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 numerous inherited metabolic disorders (IMDs).

Inherited disorders 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 IMDs, 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. These guidelines are mostly specific to the analysis of amino acids by Ion Exchange Chromatography. Limitations and methodologies 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 (IMDs).

These guidelines have also been reviewed and updated to include additional information on the requirement for medical laboratories (ISO 15189) accreditation.

Since the publication of these guidelines in 2007, we have seen clinical laboratories move to the use of liquid chromatography tandem mass spectrometry (LC-MS/MS) as the method of choice for plasma amino acid profiling. In addition, new disorders have been reported, along with new phenotypes of existing disorders being described, in particular the amino acid synthesis deficiency disorders.

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. Metabolic acidosis or lactic acidaemia
4. Alkalosis
5. Ketosis
6. Metabolic decompensation
7. Unexplained global 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. 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)

Analysis of amino acids in plasma, dried blood spots (DBS), urine and CSF all have a role in the diagnosis and monitoring of patients with disorders of amino acid metabolism and other IMDs. Before requesting amino acid investigations, consideration should be given to the choice of sample type and other pre-analytical variables that can influence the results and their interpretation.

B.1 Qualitative Analysis

The use of qualitative methods for the analysis of amino acids is not recommended as they will only detect marked changes in amino acid concentrations. In addition, amino acids co-migrate; therefore decreases, or mild increases of individual amino acids, may not be visible and can be missed.

B.2 Quantitative Analysis

The presence of both an amino group and a carboxyl group enables amino acids to be separated and identified by chromatography. Several analytical methods are used in clinical laboratories to quantitate free amino acids in bio-fluids. The most common method is that of ion-exchange chromatography (IEC) with post-column derivatisation with ninhydrin and UV detection. Ninhydrin reacts with primary amines to form products that are detectable at 570nm and secondary amines

to form products detectable at 440nm. In addition, reverse phase High Performance Liquid Chromatography (HPLC) with UV or fluorescence detection, Liquid Chromatography-mass spectrometry (LC-MS) and LC-MS/MS are also used to quantify free amino acids in physiological samples. Quantitation of compounds is by comparison to amino acid standards of known concentration.

LC-MS/MS is also used in many clinical laboratories in the UK to measure individual or small groups of amino acids for patient monitoring rather than to quantitate full physiological amino acid profiles. However, in the last few years there has been a shift in the use of LC-MS/MS for the analysis of full physiological amino acid profiles using stable isotope internal standards. [1]

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 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 (NKH), disorders of serine biosynthesis and other disorders of amino acid synthesis.[2] CSF to plasma ratios of amino acids is more informative than an isolated CSF sample. A paired plasma sample should be obtained within two hours as dietary influences can affect the plasma results.

Appendix 3 shows the changes in amino acid concentrations in plasma, urine and CSF seen in amino acid disorders, ***Appendix 4*** 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 Profile for the diagnosis of amino acid disorders

An amino acid profile (as recommended by the MetBioNet Stakeholders)[3] 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**

Amino acid profile for the diagnosis of amino acid disorders	
Alanine	Leucine
Alloisoleucine	Lysine
Arginine	Methionine
Argininosuccinic acid	Ornithine
Asparagine	Phenylalanine
Citrulline	Phosphoethanolamine
Cystine	Proline
Glutamic acid	Serine
Glutamine	Saccharopine
Glycine	Sulphocysteine***
Histidine	Taurine
Homocystine*	Threonine
Homocitrulline**	Tyrosine
Isoleucine	Valine

* Plasma free homocystine analysis shows poor sensitivity for the diagnosis of homocystinuria. Plasma total homocysteine should be used as the first line test for the diagnosis of the homocystinurias.[4]

** Homocitrulline co-elutes with methionine on IEC. A urine sample should be analysed to exclude a diagnosis of HHH syndrome.

*** Sulphocysteine may not be detectable in plasma using routine methods in sulphite oxidase and molybdenum co-factor deficiencies.[5,6] A urine and/or CSF sample should be analysed to exclude a diagnosis.

C.2 Plasma and bloodspot amino acids for patient monitoring

Metabolic control in patients with previously diagnosed amino acid disorders requires analysis of the affected metabolites. Based on current opinion, the above profile shown in Table 1 is adequate for monitoring the nutritional status of patients, including those on low protein diets.[7] A limitation to the utility of DBS specimens for monitoring IMD patients is the lack of commercially available matrix matched certified reference material for the various analytes in DBS specimens on which to standardise laboratory tests. It should be noted that there are significant differences between results obtained for plasma and dried blood amino acid concentrations e.g. bloodspot phenylalanine concentrations are reported to be 10-30% lower than plasma concentrations.[8]

Clinical laboratories should undertake rigorous evaluation studies to assess the bias between plasma and DBS amino acid concentrations in their own laboratory methods to ensure meaningful comparison of patient results to the recommended target treatment-ranges.

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 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. [4]

Notes:

- Serum is not recommended as blood needs to clot at room temperature during which, there is deamination (asparagine to aspartic acid and glutamine to glutamic acid), increases in threonine and phenylalanine, loss of sulphur containing amino acids and release of oligopeptides.[7]
- 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.[7]
- Haemolysis must be avoided because it will cause ***increases*** in serine, glycine, taurine, phosphoethanolamine, aspartic acid, glutamic acid, ornithine and ***decreased*** arginine.[7]
- Delayed separation or leucocyte and platelet contamination will cause ***increased*** serine, glycine, taurine, phosphoethanolamine, ornithine, glutamic acid and ***decreased*** arginine, homocystine, cystine.[7]
- 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 and tyrosinaemia monitoring by liquid blood specimens posted in from home.[9]
- Amino acids are more stable in deproteinised plasma stored frozen than in frozen native plasma.[10, 11]

- 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. [12]
- At temperatures >35°C, glutamine is converted to the ninhydrin negative compound pyroglutamic acid. It is therefore important to ensure that specimens are stored at 4°C 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.[13]

D.2 Urine

Urine samples should be collected into preservative free bottles. Random urine specimens are recommended and results should be normalised to creatinine concentrations.[14, 15] Faecal contamination must be avoided. Specimens should be frozen immediately and transported 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 (see notes below) and a diagnostic abnormality could potentially be 'missed'. A repeat urine should be requested if there is any evidence of specimen deterioration (i.e. urine pH>8.5).

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 and this may produce erroneous results for various amino acids.

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.[16] 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 ß-alanine from aspartic acid.[17]
- Many drugs and metabolites produce ninhydrin positive peaks e.g. antibiotics, paracetamol, penicillamine. It is important that laboratory staff are aware of where these compounds elute and how they interfere 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.[18]

- Some dietary products lead to abnormal amino acids e.g. heat-treated milk products produce homocitrulline, Chix (comminuted chicken feed) is high in the di-peptides carnosine and anserine.

D.3 Cerebrospinal fluid

CSF should be collected into preservative free bottles; however, fluoride oxalate and lithium heparin tubes may 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. CSF proline concentrations should be undetectable and increased concentrations indicate contamination of the CSF sample with blood. Ideally, a contemporaneous plasma specimen should be collected and analysed. The CSF to plasma ratio should be calculated for the various diagnostic amino acids (See section F).

D.4 Dried blood spots

Blood spots should be collected from free-flowing blood. A single hanging drop of blood of adequate size to fill the printed circle should be applied to the filter paper as over or under filling the pre-printed circle affects the volume of blood in the sub-punch that is used for analysis. Blood should be applied to the front of the card only and the blood should penetrate through to the back of the paper. Blood spots should be left to dry naturally. Small samples (<8mm diameter), multi-layering, multi-spotting, compression of the specimens and haematocrit can adversely affect the concentration of the analytes within the DBS leading to inaccurate results.[8,19] Overcoming blood spot specimen volume, quality, and haematocrit issues could potentially be achieved by the use of blood collection devices that collect defined volumes of liquid blood for sampling and such devices should be validated before being utilised clinically.

E. Analytical

These guidelines are primarily based on the use of ion exchange chromatography for a full amino acid profile. However, they are broadly applicable to other methods such as HPLC, LC-MS or LC-MS/MS.

E.1 Standardisation

- The number of calibration levels used within the method employed should be established.

- The calibrator(s) should be an aqueous solution at a concentration appropriate for each amino acid quantified.

- The frequency of calibration should be based on the stability of the analytical system / methodology 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.

- Performance should be checked after any change e.g. new ninhydrin, column or lamp, to check the response factor is correct.

- Reagent blanks should be analysed occasionally to monitor baseline.

E.2 Sample Preparation

- Urine samples may require dilution in order to ensure that the amino acids are within the analytical range of the instrument. Plasma samples may also need dilution where amino acid concentrations are outside the linear range. Note results obtained via IEC 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(s) may be included in the deproteinising solution.

- CSF samples contain lower concentrations of amino acids relative to plasma, and therefore an increased injection volume to improve sensitivity should be considered.

E.3 Internal standards

- At least one internal standard should be used.
- The 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.
- Mass-spectrometry based methods should utilise stable isotope analogues.
- A fixed amount of internal standard should be added to all samples including QC, EQA and standards prior to analysis.
- The peak area / abundance of the internal standard should be recorded and monitored to assess assay performance.
- See ***Table 2***. below for examples of internal standards used in IEC methods:

Table 2. **Commonly used internal standards for IEC methods**

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. Cys-Penicillamine & Cys-Tiopronin mixed disulphides co-elute under Nle.
Norvaline	Nva	Near valine	
S-2-aminoethyl – L-cysteine	AEC	Near ornithine	

E.4 Internal Quality Control (IQC)

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

- An appropriate number and levels of IQC material should be employed to monitor assay performance. Limits of assay acceptability should be established.

- IQC material should be analysed with every batch of patient samples and after any maintenance changes. Results should be recorded and monitored.

- IQC materials at different levels are commercially available.

E.5 External Quality Assessment

Laboratories should participate in external quality assessment programmes e.g. ERNDIM (www.**erndim**qa.nl) for the quantitative and proficiency schemes and UKNEQAS (www.uk**neqas**.org.uk), to ensure both the analytical and clinical interpretation proficiencies are monitored.

E.6 Assay performance

European consensus has established that one of the ways in which to derive performance specifications of an assay is to use the biological variability of the analyte of interest. The study of Corte Z *et al* 2010 [20] provides biological variation data for free amino acids in which to derive objective goals for minimum assay performance (imprecision, bias and total error). The analytical goals for each of the amino acids are listed in the table in ***Appendix 6***.

E.7 Linearity and limit of detection

- The linear range and limit of detection for each amino acid should be determined and reviewed periodically.

E.8 Special considerations

There are some unusual amino acids / compounds which are helpful in certain disorders and laboratories should be familiar with the characteristics of these compounds in their analytical method:

- Aspartylglucosamine in aspartulglucosaminuria. Aspartylglucosamine runs near/with PEA on IEC systems.
- Proline containing dipeptides in prolidase deficiency. These are detected as broad peaks by IEC with a large peak due to the alanine-proline dipeptide.[21]
- FIGLU (formiminoglutamic acid) shows a diffuse peak between alanine and cysteine using IEC.

F. Reference Intervals, Clinical Decision Limits & Interpretation of Amino Acid Profiles

It is the recommendation of this working group that individual laboratories should generate their own reference intervals or appropriately validate existing / published reference intervals.

The clinical interpretation of an amino acid profile is based, not only on concentrations of the individual amino acids, but also on the assessment of the entire profile. Interpretation of a profile through the simple relation of individual amino acid concentrations to their reference intervals may be misleading if their contribution to the overall pattern is ignored. Patients with disorders can present with amino acid concentrations within their respective reference intervals.

A CSF to plasma glycine ratio >0.08 is considered diagnostic for NKH. However, mildly affected patients have been reported with ratios between 0.04 – 0.1.[22] Age related multicentre reference intervals for CSF serine have been established,[23] (***Appendix 7***) to exclude disorders of serine biosynthesis.

The use of decision support techniques and machine learning tools have been used to support decision making for amino acid interpretation with a high degree of accuracy.[24,25]

G. Reporting Amino Acid Results

These recommendations are in line with the UK Accreditation Service (UKAS) based on the Medical Laboratories – requirements for quality and competence (ISO 15189) and the Royal College of Pathologists 'Code of practice for clinical biochemists/chemical pathologists and clinical biochemistry services' March 2011 (www.rcpath.org). Results should 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 authorisation 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) and 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 number 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.
    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 (including date & time).
        v. Contact details for further discussion if required

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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	605814 603471	Citrin	Citrin deficiency NICCD
Argininosuccinic aciduria	207900	Argininosuccinic acid lyase	ASA
Argininaemia	207800	Arginase	
Lysinuric Protein Intolerance (Dibasic aminoaciduria II)	222700	Dibasic amino acid transporter	LPI
Hyperornithinaemia, Hyperammonaemia, Homocitrullinuria	238970	Mitochondrial ornithine translocase	HHH Syndrome
Hyperornithinaemia,	258870	Ornithine aminotransferase (OAT)	Gyrate Atrophy, HOGA
Glutamine deficiency	610015	Glutamine synthetase	
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	
Hypermethioninemia's	250850 180960 606664	Methionine adenosyltransferase S-adenosyl hydrolase deficiency Glycine N-methyltransferase deficiency	MAT SAH GNMT

	614300	Adenosine kinase deficiency	ADK
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	618850	Mitochondrial branched chain aminotransferase 2	
Branched-chain keto acid dehydrogenase kinase deficiency	614923	Branched-chain keto acid dehydrogenase kinase (BCKDKD)	
Maple syrup urine disease, mild variant	615135	Protein phosphatase, magnesium/manganese-dependent, 1k; ppm1k	
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**

Asparaginase deficiency	615574	Asparaginase synthetase	
Nonketotic Hyperglycinaemia	605899	Glycine cleavage enzyme system	NKH
Histidinaemia	235800	Histidine ammonia lyase	
3-Phosphoglycerate Dehydrogenase Deficiency	601815	3-Phosphoglycerate Dehydrogenase	3-PHGDH
Phosphoserine aminotransferase deficiency	610992	Phosphoserine aminotransferase	PSAT
Phosphoserine phosphatase deficiency	172480	Phosphoserine phosphatase	PSP
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	↑Aad	
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	
Branched-chain keto acid dehydrogenase kinase deficiency	↓ Leu, ↓Ile, ↓Val		
Asparagine synthase deficiency	N/↓ Asn		N/↓ Asn
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
Glutamine synthase deficiency	↓Gln, ↓Glu		↓Gln, ↓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, ↑Pro		

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	
Maple syrup urine disease, mild variant due to PP1MK Deficiency	↑Leu, ↑Ile, ↑Val, ↑Aile (but lower than above)		
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	
Serine biosynthesis disorders	↓Ser, ↓Gly		↓Ser, ↓Gly
Δ^1Pyrroline-5-Carboxylate Synthetase Deficiency	↓Pro, ↓Orn, ↓Cit, ↓Arg		
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 Aaminoaciduria, ↑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 & Fanconi-Bickel Disease	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
Hypophosphatasia	↑PEA
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 *
Adenosine deaminase deficiency			↑ Gln, ↑Met
Cobalamin Disorders	↑Hcy, ↓Meth, (↑Gly)	↑Hcy, ↑Gly	
Creatine Deficiency (GAMT[a])	↑Orn, ↓Arg, ↓Lys		
Ethylmalonic acidaemia	↑Aile	↑Aile	
D-Glyceric aciduria	↑Gly	↑ Gly	↑ Gly
Hypophosphatasia	↑PEA	↑PEA	
E3-Lipomide dehydrogenase deficiency	↑Aile	↑Aile	
Lipoic acid biosynthesis disorders	↑Gly		↑Gly
Methylmalonate semialdehyde dehydrogenase def		↑ß-alanine	
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

Appendix 6. Performance goals for assay performance[20]

	Desirable goal for imprecision (%)	Desirable goal for Bias (%)	Desirable Total Error (%)
Taurine	**15.3**	**13.4**	**38.6**
Asparginine	**6.1**	**7.6**	**17.7**
Threonine	**8.9**	**9.4**	**24.1**
Serine	**6.4**	**11.2**	**21.7**
Aspartic acid	**15.6**	**15.8**	**41.7**
Glutamic Acid	**23.1**	**23.1**	**61.0**
Glutamine	**6.0**	**6.3**	**16.2**
Glycine	**5.9**	**10.5**	**20.2**
Alanine	**7.3**	**14.4**	**26.5**
Citrulline	**10.7**	**12.2**	**47.2**
Valine	**5.3**	**10.4**	**19.1**
Methionine	**7.3**	**11.5**	**23.6**
Isoleucine	**7.8**	**12.0**	**24.9**
Leucine	**7.4**	**11.6**	**23.8**
Phenylalanine	**4.7**	**10.4**	**18.2**
Ornithine	**9.2**	**14.5**	**29.7**
Lysine	**5.7**	**10.0**	**19.4**
Histidine	**4.9**	**7.2**	**15.2**
Tryptophan	**11.4**	**38.6**	**48.0**
Arginine	**9.7**	**9.8**	**25.8**
Proline	**10.4**	**26.4**	**40.1**
Tryrosine	**5.2**	**15.5**	**24.1**

Appendix 7. Multi-centre age related reference intervals for CSF serine.[23]

Age group	Predicted mean (μmol/L)	Reference intervals (Mean ± 1.96SD) (μmol/L)
1 week	59	35–82
2 weeks	56	33–78
3 weeks	54	32–76
1 month	52	31–74
2 months	49	29–70
3 months	47	28–67
6 months	44	26–63
9 months	43	24–60
1 year	41	24–59
1.5 years	40	23–56
2 years	38	22–54
3 years	37	21–52
5 years	34	20–48
10 years	31	18–44
15 years	29	17–41