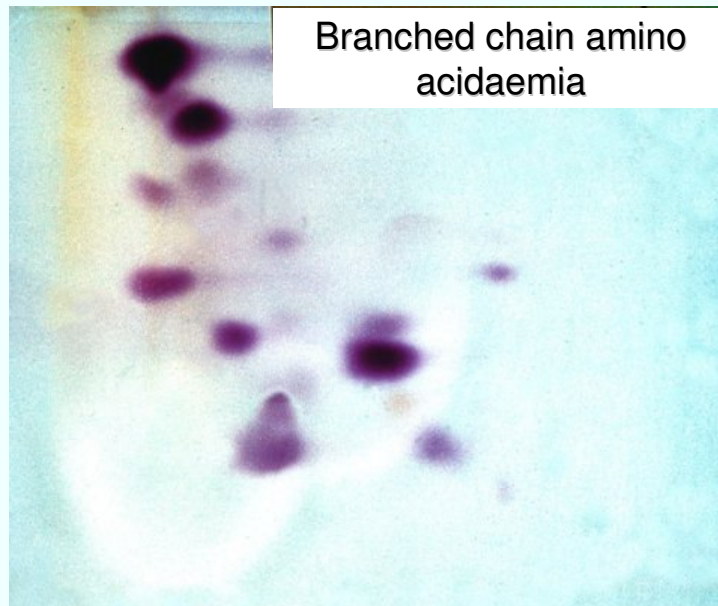
Aminoacids- High-voltage electrophoresis Urine from patients with various disorders



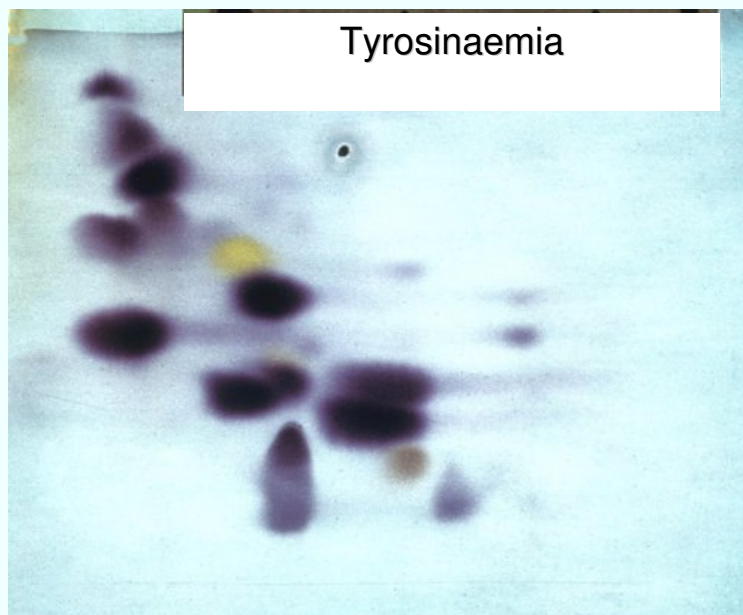
2-Dimensional thin layer chromatography of urine



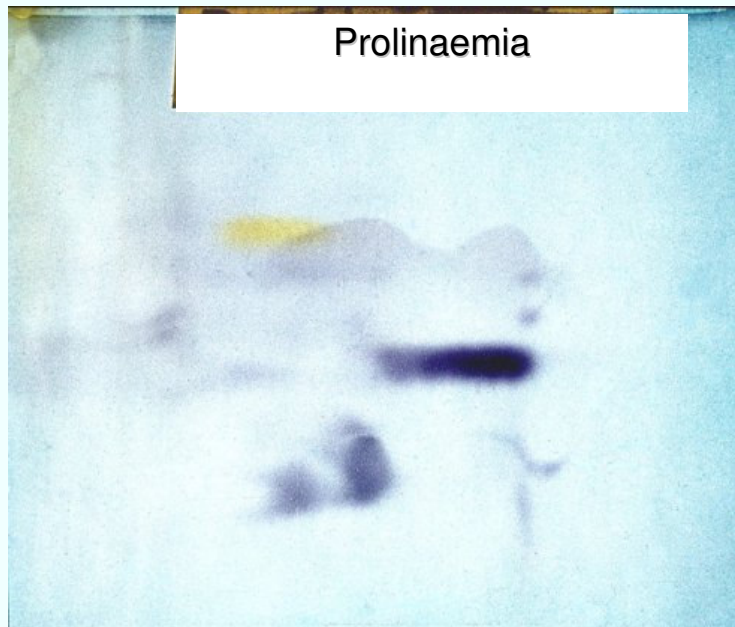
2-Dimensional thin layer chromatography of urine



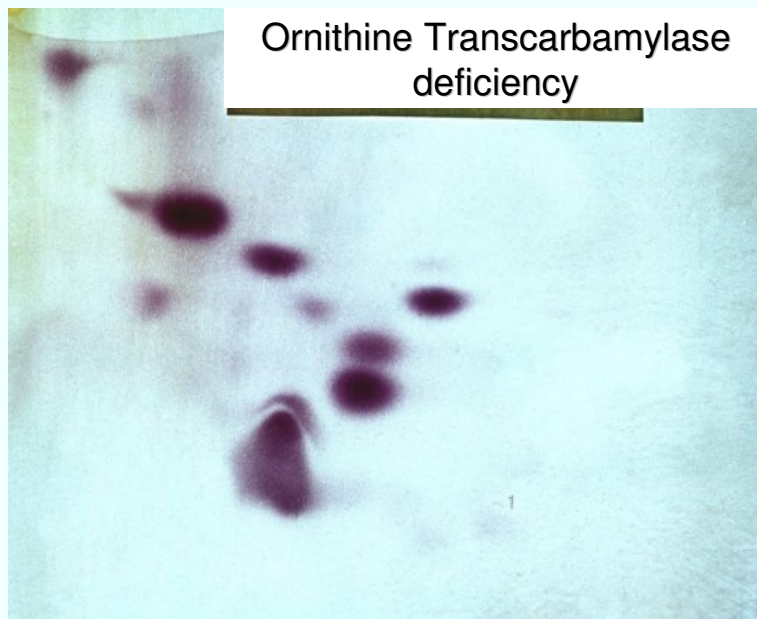
2-Dimensional thin layer chromatography of urine



2-Dimensional thin layer chromatography of urine

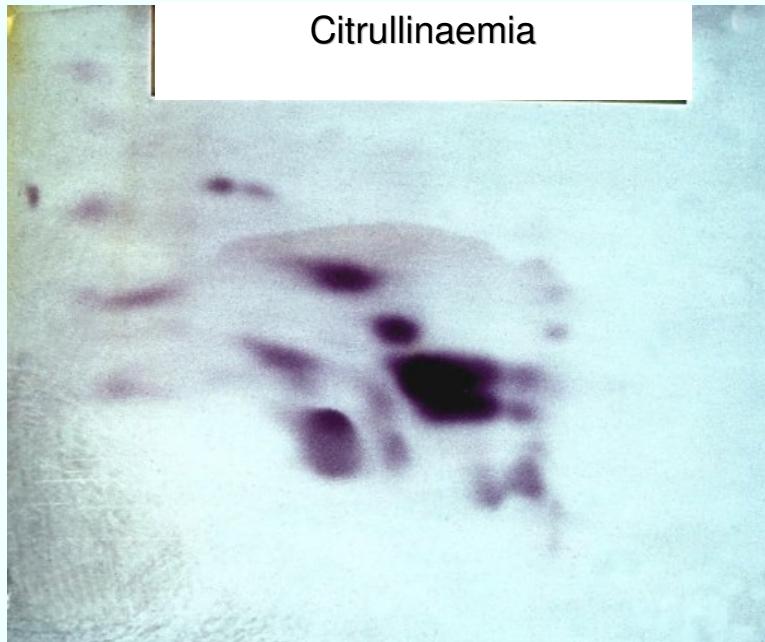


2-Dimensional thin layer chromatography of urine



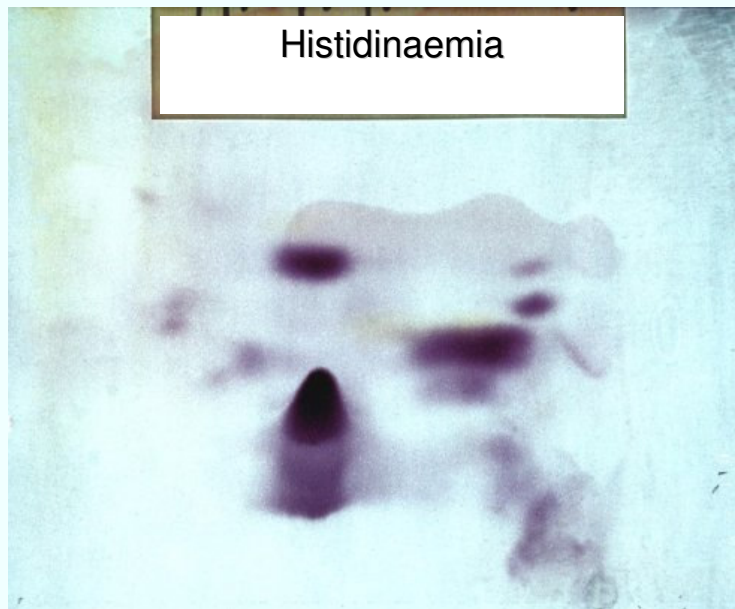
2-Dimensional thin layer chromatography of urine

Citrullinaemia



2-Dimensional thin layer chromatography of urine

Histidinaemia



Methods for Diagnosis of Amino Acid Disorders

Semi quantitative (Urine)

One-, two-dimensional paper chromatography
thin layer-chromatography
Electrophoresis,

Ninhydrin detection

Specific reagents

Iodoplatinate Sulphur amino acids
Ehrlich's reagent Citrulline, tryptophan and derivatives
Pauly reagent Imidazoles, phenolic acids

Quantitative Methods for Diagnosis of Amino Acid Disorders

Amino Acids in Plasma, Urine, CSF

- Automated Ion-Exchange Chromatography, Ninhydrin
- Tandem MS
- Gas Chromatography
- High performance liquid Chromatography
 - Full spectrum precolumn derivatisation
 - Specific Phenylalanine/ Tyrosine (blood spots)
 - Total homocysteine (mild hyperhomocysteinaemia)
- Specific assays

Fluorimetric	Phenylalanine
Enzyme	Phenylalanine
Immunoassays	Homocysteine

Amino Acid Analysis: Sample Preparation

Urine, CSF (0.5 - 1.0 ml) collect in plain tubes.

Store frozen, -20 °C if longer -70 °C

Urine overnight, 24 hour or early morning

Plasma Blood (1 ml.)by venepuncture, lithium heparin tube,
overnight fast / pre-prandial

Capillary thorough cleansing of skin

Centrifuge 2000 g best at 4 °C.

Deproteinise within 30 minutes of collection with sulphocalicylic
acid / store SNF at -20 °C (-70 °C)

Plasma stored at -20 °C up to 2months, if longer -70 °C but
cystine / homocystine lost.

Biochrom Amino Acid Analyser

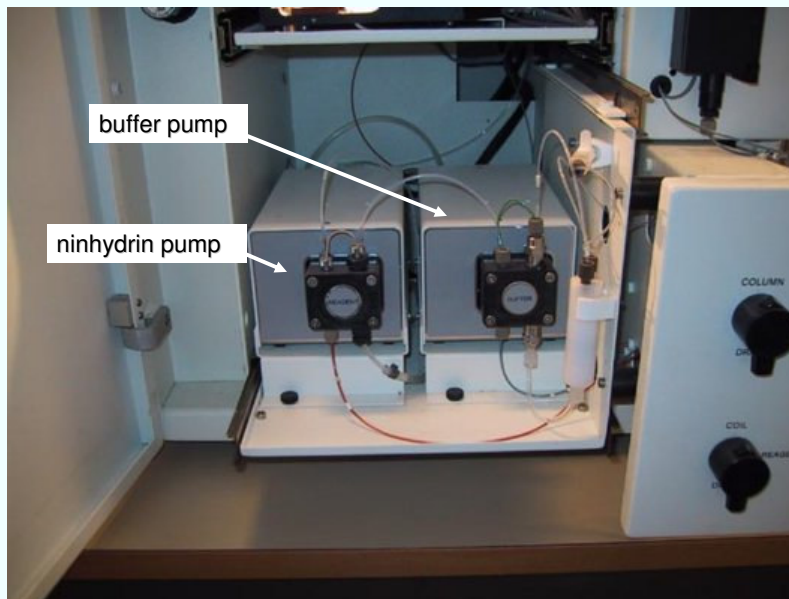


Biochrom Amino Acid Analyser

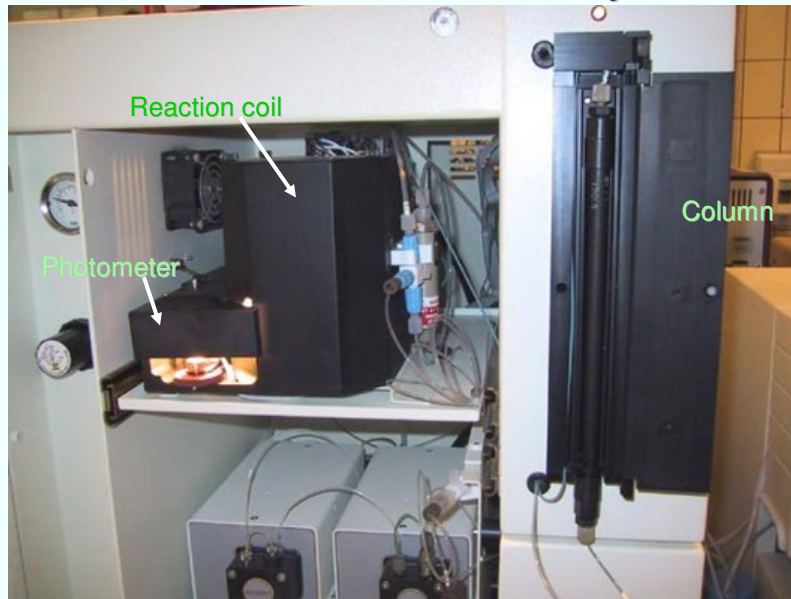
Autosampler cooled



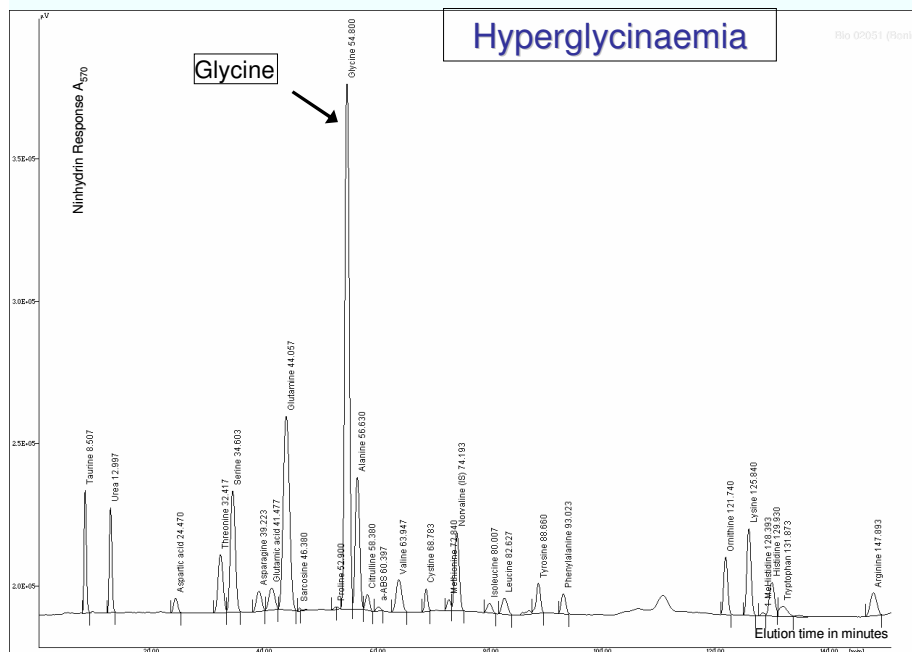
Biochrom Amino Acid Analyser



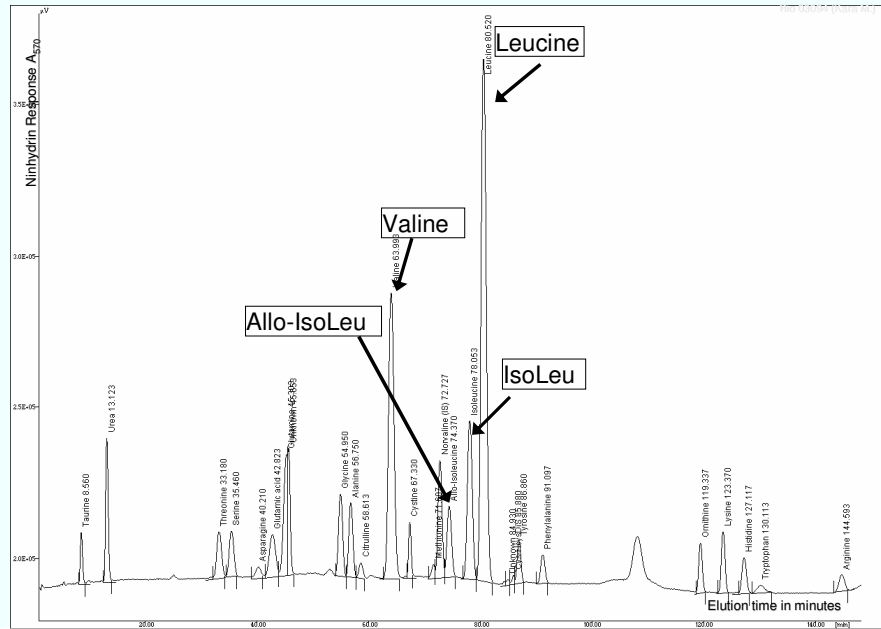
Biochrom Amino Acid Analyser



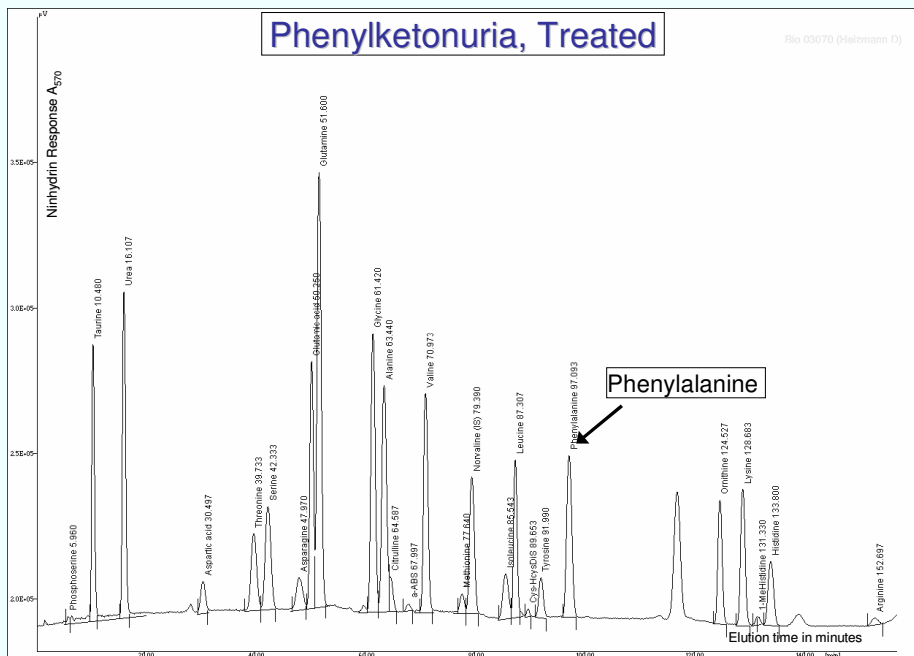
Ion-exchange chromatogram 1: Plasma



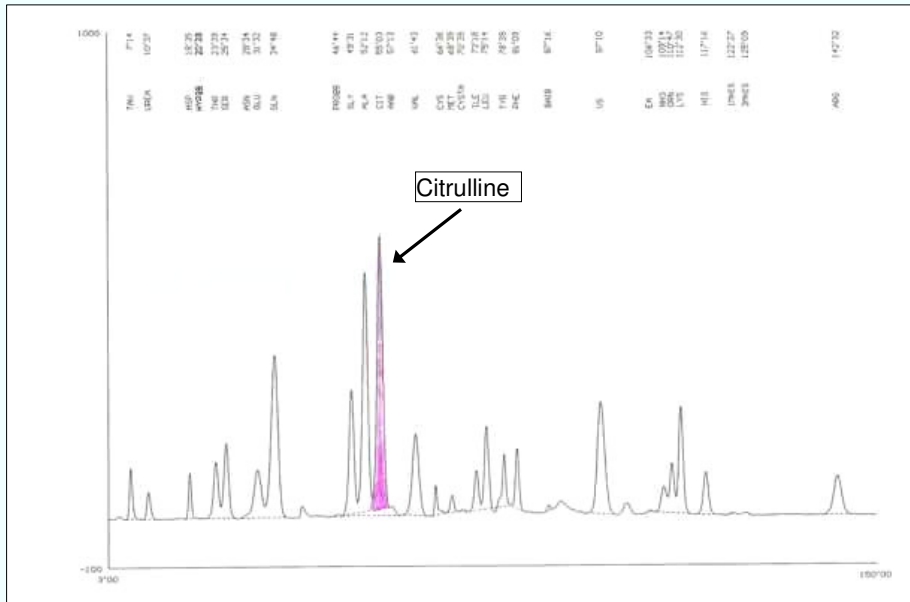
Ion-exchange chromatogram 2: Plasma Branched-chain aminoacidaemia



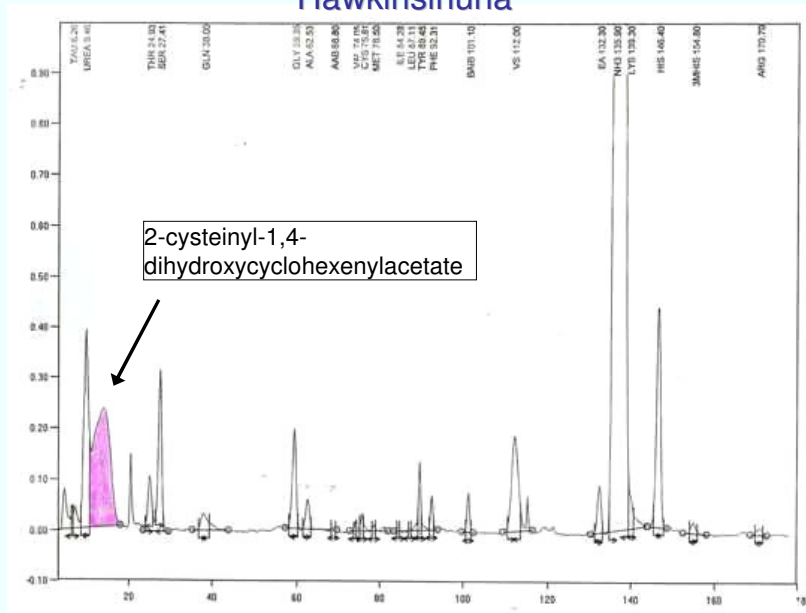
Ion-exchange chromatogram 3: Plasma Phenylketonuria, Treated



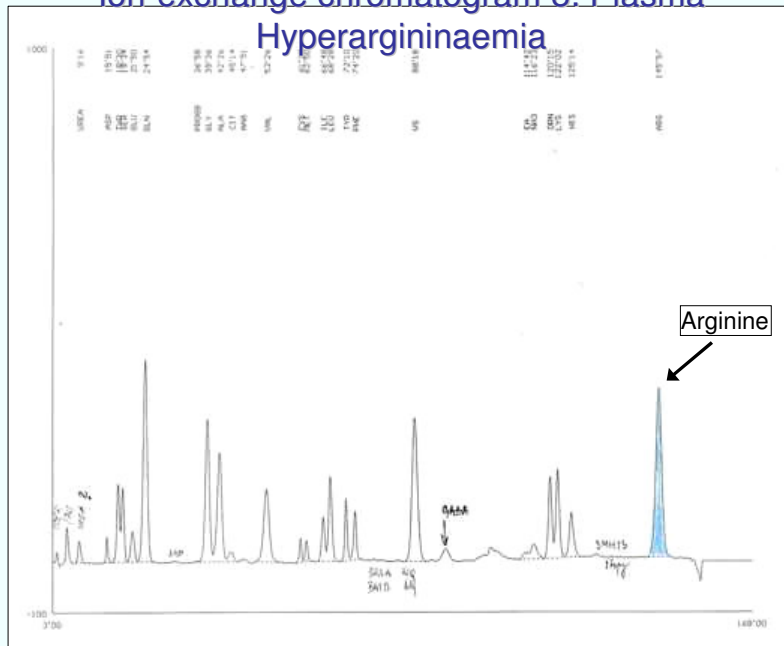
Ion-exchange chromatogram 6: Plasma Citrullinaemia



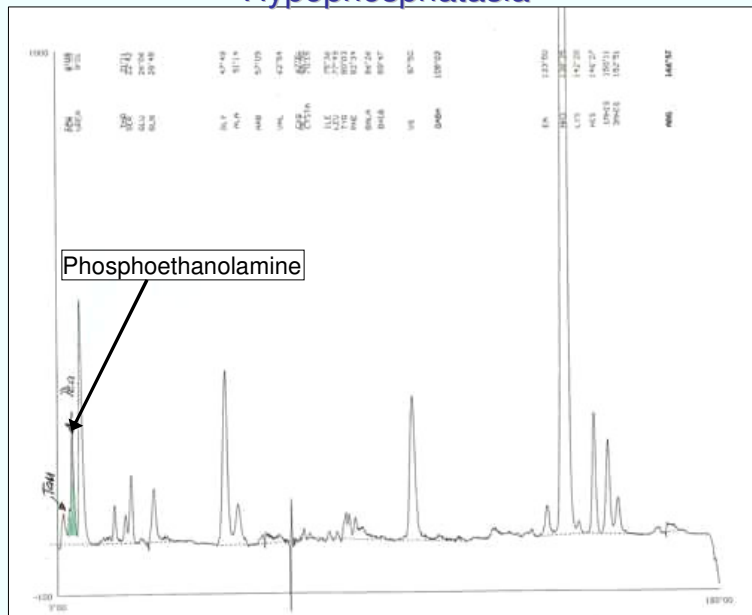
Ion-exchange chromatogram 7: Urine Hawkinsinuria



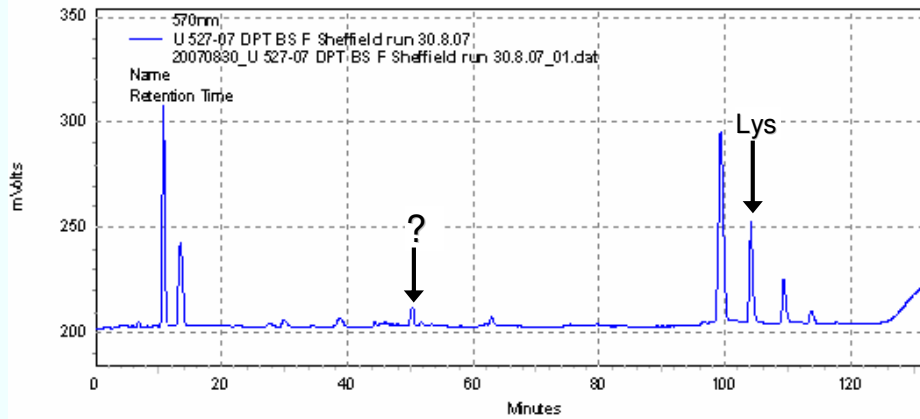
Ion-exchange chromatogram 8: Plasma Hyperargininaemia



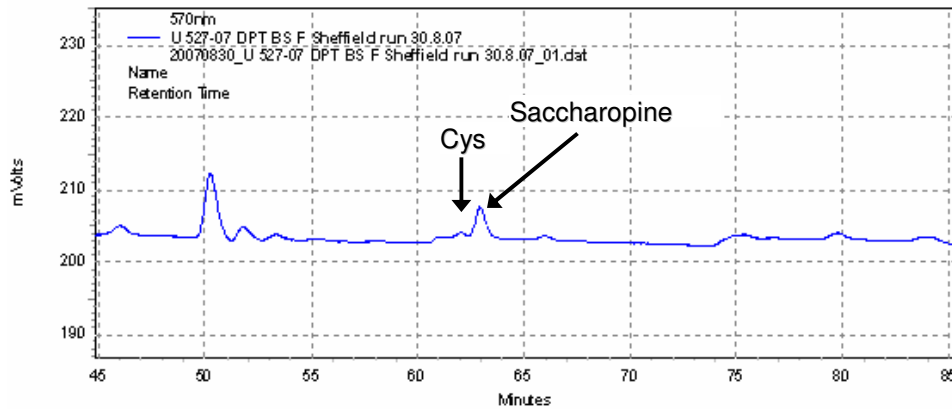
Ion-exchange chromatogram 10: Urine Hypophosphatasia



Amino acids: Basel DPT 2007 Sample 5



Amino acids: Basel DPT 2007 Sample 5 Expanded Scale



Pitfalls in Diagnosis of Amino Acid Disorders

Interferences and Influences

Diet

Specific effects e.g. red meat, poultry carnosine

anserine 1-methylhistidine, Balanine

Gelatine rich foods proline, hydroxyproline glycine

IV alimentation

hyperaminoacid-aemia, -uria

Drugs

paracetamol phenylalanine, lysine in aspirin preps.

Antibiotics

penicillin derivatives

Valproate

glycine, alanine

Pitfalls in Diagnosis of Amino Acid Disorders

Interferences and Influences

Sample handling artefacts

Urine Bacterial contamination (pH > 7, Nitrite test positive)

glutamine → glutamic acid, asparagine → aspartic acid

losses of e.g. glycine, alanine, proline,

hippuric acid → glycine

cystathionine → homocystine

Faecal contamination

increases of all amino acids

especially proline, glutamic acid

branched chain amino acids

Pitfalls in Diagnosis of Amino Acid Disorders

Sample handling artefacts ↑ ↓

Plasma sample processing

Haemolysis

general increase, aspartic acid ↑, glutamic acid ↑
glutathione ↑

Delay in separation

homocysteine increases, arginine → ornithine

Leukocyte, platelet contamination

taurine ↑

Storage of plasma (even frozen)

glutamine → glutamic acid, asparagine → aspartic acid

Delay in deproteinization

cystine and homocystine bind to protein (also in urine with protein)



ERNDIM =  quality

We provide 9 external quality control schemes for:

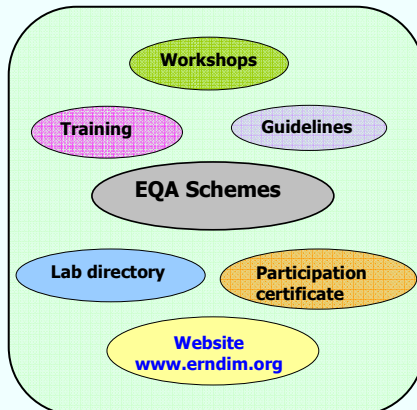
- Quantitative Amino Acids
- Special Assays in Urine
- Special Assays in Serum
- Quantitative Organic Acids in Urine
- Qualitative Organic Acids
- Quantitative Purines / Pyrimidines in Urine
- Acyl Carnitines
- Cystine in White Cells
- Diagnostic Proficiency Schemes

Number of participants range from 28 to 200 per scheme.

Schemes are organized according to the latest scientific and regulatory standards.

We have 1050 scheme subscriptions with 270 participants from mainly European countries but also worldwide.

The ERNDIM board, scientific advisor board and executive committee work together to strive for improvement of laboratory services to the benefit of our patients.



Partners

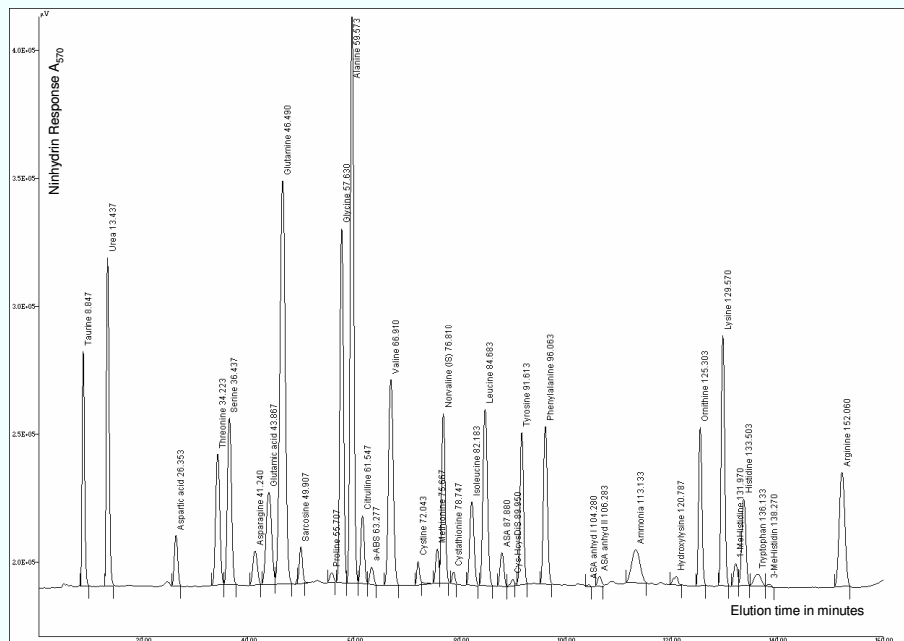


SSIEM

ERNDIM EQA Schemes

Schemes	Samples	Reporting Web-Site
Qualitative organic acids	Abnormal patient and control urines	Diagnosis & recommendations
Quantitative organic acids	8 freeze dried urines 15 organic acids added	OA concentrations
Quantitative amino acids	8 freeze dried plasmas 25 std. + 4 special amino acids	AA concentrations
Special assays urine	8 freeze dried urines	concentrations
Special assays plasma	8 freeze dried plasmas	concentrations
Purines / Pyrimidines	8 freeze dried urines	concentrations
Proficiency Test	5 abnormal urines/year various abnormalities	Diagnosis recommendations Meeting at SSIEM
Cystine (WBC)	8 freeze dried plasmas added cystine +protein	Cystine / protein concns.

Ion-exchange chromatogram: ERNDIM Sample No. 79



ERNDIM Amino acid QC scheme:

Annual Report

Annual Report Amino Acids 2001										
Analyte	Accuracy (Mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (% added analyte)		Data all labs	
	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Nr. of Labs	Inter Lab CV
	Alanine	735	774	6.1%	4.6%	0.9898	0.9918	88%	98%	158
alpha-Aminobutyric acid	23.8	25.3	2.1%	7.9%	0.9889	0.9667	87%	99%	141	13.5
Arginine	277	288	8.1%	4.7%	0.9852	0.9926	92%	96%	157	8.1
Argininosuccinic acid	153	153	41.0%	15.3%	0.9016	0.9839	82%	70%	112	218.5
Asparagine	91.1	67.6	10.1%	8.3%	0.9469	0.9554	125%	95%	136	23.5
Aspartic Acid	45.3	61.3	6.8%	6.8%	0.9768	0.9723	65%	92%	152	16.5
Citrulline	95.4	100	5.6%	6.1%	0.9970	0.9941	89%	97%	149	9.8
Cystathionine	10.4	12.3	11.3%	15.4%	0.9596	0.9341	82%	104%	130	29.9
Cystine	20.6	20.1	14.8%	9.3%	0.9491	0.9865	43%	48%	143	19.1
Glutamic acid	190	204	9.5%	6.0%	0.9779	0.9894	93%	98%	153	9.7
Glutamine	903	960	5.7%	5.8%	0.9923	0.9925	86%	94%	156	9.8
Glycine	465	476	4.9%	4.5%	0.9923	0.9896	88%	92%	157	7.6
Histidine	133	140	4.6%	5.9%	0.9934	0.9842	87%	94%	154	10.6
Histidine 1-Methyl	38.3	41.0	15.2%	9.9%	0.9662	0.9834	81%	87%	135	33.5
Histidine 3-Methyl	3.88	4.90	81.1%	29.2%	n.a.a.	n.a.a.	n.a.a.	n.a.a.	128	54.1
Hydroxylysine	14.1	13.6	7.5%	12.5%	0.9730	0.9157	87%	93%	113	58.5
Hydroxyproline	31.6	35.2	17.4%	10.7%	0.9716	0.9691	91%	85%	129	20.6
Isoleucine	147	150	7.3%	4.8%	0.9912	0.9940	89%	93%	157	8.2
Leucine	324	335	7.0%	5.1%	0.9927	0.9939	90%	93%	157	8.0
Lysine	308	312	7.0%	4.5%	0.9862	0.9915	100%	104%	157	8.3
Methionine	40.6	45.1	9.6%	6.3%	0.9873	0.9873	83%	95%	156	9.5
Ornithine	174	177	6.4%	5.0%	0.9922	0.9938	92%	96%	154	8.0
Phenylalanine	263	273	6.7%	4.1%	0.9944	0.9969	85%	91%	157	7.4
Proline	344	310	8.4%	6.3%	0.9077	0.9026	118%	95%	142	9.5
Sarcosine	255	244	19.4%	8.6%	0.9688	0.9914	91%	97%	125	35.0
Serine	234	241	5.1%	5.1%	0.9889	0.9874	88%	95%	157	7.4
Taurine	202	210	13.6%	5.5%	0.9696	0.9950	87%	93%	153	8.2
Threonine	199	207	6.5%	4.6%	0.9866	0.9911	86%	94%	156	7.3
Tyrosine	233	240	7.5%	4.8%	0.9939	0.9969	86%	90%	157	8.6
Valine	379	389	6.1%	4.7%	0.9892	0.9916	91%	97%	157	7.1
Overall	211	217	12.1%	7.7%	0.9762	0.9795	88%	92%	146	22.8

n.a.a. = No analyte added, thus no linearity and recovery calculated

Annual comment of scheme organiser and scheme advisor [Click to download](#)

ERNDIM Amino acid QC scheme: Annual Report

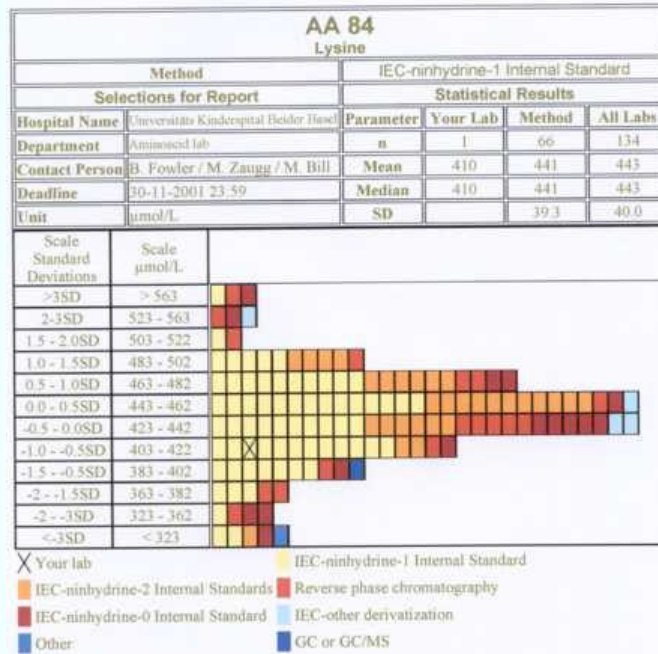
Detail

Annual Report Amino Acids 2001										
Analyte	Accuracy (Mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (% added analyte)		Data all labs	
	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Nr. of Labs	Inter Lab CV
Alanine	735	774	6.1%	4.6%	0.9898	0.9918	88%	98%	158	7.6
alpha-Aminobutyric acid	23.8	25.3	2.1%	7.9%	0.9889	0.9667	87%	99%	141	13.5
Arginine	277	288	8.1%	4.7%	0.9852	0.9926	92%	96%	157	8.1
Argininosuccinic acid	153	153	41.0%	15.3%	0.9016	0.9839	82%	70%	112	218.5
Asparagine	91.1	67.6	10.1%	8.3%	0.9469	0.9554	125%	95%	136	23.5
Aspartic Acid	45.3	61.3	6.8%	6.8%	0.9768	0.9723	65%	92%	152	16.5
Citrulline	95.4	100	5.6%	6.1%	0.9970	0.9941	89%	97%	149	9.8
Cystathionine	10.4	12.3	11.3%	15.4%	0.9596	0.9341	82%	104%	130	29.9
Cystine	20.6	20.1	14.8%	9.3%	0.9491	0.9865	43%	48%	143	19.1
Glutamic acid	190	204	9.5%	6.0%	0.9779	0.9894	93%	98%	153	9.7
Glutamine	903	960	5.7%	5.8%	0.9923	0.9925	86%	94%	156	9.8
Glycine	465	476	4.9%	4.5%	0.9923	0.9896	88%	92%	157	7.6
Histidine	133	140	4.6%	5.9%	0.9934	0.9842	87%	94%	154	10.6
Histidine 1-Methyl	38.3	41.0	15.2%	9.9%	0.9662	0.9834	81%	87%	135	33.5
Histidine 3-Methyl	3.88	4.90	81.1%	29.2%	n.a.a.	n.a.a.	n.a.a.	n.a.a.	128	54.1
Hydroxylysine	14.1	13.6	7.5%	12.5%	0.9730	0.9157	87%	93%	113	58.5
Hydroxyproline	31.6	35.2	17.4%	10.7%	0.9716	0.9691	91%	85%	129	20.6
Isoleucine	147	150	7.3%	4.8%	0.9912	0.9940	89%	93%	157	8.2
Leucine	324	335	7.0%	5.1%	0.9927	0.9939	90%	93%	157	8.0
Lysine	308	312	7.0%	4.5%	0.9862	0.9915	100%	104%	157	8.3
Methionine	40.6	45.1	9.6%	6.3%	0.9873	0.9873	83%	95%	156	9.5
Ornithine	174	177	6.4%	5.0%	0.9922	0.9938	92%	96%	154	8.0
Phenylalanine	263	273	6.7%	4.1%	0.9944	0.9969	85%	91%	157	7.4
Proline	344	310	8.4%	6.3%	0.9077	0.9026	118%	95%	142	9.5
Sarcosine	255	244	19.4%	8.6%	0.9688	0.9914	91%	97%	125	35.0
Serine	234	241	5.1%	5.1%	0.9889	0.9874	88%	95%	157	7.4
Taurine	202	210	13.6%	5.5%	0.9696	0.9950	87%	93%	153	8.2
Threonine	199	207	6.5%	4.6%	0.9866	0.9911	86%	94%	156	7.3
Tyrosine	233	240	7.5%	4.8%	0.9939	0.9969	86%	90%	157	8.6
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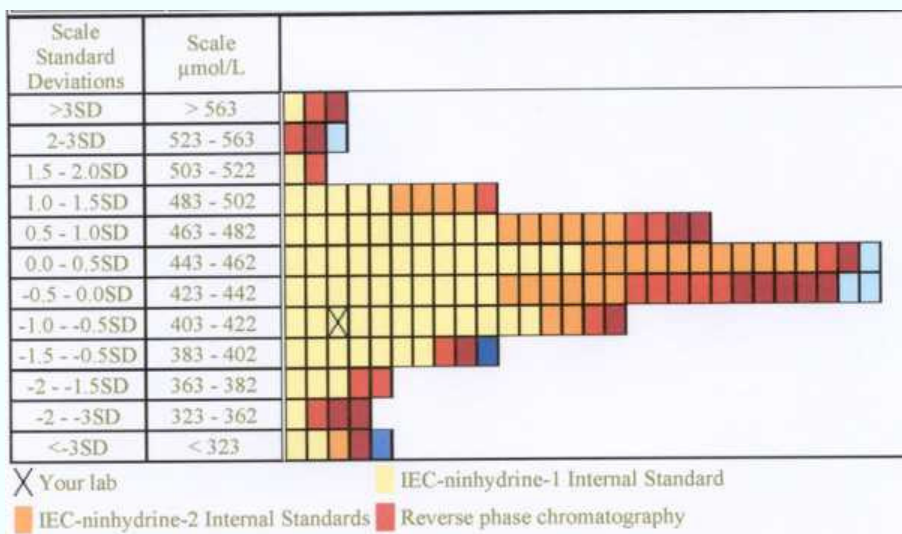
ERNDIM
Amino acid
QC
scheme:

Annual
Report

Individual
Amino Acid



ERNDIM Amino acid QC scheme: Annual Report
Individual Amino Acid: Close up



ERNDIM Proficiency Scheme: Design

Clinical picture

Lab results and interpretation

Preinvestigations

Amino acid analysis

Organic acid analysis

Purines/pyrimidines analysis

Mucopolysaccharide analysis

Other analyses performed

Conclusion (diagnosis)

Advice for follow up investigations

Advice to attending clinician

ERNDIM Proficiency Scheme: Sample B

Clinical picture

The patient was born in December 2000 and had liver failure and tubulopathy when initially presented.

? Which investigations

ERNDIM Proficiency Scheme: Sample B

Pre-investigations (number of labs 19 / 20)

Amino acid analysis (number of labs 19)

Organic acid analysis (n = 18)

Purines/pyrimidines analysis (3)

Mucopolysaccharide analysis (3)

Other analyses performed

ERNDIM Proficiency Scheme: Sample B

Pre-investigations (number of labs 19 / 20)

Creatinine 1.0-2.5, mean 1.3mmol/L, pH 6-9 (8.0)

nitrite +ve (11/20), all others negative

Amino acid analysis (number of labs 19)

Tyrosine ↑ (17), Glycine ↑ (15), Methionine ↑ (12), Normal 1

Organic acid analysis (n = 18)

See Chromatogram

Purines/pyrimidines analysis (3)

Normal

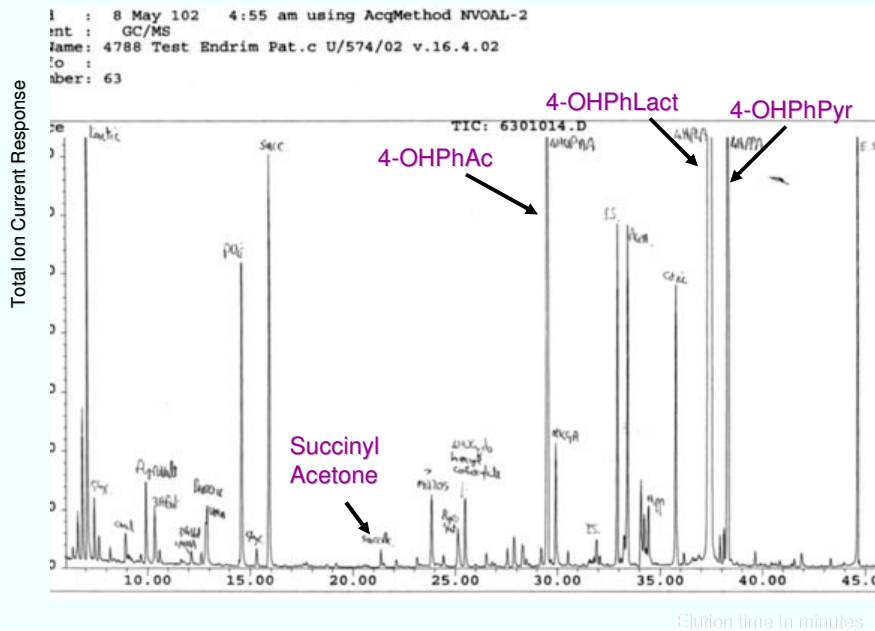
Mucopolysaccharide analysis (3)

Normal

Other analyses performed

oxalate (n=1)

GC-MS chromatogram: Case B



ERNDIM Proficiency Scheme: Sample B

Crucial Analysis

Succinyl Acetone or related compounds by GC/MS or photometric method.
 Presence of tyrosine derivatives only partially correct since it cannot differentiate other causes of disturbed tyrosine metabolism

Organic acid analysis	n
↑ 4-OH-phenyllactate	17
↑ 4-OH-phenylpyruvate	16
↑ 4-OH-phenylacetate	16

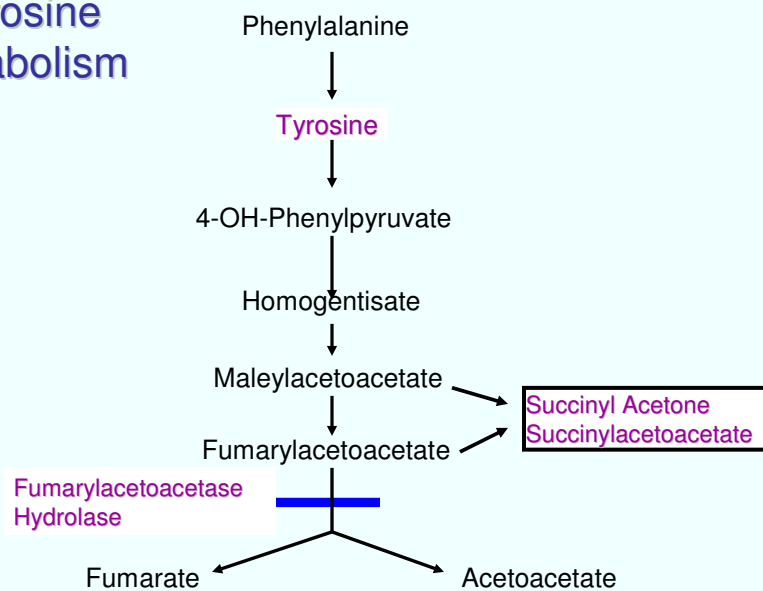
Succinyl acetone or derivative positive n=15

ERNDIM Proficiency Scheme: Sample B

Conclusion (diagnosis)	n
Tyrosinaemia type I	17
Tyrosinaemia probably type I	1
Tyrosinaemia type I or hepatorenal tyrosinaemia	1
hepatorenal tyrosinaemia	1

Tyrosinaemia type I

Tyrosine Metabolism



ERNDIM Proficiency Scheme: Sample B

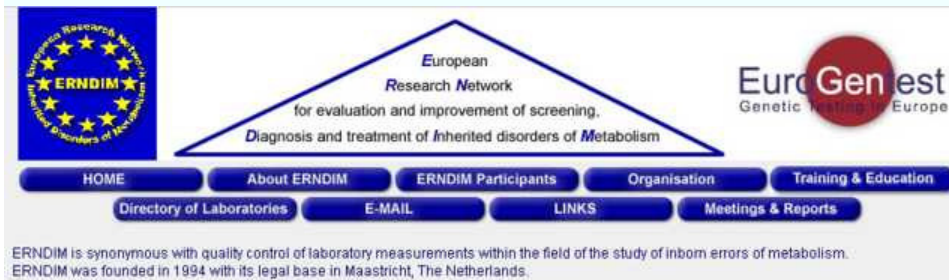
Advice for follow up investigations	n
• organic acids in urine	4
• succinylacetone in urine	4
• plasma amino acids	12
• fumarylacetoacetase in leuco / fibroblasts	12
• mutation analysis	9
• α -foetoprotein	10
• routine biochemical parameters	5
• δ -aminolaevulinic acid in urine	6
• clotting factors	6

ERNDIM Proficiency Scheme: Sample B

Advice to attending clinician	
• NTBC therapy	17
• restricted Tyrosine, Phenylalanine intake	17
• liver transplantation	10
• family studies	2
• genetic counselling	4
• possible prenatal diagnosis	4

Expert View

- confirm diagnosis by enzyme or mutation
- dietary treatment + NTBC
- monitor possible developing hepatoma, or liver transplant possibility.



ERNDIM is synonymous with quality control of laboratory measurements within the field of the study of inborn errors of metabolism. ERNDIM was founded in 1994 with its legal base in Maastricht, The Netherlands.

Support grants

We remind you of the two support grants:

[Training grants](#) to allow trainees, to spend a short visit to a European ERNDIM approved laboratory to gain experience and specialised knowledge

[Support grants](#) to allow laboratories from European countries suffering severe financial restrictions to participate in ERNDIM QA schemes.



