METBIONET GUIDELINES FOR AMINO ACID ANALYSIS.

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INTRODUCTION

Amino acids play a role as primary components of proteins, metabolic intermediates and as a source of energy. They are present in virtually all metabolic and cellular functions and are implicated in several metabolic defects.

Inherited defects of amino acid metabolism are clinically and biochemically heterogeneous with a variable and disease specific course. Their combined incidence is approximately 1:6000. Characteristic amino acid profiles or the presence of low or normally undetectable amino acids may lead to, or suggest, a diagnosis. Once diagnosed, patients with these disorders, and others with inherited metabolic defects, require monitoring of amino acids to assess metabolic control and nutritional status.

This document, produced by the MetBioNet Amino Acid Working Group, aims to set out the ideal conditions for amino acid analysis in a variety of sample types and clinical situations. Limitations and methodology are discussed. There are notes to aid interpretation of artefactual changes in the sample and tables listing expected amino acid deviations from normal in a variety of inherited metabolic defects.

A review and extension of the guidelines is intended to optimise the analytical guidelines and to provide recommendations for amino acid reference ranges once the results of work currently in progress become available i.e. the European Research Network for the Evaluation and Improvement of Screening Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM) Laboratory survey of amino acid measurement and the MetBioNet Amino Acid Working Group reference range study.

See ***Appendix 1.*** for a list of amino acid abbreviations used in this document.

A. Clinical Indications for Amino Acid analysis

The clinical presentation of metabolic disorders may be variable, non-specific and can occur at any age. Therefore amino acid investigations should be considered if any of the following are present.

1. Lethargy, coma, seizures or vomiting in a neonate
2. Hyperammonaemia
3. Ketosis
4. Metabolic acidosis or lactic acidaemia
5. Alkalosis
6. Metabolic decompensation
7. Unexplained developmental delay or developmental regression
8. Polyuria, polydipsia and dehydration
9. Unexplained liver dysfunction
10. Unexplained neurological symptoms
11. Abnormal amino acid results on newborn screening programme
12. Previous sibling with similar clinical presentation
13. Clinical presentation specific to an amino acid disorder

Clinical information should be supplied when requesting metabolic investigations.

An index of the groups of disorders of Amino Acid Metabolism is given in ***Appendix 2.*** The amino acid abnormalities found in the inherited disorders of amino acid metabolism, in renal amino acid transport and resorption defects and in other metabolic conditions are outlined in ***Appendices 3, 4 and 5.***

B. Type of analysis (qualitative or quantitative)

Amino acid analyses in the biological fluids, blood, urine and CSF all have a role in the diagnosis and monitoring of aminoacidopathies and other metabolic disorders. Before requesting amino acid investigations, consideration should be given to the choice of sample type and whether qualitative or quantitative analysis is necessary.

B.1 Qualitative Analysis

Qualitative analysis, or general screening, by methods such as thin layer chromatography will only detect marked changes in amino acid concentrations. Amino acids co-migrate; therefore decreases, or mild elevations of individual amino acids, are not visible and can be missed. Some amino acids e.g. homocysteine are not easily detectable by these methods. Interpretation of chromatograms is subjective and can be misleading. Therefore users must be aware of the limitations of their method of choice.

If qualitative analysis is performed then plasma is the preferred sample type. Screening of urine samples will detect defects in renal transport of amino acids, but for other aminoacidopathies the interpretation can be difficult, as amino acid excretion is variable and subject to interference from medication. The presence of a generalised aminoaciduria can be useful as an indication of many metabolic disturbances ***(Appendix 3).*** However screening of urine alone should be discouraged.

Qualitative analysis should be reported with a caution that it is a screening test and only gross abnormalities can be excluded. If clinical indications are suggestive of an aminoacidopathy then quantitative analysis should be performed.

B.2 Quantitative Analysis.

The presence of both an amino group and a carboxyl group enables amino acids to be separated and identified by some form of chromatography, most commonly ion-exchange with ninhydrin detection. Liquid chromatography with tandem mass spectrometry can also be used to quantitate amino acids and is currently used in the UK to measure individual or small groups of amino acids rather than to quantitate full physiological amino acid profiles. Quantitation of compounds is by comparison to amino acid standards of known concentration.

For quantitative amino acid analysis plasma is the most informative and therefore is the preferred sample type. It is important to note however that disorders of renal amino acid transport e.g. cystinuria will be missed if a plasma sample alone is analysed. For the diagnosis of this group of disorders quantitation of urinary amino acids should be performed. Amino acids are reported relative to the creatinine level to compensate for urine concentration. Quantitative amino acid analysis in CSF samples is useful for the investigation of neurological disorders and essential for the diagnosis of non ketotic hyperglycinaemia. CSF/Plasma ratio of amino acids is more informative than an isolated CSF sample. A paired plasma sample should be obtained within two hours.

Appendix 3 shows the changes in amino acid concentrations in plasma, urine and CSF seen in amino acid disorders, ***Appendix .*** highlights the expected findings in disorders in which amino acid abnormalities are predominantly found in urine and ***Appendix 5*** the changes seen in other metabolic conditions which may be indicated using quantitative amino acid analysis.

C. Profile for Quantitation of Amino Acids

C.1 Plasma profile for diagnosis of amino acid disorders

An amino acid profile (as recommended by the MetBioNet Stakeholders[5]) capable of identifying the majority of inherited disorders of amino acid metabolism and other metabolic defects (listed in ***Appendices 3., 4. and 5***.), is given in ***Table 1.***

Table 1. **Amino acid profile**

A plasma profile for the diagnosis of amino acid disorders	
Alanine	Leucine
Alloisoleucine	Lysine
Arginine	Methionine
Argininosuccinic acid	Ornithine
Citrulline	Phenylalanine
Cystine	Proline
Glutamic acid	Serine
Glutamine	Sulphocysteine**
Glycine	Taurine
Histidine	Threonine
Homocysteine*	Tyrosine
Isoleucine	Valine

* Plasma total homocysteine is not detected by routine methods and plasma free homocystine analysis shows poor sensitivity for the diagnosis of mild forms of homocystinuria[2].

** Sulphocysteine may not be detectable in plasma using routine methods in sulphite oxidase and molybdenum co-factor deficiencies[3,4].

C.2 Plasma profile for monitoring

Metabolic control in patients with previously diagnosed amino acid disorders requires analysis of the affected metabolites (e.g. alloisoleucine, isoleucine, leucine and valine in maple syrup urine disease). Based on current opinion, the above profile is adequate for monitoring the nutritional status of patients including those on low protein diets[5].

C.3 Urine profile for diagnosis of renal tubulopathies

Diagnosis of disorders of amino acid transport and other renal tubulopathies requires analysis of amino acids in urine (see ***Appendix 4.***). The amino acid profile in ***Table 1*** is adequate for the diagnosis and monitoring of these disorders.

D. Specimen collection

D.1 Blood

Lithium heparin venous plasma is the preferred specimen type. In general, unless specified for a particular reason or clinical question, specimens should be collected in the pre-prandial state. Timing of the specimen in relation to feeds and a list of drug therapy should be provided to aid interpretation of results. The specimen should be separated promptly taking care to avoid disturbing the buffy coat. Plasma should be stored and transported deep frozen. Prompt separation and deproteinisation is essential for accurate measurement of (free) sulphur containing amino acids. Total homocysteine can be measured as an alternative to free homocystine, although specimens still require prompt separation.

Notes:

- Serum should not be used because blood needs to clot at room temperature during which there may be deamination (asparagine to aspartic acid and glutamine to glutamic acid), loss of sulphur containing amino acids and release of oligopeptides[5].
- EDTA plasma is recommended in some centres as the specimen of choice. The older literature reports ninhydrin positive artefacts in EDTA plasma but modern tubes do not seem to have this problem[5].
- Haemolysis must be avoided because it will cause ***increases*** in serine, glycine, taurine, phosphoethanolamine, aspartic acid, glutamic acid, ornithine and ***decreased*** arginine[5].
- Delayed separation or leucocyte and platelet contamination will cause ***increased*** serine, glycine, taurine, phosphoethanolamine, ornithine, glutamic acid and ***decreased*** arginine, homocystine, cystine[5].
- Phenylalanine and tyrosine increase if specimen separation is delayed – this effect is more pronounced at normal physiological concentrations than at higher concentrations and has implications for PKU monitoring by liquid blood specimens posted in from home[6].
- Amino acids are more stable in deep frozen deproteinised plasma than in deep frozen native plasma[7,8].
- Capillary blood may be used with careful cleaning of the skin prior to specimen collection provided the blood is flowing freely. If excessive pressure is required, the artefacts of haemolysis may be observed.
- Free tryptophan may be lost when using sulphosalicylic acid as deproteinising agent; trichloroacetic acid is the deproteinising agent of choice for this amino acid[48].
- At temperatures greater than 35^{o}C, glutamine is converted to the ninhydrin negative compound pyrrolidone carboxylic acid. It is therefore important to ensure that specimens are kept cool before analysis including after deproteinisation and whilst in an autosampler prior to loading onto an analyser.
- Sodium metabisulphite, found in some intravenous preparations as a preservative, can cause the conversion of cystine to sulphocysteine[9].

D.2. Urine

Where urine is the specimen of choice it should be collected into preservative free bottles. In practice random urine specimens are acceptable because urine creatinine can be used to normalise results[10,11]. Faecal contamination must be avoided. Specimens should be frozen immediately and transported deep frozen. If there is likely to be a significant delay and it is not possible to freeze the specimen, merthiolate or thymol may be used as a preservative. It is ESSENTIAL that specimen quality is checked by testing for nitrite and pH. If a specimen shows signs of deterioration, some amino acids may be falsely low and a diagnostic abnormality could potentially be 'missed'. A repeat urine should be requested if there is any evidence of specimen deterioration.

Amino acid concentrations in urine show more variation than in plasma due to differences in renal function and diurnal variation. There is also more interference from drugs and drug metabolites.
Notes

- Results obtained in very dilute urine specimens (creatinine < 1.0 mmol/L) should be interpreted with caution and a repeat specimen should be considered.
- Specimen deterioration causes decreased serine, increased or decreased alanine, increased glycine, decarboxylation of glutamic acid to form γ-aminobutyric acid, breakdown of phosphoethanolamine to ethanolamine and phosphate, breakdown of cystathionine to homocystine and hydrolysis of peptides causing increased proline[12]. A repeat specimen should be requested if there is any evidence of specimen deterioration.
- Faecal contamination causes increased proline, glutamic acid, branched chain amino acids but not hydroxyproline. Faecal bacteria can produce γ-aminobutyric acid from glutamic acid and β-alanine from aspartic acid[13].
- Many drugs and metabolites produce ninhydrin positive peaks e.g. antibiotics, paracetamol, penicillamine. It is important to be aware of how these run on the analytical system being used[14].
- Some drugs interfere with amino acid metabolism and cause apparent amino acid abnormalities e.g. valproate causes increased glycine, vigabatrin causes increased β-alanine and γ-aminobutyric acid, asparaginase causes increased aspartic acid[14].
- Some dietary products lead to abnormal amino acids e.g. heat treated milk products produce homocitrulline, Chix (comminuted chicken) feed is high in the dipeptides carnosine and anserine.

D.3 Cerebrospinal fluid

CSF should be collected into preservative free bottles; however fluoride oxalate and lithium heparin tubes may also be used. CSF should be stored frozen if not immediately analysed. Specimens contaminated with blood should not be analysed because most amino acids are present in blood at much higher concentrations than in CSF. Ideally a simultaneous plasma specimen should be analysed and the CSF: plasma ratio calculated for individual amino acids.

D.4 Dried bloodspots.

Bloodspots should be collected from free flowing blood spotted onto newborn screening bloodspot card. Bloodspots should be left to dry naturally before placing in glassine sleeve.

E. Analytical

The analytical guidelines will be reviewed following the publication of the results of the ERNDIM amino acid questionnaire.

These guidelines are primarily based on the use of ion exchange chromatography for a full amino acid profile. This is currently the most widely used method but the guidelines may be applied where relevant when using other methods such as HPLC or UPLC. GCMS methods are available but are not widely used in the clinical setting. Tandem Mass Spectrometry is also available but is generally used for selected amino acids only e.g. phenylalanine and tyrosine for monitoring patients with phenylketonuria.

E.1 Standardisation

- For validated methods a single point calibration may be used.
- The calibrator should be an aqueous solution at a concentration appropriate for the analytical method employed, typically between 100 - 250umol/L, for each amino acid quantified.
- The frequency of calibration should be based on the stability of the analytical system used and will be determined through the practice of good quality assurance.
- Calibrators should include any amino acids that will be quantified. Glutamine (and asparagine if measured) should be added immediately before calibration.

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- Performance should be checked after any change e.g. new ninhydrin, column or lamp, to check response factor is correct.
- Reagent blanks should be analysed occasionally to monitor baseline.

E.2 Sample Preparation

- Urines may require dilution to bring amino acids into the analytical range of the instrument. Plasma samples may also need dilution where amino acid concentrations are outside the linear range. Note results which are over-range on the 570nm channel may be within the linear range on the 440nm channel.
- Samples require deproteinisation prior to analysis.
- Internal standard may be included in the deproteinising solution.
- As CSF is likely to contain low concentrations of amino acids, an increased injection volume to improve sensitivity should be considered.

E.3 Internal standards

- At least one internal standard should be used.
- Internal standard peak should not interfere with other amino acid peaks.
- The internal standard should be an amino acid which does not naturally occur in the sample.

- A fixed amount of internal standard should be added to all samples including QC, EQA and standards prior to analysis.

- Peak area or height of internal standard should be recorded and assessed for each analysis.

- See ***Table 2.*** below for examples of internal standards used in ion exchange chromatography:

Table 2. Commonly used internal standards

Full name	Abbreviation	Position	Interference
D-Glucosaminic acid	GSAA	Between urea and aspartate	
Norleucine	Nle	After leucine	May interfere with mixed disulphide and argininosuccinic acid
Norvaline	Nva	Near valine	
S-2-aminoethyl – L-cysteine	AEC	Near ornithine	

E.4 Internal Quality Control

- QC material should be of a comparable matrix (plasma, urine, CSF) and concentration to the samples being analysed.

- QC samples should be analysed regularly and after any maintenance changes e.g. with each bottle of ninhydrin.

- Results should be recorded and any falling outside 2 standard deviations should be investigated.

- QC samples may be a commercial product where available or pooled patient samples which may be enriched with other amino acids. Material may be obtained from ERNDIM.

E.5 External Quality Control

- Laboratories should participate in external quality assurance programmes
  E.g. ERNDIM www.**erndim**qa.nl or UKNEQAS www.uk**neqas**.org.uk

E.6 Precision

- An inter assay CV of <10% should be achievable for most amino acids.

F. Reference Intervals

Collation of data is still ongoing.

G. Reporting Amino acid results

These recommendations are in line with the Clinical Pathology Accreditation (UK) Ltd 'Standards for the Medical Laboratory' (www.cpa-UK.co.uk) and Royal College of Pathologists 'Code of Practice for clinical biochemists (chemical pathologists) and clinical biochemistry services' May 2005 (www.rcpath.org).

The results of plasma amino acids must be reported in a timely manner with the inclusion of relevant interpretive comments and with clinical liaison as appropriate. Only appropriately qualified and trained individuals should perform interpretation and clinical authorization unsupervised. Urgent or abnormal results that may affect patient management should be telephoned to the requesting clinical team as appropriate.

The written report is a permanent record of the investigation undertaken. It should provide all the necessary information (see below) should be clear, unambiguous and succinct. The report should include the following information:-

1. Laboratory information
    a) Name and address of performing laboratory
    b) Contact telephone no. of reporting laboratory

2. Patient and specimen information
    a) Unequivocal identification of the patient. This includes a unique patient identifier which for England and Wales is the NHS number, a mandatory requirement on all patient records from September 18th 2009 (National Patient Safety Agency Safer Practice Notice NPSA/2009/SPN002).
    b) Requesting clinician
    c) Specimen type
    d) Date & time of:
        i. Sample collection
        ii. Sample receipt
        iii. Report
3. Results
    a) A brief description of methodology used
    b) Results reported with appropriate age related reference ranges
    c) Abnormal results highlighted
    d) Interpretive comments (see below)
    e) Status of report when appropriate e.g. copy, interim, supplementary

4. Interpretive comments
    a) When no significant abnormalities are detected this should be indicated. It may be necessary to include qualifiers including:
        i. An explanation for any non significant variation from normal
        ii. Methodological limitations e.g. Urine amino acid analysis & homocystinuria
        iii. Nature of sample e.g. dilution, delayed separation and analysis
        iv. Clinical state –e.g. suitably stressed, diet

b) When abnormal results are detected, a detailed interpretation should include
    i. A brief description of the abnormalities
    ii. A possible diagnosis/differential diagnosis with reference to available clinical information if relevant
    iii. Recommendations for additional biochemical testing including confirmatory studies (enzyme assay, molecular analysis) and family testing if appropriate
    iv. A record of results being communicated directly if telephoned
    v. Contact details for further discussion if required
    vi. Dated and recorded

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Appendix 1. Abbreviations used for amino acids

	Abbreviation	Amino Acid		Abbreviation	Amino Acid
	Aad	αAminoadipic acid		His	Histidine
	Abu	Aminobutyric acid		Hyp	Hydroxyproline
	Aile	Alloisoleucine		Hyl	Hydroxylysine
	Ala	Alanine		Ile	Isoleucine
	Ans	Anserine		Leu	Leucine
	Arg	Arginine		Lys	Lysine
	Asn	Asparagine		Met	Methionine
	Asa	Argininosuccinic acid		Nle	Norleucine
	Asp	Aspartic Acid		Nva	Norvaline
	ß-Ala	ß-Alanine		Orn	Ornithine
	ß-Aiba	ß-Aminoisobutyric acid		Pea	Phosphoethanolamine
	Car	Carnosine		Phe	Phenylalanine
	Cysta	Cystathionine		Pip	Pipecolic acid
	Cys	Cysteine		Pro	Proline
	Cys_2	Cystine		Sac	Saccharopine
	GABA	Gamma aminobutyric acid		Sar	Sarcosine
	Glu	Glutamic acid		Scys	Sulphocysteine
	Gln	Glutamine		Ser	Serine
	Gly	Glycine		Tau	Taurine
	Hcit	Homocitrulline		Thr	Threonine
	Hcy	Homocysteine		Trp	Tryptophan
	Hcy_2	Homocystine		Tyr	Tyrosine
	Hcy-Cys	Homocysteine - Cysteine Mixed Disulphide		Val	Valine

Appendix 2. Index of Groups of Disorders of Amino Acid Metabolism

A. UREA CYCLE And Related Disorders			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Ornithine Transcarbamylase Deficiency	311250	Ornithine transcarbamylase	OTC, OCT
N-Acetylglutamate Synthase Deficiency	237310	N-Acetylglutamate synthase	NAGS
Carbamoyl Phosphate Synthase Deficiency	237300	Carbamoyl phosphate synthase	CPSI
Citrullinaemia Type I	215700	Argininosuccinic acid synthase	
Citrullinaemia Type II	603814 603471	Citrin	Citrin deficiency NICCD
Argininosuccinic aciduria	207900	Argininosuccinic acid lyase	ASA
Argininaemia	107830	Arginase	
Lysinuric Protein Intolerance (Dibasic aminoaciduria II)	222700	Dibasic amino acid transporter	LPI
Hyperornithinaemia, hyperammonaemia, homocitrullinuria	238970	Mitochondrial ornithine translocase	HHH Syndrome
Ornithinaemia,	258870	Ornithine aminotransferase (OAT)	Gyrate Atrophy, HOGA
Hypo-ornithinaemia		Ornithine aminotransferase (OAT)	Neonatal Gyrate Atrophy
B. Phenylalanine and Tyrosine Metabolism			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Phenylketonuria Classical and Mild Forms	261600	Phenylalanine hydroxylase	PKU
Dihydropteridine Reductase Deficiency	261630	Dihydropteridine reductase	DHPR
Tyrosinaemia Type I	276700	Fumarylacetoacetate lyase	
Tyrosinaemia Type II	276600	Tyrosine aminotransferase	Oculocutaneous Tyrosinaemia
Tyrosinaemia Type III	276710	4-Hydroxyphenylpyruvate dioxygenase	
C. Methionine and Sulphur metabolism			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Homocystinuria	236200	Cystathionine ß-synthase	HCU
5,10-Methylenetetrahydrofolate Reductase Deficiency	236250	5,10-Methylenetetrahydrofolate reductase	MTHFR
Methylmalonic Acidaemia - Homocystinuria	236270	Methionine synthase reductase	
Hypermethioninaemia	250850	Methionine adenosyltransferase	
Cystathioninuria	219500	Cystathioninase	
Sulphite Oxidase Deficiency	606887	Sulphite oxidase	
Molybdenum Cofactor Defect	252150	Molybdopterin synthase	

D. Proline and Hydroxyproline metabolism			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Hyperprolinaemia Type I	239500	Proline oxidase	
Hyperprolinaemia Type II	239510	Δ^1Pyrroline-5-carboxlate-dehydrogenase	
Δ^1Pyrroline-5-carboxlate synthase Deficiency	138250	Δ^1Pyrroline-5-carboxlate synthase	
Hyperhydroxyprolinaemia	237000	4-Hydroxyproline oxidase	
E. Branched Chain Amino Acid metabolism			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Maple Syrup Urine Disease (Branched Chain ketoaciduria)	248600	Branched chain α-ketoacid dehydrogenase complex (BCKD)	MSUD
Hypervalinaemia or Hyperisoleucine-hyperleucinaemia	277100	Mitochondrial branched chain aminotransferase 2	
F. Lysine metabolism			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Hyperlysinaemia	238700	Lysine: α-ketoglutarate reductase	
Saccharopinuria	268700	α-Aminoadipic semialdehyde synthase	
G. ß and γ Amino Acid metabolism			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Hyper-ß-Alaninaemia	237400	ß-Alanine-α-ketoglutarate aminotransferase	
Hyper-ß-Aminoisobutyric aciduria	210100	3-Aminoisobutyrate: pyruvate aminotransferase	
GABA transaminase Deficiency	137150	4-Aminobutyrate transferase	
4-Hydroxybutyric aciduria	271980	Succinic semialdehyde dehydrogenase	
Carnosinuria	212200	Carnosinase	
Homocarnosinuria	216130		
H. Miscellaneous			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Nonketotic Hyperglycinaemia	605899	Glycine cleavage enzyme system	NKH
Histidinaemia	235800	Histidine ammonia lyase	
3-Phosphoglycerate Dehydrogenase Deficiency	601815	3-Phosphoglycerate Dehydrogenase	PHGDH
Sarcosinaemia	268900	Sarcosine dehydrogenase	
I. Renal Tubular Aminoacidopathies			
Disease	**OMIM Number**	**Defective Enzyme/Protein**	**Other Terminology**
Cystinuria Type I	220100	Renal dibasic amino acid transporter:heavy subunit	COAL
Cystinuria Type II & III	220100	Renal dibasic amino acid transporter: light subunit	
Iminoglycinuria	242600	Renal transporter of proline, hydroxyproline and glycine	
Hartnup disorder	234500	Neutral amino acid transporter	

Appendix 3.

Diagnosis of Inherited Disorders of Amino Acid Metabolism by Amino Acid Analysis

Condition	Quantitative Plasma	Quantitative Urine	Quantitative CSF *
αAminoadipic aciduria	↑Aad	↑Aada	
Argininaemia	↑Arg, (↑Gln)	↑Cys, ↑Orn, ↑Arg, ↑Lys	
Argininosuccinic aciduria	↑Asa, ↑Gln, ↑Cit, (↓Arg)	↑Asa, ↑Cit	
ß-Alaninaemia	↑ß-Ala, ↑ß-Aiba, ↑GABA	↑GABA, ↑ß-Ala, ↑Tau	
Carbamoyl Phosphate Synthase deficiency	↑Gln, ↓Cit, ↓Arg, (↑Ala)	↑Gln	
Carnosinaemia	↑Car, (↑Ans)	↑Car, (↑Ans)	
Citrullinaemia Type I	↑Cit, ↑Gln, (↓Arg)	↑Cit, ↑Gln	
Citrullinaemia Type II (Citrin Def)	(↑Cit), (↑Orn), (↑Thr), (↑Arg), (↑Lys)	(↑Cit), (↑Orn), (↑Thr), (↑Arg), (↑Lys)	
Cystathioninase Deficiency	↑Cystathionine	↑Cystathionine	
E3 dehydrogenase deficiencies	↑Leu, ↑Ile, ↑Val, ↑Aile, ↑Ala	↑Leu, ↑Ile, ↑Val, ↑Aile, ↑Ala	
GABA transaminase deficiency	↑GABA, ↑ß-Ala	↑GABA, ↑ß-Ala	↑GABA, ↑ß-Ala
Glutamic Acidaemia	↑Glu		↑Glu
HHH Syndrome	↑Orn, (↑Gln), (↓Arg), (↓Lys)	↑Hcit, ↑Orn	
Histidinaemia	↑His	↑His	
Homocystinuria (Cystathionine β-Synthase Def)	↑Hcy, ↑Meth, ↑Hcy-Cys, ↓Cys	↑Hcy_2 , ↑Meth	
Hydroxyprolinaemia	↑Hyp	↑Hyp, ↑Pro, ↑Gly	
Hyperlysinaemia	↑Lys	↑Lys, (↑Orn), (↑Cys)	
Hypermethioninaemia (MAT)	↑Meth, (↑Hcy)	(↑Meth)	
Hypermethioninaemia (SAH)	↑Meth, ↑Hcy	(↑Meth)	
Hyperornithinaemia (Gyrate Atrophy)	↑Orn, ↓Lys, ↓Gln, ↓Arg	↑Orn, ↑Lys, ↑Arg, ↑Cys	
Hyperornithinaemia - Neonatal Gyrate Atrophy	↓Orn,↓Arg, ↑Gln		

* CSF analysis where required for diagnosis. In other disorders CSF amino acids will reflect the variation of plasma amino acids

Diagnosis of Inherited Disorders of Amino Acid Metabolism by Amino Acid Analysis (continued)

Condition	Quantitative Plasma	Quantitative Urine	Quantitative CSF
Hyperprolinaemia Type I	↑Pro	↑Pro, ↑Hyp, ↑Gly	
Hyperprolinaemia Type II	↑Pro	↑Pro, ↑Hyp, ↑Gly	
Hypervalinaemia	↑Val	↑Val	
Lysinuric Protein Intolerance	↑Gln, (↓Lys), (↑Cit), (↓Arg), (↓Orn)	↑Lys, ↑Arg, ↑Orn, ↑Gln, (↑Cys)	
Maple Syrup Urine Disease	↑Leu, ↑Ile, ↑Val, ↑Aile, ↓Ala	↑Leu, ↑Ile, ↑Val	
5, 10-Methylene Tetrahydrofolate Reductase Def	↑Hcy, (↓Meth)	↑Hcy_2	
Molybdenum Cofactor Deficiency	↑Scys, ↓Cys, ↓Hcy, (↑Tau)	↑Scys, ↑Tau	
N-Acetylglutamate Synthase Deficiency	↑Gln, (↓Cit), (↓Arg)	↑Gln	
Non Ketotic Hyperglycinaemia	↑Gly	↑Gly	↑CSF/Plasma Gly ratio
Ornithine Transcarbamylase Deficiency	↑Gln, ↓ Arg, ↓Cit, ↑Ala	↑Gln	
Phenylketonuria	↑Phe, ↓Tyr	↑Phe	
3-Phosphoglycerate Dehydrogenase Deficiency	↓Ser, ↓Gly		↓Ser, ↓Gly
3-Phosphoserine Phosphatase Deficiency	↓Ser, ↓Gly		↓Ser, ↓Gly
Δ^1Pyrroline-5-Carboxylate Synthetase Deficiency	↓Pro		
Saccharopinuria	↑Sac, ↑Cit, ↑Hcit, ↑Lys	↑Sac, ↑Cit, ↑Hcit, ↑Lys	
Sarcosinaemia	↑Sar	↑Sar	
Sulphite Oxidase Deficiency	↑Scys, ↓Cys, ↓Hcy, (↑Tau)	↑Scys, ↑Tau	
Tryptophanuria	↑Trp	↑Trp	
Tyrosinaemia Type I	↑Tyr, (↑Phe), (↑Meth)	Generalised Aminoaciduria, δ-aminolevulinic acid	
Tyrosinaemia Type II	↑Tyr	↑Tyr	
Tyrosinaemia Type III	↑Tyr	↑Tyr	

* CSF analysis where required for diagnosis. In other disorders CSF amino acids will reflect the variation of plasma amino acids

Appendix 4. Disorders in which amino acid abnormalities are predominantly found in urine

Condition	Quantitative Urine
Aspartylglycosaminuria	Aspartylglucosamine
Cystinosis	Generalised Aminoaciduria
Cystinuria	↑Cys, ↑Orn, ↑Arg, ↑Lys
Dicarboxylic Aminoaciduria	↑Glu, ↑Asp
Fanconi Syndrome	Generalised Aminoaciduria
Fructose Intolerance	Generalised Aminoaciduria
Galactosaemia (Classical)	Generalised Aminoaciduria
Glutamylcysteine Synthase Deficiency	Generalised Aminoaciduria
Hartnup's Disorder	↑Neutral Amino Acids
Lowe Syndrome	Generalised Aminoaciduria
Lysinuric Protein Intolerance	↑Lys, ↑Arg, ↑Orn, ↑Gln, (↑Cys)
Prolidase Deficiency	Proline containing di- and tri-peptides
Renal Iminoglycinuria	↑Pro, ↑Hyp, ↑Gly
Rickets (Vitamin D Dependent)	Generalised Aminoaciduria
Wilson's Disease	Generalised Aminoaciduria

Appendix 5. Indicators of Other Metabolic Disorders by Amino Acid Analysis

Condition	**Quantitative Plasma**	**Quantitative Urine**	**Quantitative CSF *** ***Where required for diagnosis. In other disorders csf amino acids will reflect the variation of plasma amino acids***
Cobalamin Disorders	↑Hcy, ↓Meth, (↑Gly)	↑Hcy, ↑Gly	
Creatine Deficiency (GAMT[a])	↑Orn, ↓Arg, ↓Lys	Generalised Aminoaciduria	
Hypophosphatasia	↑Pea	↑Pea	
Mitochondrial Disorders	↑Ala, ↑Pro, (↑Gly), (↑Sar)	(Generalised Aminoaciduria)	
Organic Acidaemias (MMA[b], PA[c], IVA[d])	↑Gly	↑Gly	
Peroxisomal Disorders	↑Pip (***not easily detectable)***	↑Pip (***not easily detectable)***	
Pterin Deficiencies	↑Phe, ↓Tyr	↑Phe, ↓Tyr	↑Phe/Tyr ratio
Pyridoxal Phosphate Deficiency	↑Thr, ↑Gly		↑Thr, ↑Gly
Pyruvate Carboxylase Deficiency	↑Ala, (↑Cit), (↑Lys), (↑Pro)		

* CSF analysis where required for diagnosis. In other disorders CSF amino acids will reflect the variation of plasma amino acids

[a]Guanidinoacetate Methyltransferase

[b]Methylmalonic Acidaemia

[c]Propionic Acidaemia

[d]Isovaleric Acidaemia