Urinary metabolic profiling for detection of metabolic disorders

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The Medical Services Advisory Committee (MSAC) is an independent committee which has been established to provide advice to the Minister for Health and Ageing on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC's advice does not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

The advice in this report was noted by the Minister for Health and Ageing on 8 December 2008.

This report was prepared by the Medical Services Advisory Committee with the assistance of John Gillespie, Carmel Guarnieri, Heather Phillips and Taimur Bhatti from IMS Health. The report was edited by Ann Jones. The report was endorsed by the Minister for Health and Ageing on 8 December 2008.

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Abbreviations

2-HV	2-hydroxyisovalerate
3-HB	3- hydroxybutyrate
3-HGA	3-hydroxyglutaric acid
4-MHG	4-methylhexanoylglycine
ABS	Australian Bureau of Statistics
AHMAC	Australian Health Ministers' Advisory Council
AIHW	Australian Institute of Health and Welfare
ASIEM	Australasian Society for Inborn Errors of Metabolism
ATP	adenosine triphosphate
BCKD	branched-chain 2-keto acid dehydrogenase
BG	butyrylglycine
CβS	cystathionine beta-synthase
CID	collision-induced dissociation
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
EEG	electroencephalogram
EOC/MO/TBDMS	ethoxycarbonyl/methoxime/tert-butyldimethylsilyl
FN	false negative
GA1	glutaric aciduria type I
GC/MS	gas chromatography–mass spectrometry
GCDH	glutaryl-CoA dehydrogenase
HG	hexanoylglycine
HMG	methylhexanoylglycine
HOG	hydroxyoctanoylglycine
HTA	health technology assessment

HPLC	high performance liquid chromatography
HVE	high voltage electrophoresis
IBG	isobutyrylglycine
IEM	inborn error of metabolism
IG	isocaproylglycine
IVG	isovalerylglycine
LA	lactic acidoses
MBG	methylbutyrylglycine
MBS	Medicare Benefits Scheme
MCADD	medium-chain acyl-CoA dehydrogenase deficiency
MHG	methylhexanoylglycine
μM	micromolar
MMA	methylmalonic aciduria
MSAC	Medical Services Advisory Committee
MS/MS	tandem mass spectrometry
MSUD	maple syrup urine disease
ND	not detected
NKH	non-ketotic hyperglycinaemia
NHMRC	National Health and Medical Research Council
NMDA	N-methyl-D-aspartate
NR	not reported
OTC	ornithine transcarbamylase
PDC	pyruvate dehydrogenase complex
PFB	pentaflourobenzyl
PPG	phenylpropionylglycine
PPICO	target population, prior tests, index test, comparator, outcomes
PPV	positive predictive value

QUOROM	quality of reporting of meta-analyses
RCT	randomised controlled trial
RPHPLC	reverse phase high performance liquid chromatography
SG	suberylglycine
SPE	solid phase extraction
TLC	thin layer chromatography
TMS	trimethylsilyl
UMP	urinary metabolic profiling

Executive summary

The procedure

Urinary metabolic profiling (UMP) involves analysing urine to detect altered levels of intermediate metabolites that result from incomplete metabolism of amino or organic acids. The analytical methods used to diagnose amino acid and organic acid disorders differ. In Australia, amino acids are assayed using thin layer chromatography (TLC), high voltage electrophoresis (HVE), a dedicated high performance liquid chromatograph (HPLC, amino acid analyser) or tandem mass spectrometry (MS/MS). Gas liquid chromatography with mass detection (GC/MS) is used for organic acid analysis by all Australian laboratories that conduct UMP. UMP in some form has been in use worldwide, including throughout Australia, since the 1970s

Medical Services Advisory Committee—role and approach

The Medical Services Advisory Committee (MSAC) was established by the Australian Government to strengthen the role of evidence in health financing decisions in Australia. MSAC advises the Minister for Health and Ageing on the evidence relating to the safety, effectiveness and cost-effectiveness of new and existing medical technologies and procedures and under what circumstances public funding should be supported.

A rigorous assessment of evidence is thus the basis of decision making when funding is sought under Medicare. A team from IMS Health was engaged to conduct a systematic review of literature on urinary metabolic profiling for diagnosis of inborn errors of metabolism (IEM). An advisory panel with expertise in this area then evaluated the evidence and provided advice to MSAC.

MSAC's assessment of urinary metabolic profiling for detection of inborn errors of metabolism

Clinical need

Inborn errors of metabolism (IEM) refer to a range of over 500 conditions (Winter and Buist 1998). Errors are typically caused by a deficiency in one or more metabolic enzymes. For most IEMs inheritance is autosomal recessive, but occasionally other modes of inheritance apply. Although each IEM is rare, they collectively affect many people globally. IEMs can have serious effects on growth, neurological development, movement, vision, skin, organ size and function, and behaviour. IEMs can be fatal if untreated (Raghuveer et al 2006). Most IEMs arise from defects in single genes that code for specific enzymes involved in converting one substance to another, or that are concerned with transporting proteins (Saudubray et al 2006, Martins 1999). Intermediate metabolites that cannot be broken down accumulate in the affected pathway which can have toxic effects and lead to deficiencies of substances produced at later steps in the specific metabolic pathway. In some cases, substances that cannot be metabolised

normally may be broken down via alternate pathways, leading to accumulation of metabolites associated with that alternate pathway (Martins 1999).

Diagnoses are generally made following the results of screening tests conducted at birth for those disorders detectable by current routine screening and for other disorders when symptoms are exhibited. Diagnosing specific IEMs can be challenging, not only because they are relatively rare, but also because many symptoms are not unique to particular IEMs (Winter and Buist 1998, Martins 1999).

This assessment investigates nine of the most common IEMs: cystinuria, homocystinuria (CβS deficiency), ornithine transcarbamylase (OTC) deficiency, non-ketotic hyperglycinaemia (NKH), maple syrup urine disease (MSUD), glutaric aciduria type I (GA1), methylmalonic aciduria (MMA), lactic acidoses (LA) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD). Of these, MCADD, GA1, classical (severe) MSUD, MMA, and pyridoxine-nonresponsive CβS deficiency can now be indicated for further investigation by newborn screening test results. UMP for the nine most common IEMs was investigated to make the assessment manageable, but it must be noted that one main use of a UMP is to help exclude a very wide variety of IEMs in patients presenting with non-specific symptoms.

Research questions

The research questions addressed were:

Asymptomatic newborns with screening results suggestive of metabolic disorder

To what extent is urinary metabolic profiling (UMP) of asymptomatic newborns with screening results suggestive of metabolic disorder safe, effective and cost-effective relative to alternative tests when UMP is not available?

Individuals with a clinical presentation suggestive of genetic metabolic disorder

To what extent is urinary metabolic profiling of individuals with a clinical presentation suggestive of genetic metabolic disorder safe, effective and cost-effective relative to alternative tests when UMP is not available?

At-risk family members of patients with specific genetic metabolic diseases

To what extent is urinary metabolic profiling of at-risk family members of patients with specific genetic metabolic diseases safe, effective and cost-effective relative to alternative tests when UMP is not available?

Safety

UMP is a non-invasive test performed on patients' urine samples. The UMP procedure is therefore not considered to impose safety issues for patients.

Effectiveness

A linked evidence approach was undertaken to evaluate the diagnostic effectiveness of UMP and its impact on patient management and treatment.

A total of 13 studies were identified for inclusion in the analysis.

Of the included studies, results reported by Korman et al (2007) enabled comparison of UMP with a pre-specified comparator test. Results reported by Carpenter et al (2001) and Waddell et al (2006) enabled consideration of the role of UMP in the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency (MCADD) in screened newborns. Korman et al (2007) investigated UMP for detection of glutaric aciduria type I (GA1). These studies were considered to provide significant evidence to support UMP use for diagnosing inherited metabolic disorders in newborns whose screening results are positive (Carpenter et al 2001, Waddell et al 2006) and symptomatic patients (Korman et al 2007). Because they were not designed as diagnostic test studies or direct comparisons with other tests, results provided limited evidence. In terms of assessing the diagnostic performance of UMP, the study by Korman et al (2007) was subject to selection bias because patient inclusion was based on the results of the reference standard.

The remaining 10 of the 13 identified studies enabled urine sample comparisons to be made among UMP tested patients with previously diagnosed inborn errors of metabolism (IEMs) and participants who were disease-free. Results from these studies showed that UMP can be considered a reliable form of diagnosis for the IEMs examined in this analysis, although evidence reliability was limited by study design. Inherent spectrum bias, introduced by including patients with known IEMs, and a lack of blinding of samples to researchers were common to many of these studies. The quality of the available evidence was poor overall.

A quality assurance/educational program was identified that involved sending blinded reference samples of urine from patients with known IEMs to each of the six laboratories that undertake UMP testing in Australia (ASIEM quality assurance/educational program 1992–2007. JR Harrison, personal communication, May 2008). Of the six laboratories, five correctly diagnosed all disorders, including reporting negative diagnoses for two normal urine samples containing compounds that may yield false positive results, and two samples from patients in whom no evidence for an IEM had been found. One laboratory obtained a single false negative result (for ornithine transcarbamylase deficiency in a symptomatic female carrier for this X-linked disorder). These findings appear to support the use of UMP to detect IEMs. The study was limited by the small number of samples for each IEM tested by the six laboratories. Details about the patients who supplied the samples and the technologies used by the laboratories were not provided, and criteria for test positivity were not reported.
The case-control study design precludes determination of how well UMP would perform in diagnosing IEMs when suspected and not pre-diagnosed. Based on these results it was not possible to draw categorical conclusions about the status of UMP as a highly specific and sensitive test for diagnosing IEMs examined for this assessment.

Summary of evidence for the clinical effectiveness of urinary metabolic profiling

- Carpenter et al (2001) and Waddell et al (2006) indicated that the diagnosis of MCADD in screened newborns may involve UMP by acylglycine, plasma carnitine and mutational analyses.

- Korman et al (2007) showed that there were increased levels of urinary excretion of glutaric acid and 3-hydroxyglutaric acid in patients who were symptomatic for GA1. Plasma free carnitine deficiency was marked in all patients who had elevated levels of 3-hydroxyglutaric acid and glutaric acid excretion. Mutational analysis showed that all patients had a change in the glutaryl CoA dehydrogenase gene. The diagnostic performances of UMP and plasma carnitine analysis were estimated; both tests had 100 per cent PPV for diagnosis of GA1.

- Bonham Carter et al (2000) provided evidence to indicate that UMP by gas chromatography/mass spectrometry (GC/MS) can be used to exclude diagnosis of MCADD in close family members of patients with known MCADD.

- Korman et al (2007) provided evidence that UMP by GC/MS can be used to confirm diagnosis by mutational analysis in close family members of patients with known GA1.

- Bonham Carter et al (2000), Costa et al (2000), and Pitt (1993) showed that patients with MCADD had elevated urinary levels of acylgylcines compared with controls.

- Paik et al (2005) showed that 2-hydroxyvaleric acid was the most abundant metabolite in the urine of patients with maple syrup urine disease (MSUD) compared with controls; and that glycine and methylmalonic acid levels were elevated in the urine of patients with methylmalonic aciduria (MMA) in comparison with controls.

- Urinary 3-HGA excretion was elevated in patients with GA1 in comparison with controls (Schor et al 2002).

- Compared with controls, patients with homocystinuria had elevated urinary levels of homocystine and methionine (Kuhara et al 2000).

- Patients with lactic acidosis had elevated levels of urinary excretion of 2-hydroxyisolvalerate, lactate and 3-hydroxybutyrate in comparison with controls Landaas and Jakobs 1997).

- Tandem mass spectrometry (MS/MS) was able to confirm diagnoses for patients with MCADD from known disease-free controls (Bonafé et al 2000).

- Thin layer chromatography (TLC) was able to confirm diagnoses for patients known to have cystinuria from known disease-free controls (Giugliani et al 1987).

- High performance liquid chromatography (HPLC) was able to confirm diagnoses for patients known to have non-ketotic hyperglycinaemia (NKH) (Tsai et al 1980).

- No studies were found concerning any of the technologies that assess ornithine transcarbamylase (OTC) deficiency in patients known to have the condition, or among controls.
- Results from a quality assurance/educational program showed that UMP was able to distinguish urine samples from patients with known IEMs from control samples. A single false negative result was reported for OTC deficiency.

Cost-effectiveness

The systematic literature review revealed a lack of comparative clinical evidence associated with UMP technologies currently used in Australia. Cost-effectiveness or cost-utility analyses could therefore not be conducted. A budget impact analysis was performed to estimate the potential total financial implications associated with introducing UMP technologies for detection of genetic metabolic disorders on the Medicare Benefits Schedule for three patient populations: asymptomatic newborns with positive screening results, individuals with clinical presentations suggestive of genetic metabolic disorders, and at-risk family members of patients with specific genetic metabolic disorders. The average unit cost of conducting a UMP test was estimated at $131.50. This estimate was based on information provided by four of the six laboratories presently providing UMP testing in Australia. All laboratories are associated with major teaching hospitals. According to the most recently available laboratory data, UMP test utilisation in Australia was estimated to be approximately 11,150 patients annually. Based on the unit costs and total number of UMP tests performed, the potential financial implications associated with UMP tests are estimated to be $1,466,225 ($1,070,400 to $2,140,800) annually. These estimates are not expected to increase significantly over the next five years.

Despite the absence of appropriate comparative evidence, it is important to consider the potential cost savings associated with UMP testing. In the absence of UMP testing, a number of other tests would be required to investigate suspected IEMs, resulting in increased test costs. Furthermore, alternative tests often require extended analysis and interpretation time compared with UMP testing. Therefore, potential delayed diagnosis associated with using other tests may lead to further long term health and cost consequences. Depending on the particular IEM, a delay in implementing therapy could result in severe health consequences in terms of mental and/or physical disability, with associated long term management costs.

Advice

MSAC has considered the strength of the evidence in relation to the safety, effectiveness and cost-effectiveness of urinary metabolic profiling compared with alternative investigations for the detection of metabolic disorders for the three following indications:

1. Asymptomatic newborns with a positive screening result suggestive of metabolic disorder.

2. Individuals with a clinical presentation suggestive of a metabolic disorder.

3. At-risk family members of patients with specific genetic metabolic disorder.

The evidence was limited by three factors:

- the rarity of the various metabolic disorders such that conventional high quality comparative diagnostic test studies are not possible
- the large number of metabolic disorders that can be diagnosed by urinary metabolic profiling
- that urinary metabolic profiling has been standard practice in Australia for many years.

MSAC finds that urinary metabolic profiling is either as safe or safer than alternative investigations to diagnose metabolic disorders.

MSAC finds that urinary metabolic profiling is effective in diagnosing metabolic disorders and is likely to be more effective than alternative investigations in allowing a timely diagnosis, especially in patients with undifferentiated presentations where a metabolic disorder is suspected or needs to be excluded.

Given the lack of high quality comparative evidence of effectiveness, a cost-effectiveness analysis could not be conducted and a budget impact analysis was performed. Whilst it is likely that downstream costs incurred by alternative investigations or delayed diagnosis are avoided by the use of urinary metabolic profiling, these could not be formally costed. MSAC considers it is likely that urinary metabolic profiling in carefully selected patients is cost-effective.

The current funding arrangements adequately capture the target population. MSAC advises that current public funding arrangements within the health care system should continue to be supported for this procedure.

—The Minister for Health and Ageing noted this advice on 8 December 2008—

Introduction

The Medical Services Advisory Committee (MSAC) has reviewed the use of urinary metabolic profiling, which is a diagnostic test for the detection of inborn errors of metabolism. MSAC evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Schedule in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

MSAC's terms of reference and membership are at Appendix A. MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

This report summarises the assessment of current evidence for urinary metabolic profiling for the diagnosis of inborn errors of metabolism.

Background

Summary

Urinary metabolic profiling (UMP) involves analysing urine to detect altered levels of intermediate metabolites that result from incomplete metabolism of amino or organic acids. The analytical methods used to diagnose amino and organic acid disorders differ. In Australia, amino acids are assayed using thin layer chromatography (TLC), high voltage electrophoresis (HVE), dedicated high performance liquid chromatography (HPLC, amino acid analyser) or tandem mass spectrometry (MS/MS). In all laboratories performing UMP, gas liquid chromatography with mass spectrometry detection (GC/MS) is used for organic acid analysis.

The term "inborn errors of metabolism" (IEM) refers to a range of over 500 conditions that are typically caused by a deficiency in one or more metabolic enzymes (Winter and Buist 1998). IEMs tend to be autosomal recessive, but some disorders arise from other modes of inheritance. Although individual IEMs are rare, they collectively affect many people. IEMs are potentially fatal and if untreated can impose serious impediments to growth, neurological development, movement, vision, skin, organ size and function, and behaviour (Raghuveer et al 2006). Most IEMs arise from defects in single genes that code for specific enzymes involved in converting one substance to another, or that are involved in transporting proteins (Saudubray et al 2006, Martins 1999). Intermediate metabolites that cannot be broken down accumulate in the affected pathway which can have toxic effects and lead to deficiencies in substances produced at later steps in the specific metabolic pathway. Substances that cannot be metabolised normally may be broken down via alternate pathways. In some cases this can lead to accumulation of the metabolites of those alternate pathways (Martins 1999).

Diagnoses are generally made following results of screening tests at birth for disorders detectable by current routine screening or, for other disorders, when symptoms are exhibited. Diagnosing specific IEMs can be challenging, not only because the conditions are relatively rare, but also because many symptoms are common to several of these and other conditions (Winter and Buist 1998, Martins 1999).

This assessment investigates nine of the most common IEMs: cystinuria, homocystinuria (CβS deficiency), ornithine transcarbamylase (OTC) deficiency, non-ketotic hyperglycinaemia (NKH), maple syrup urine disease (MSUD), glutaric aciduria type I (GA1), methylmalonic aciduria (MMA), lactic acidoses (LA) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD).

The procedure

Inborn errors of metabolism (IEMs) are rare congenital disorders that occur when the metabolism of one or more amino or organic acids is compromised. There are over 500 known IEMs (Winter and Buist 1998). The causes of IEMs vary, but they are generally characterised by the reduction or absence of an enzyme or enzymes responsible for the breakdown of a specific amino or organic acid, or absence or reduced availability of a transport protein.

IEMs impose significant health burdens on people with these conditions. Accumulation of intermediate metabolites can have toxic effects on the body, leading to physical and neurological deficits, and increasing risk of death. Because these intermediate metabolites are excreted in the urine, IEMs can be diagnosed or excluded by undertaking urinary metabolic profiling (UMP).

UMP involves analysing urine for raised levels of intermediate metabolites that result from the incomplete metabolism of amino or organic acids. The analytical methods used to diagnose amino and organic acids disorders differ. In Australia, amino acids are assayed using thin layer chromatography (TLC), high voltage electrophoresis (HVE) a dedicated high performance liquid chromatograph (HPLC, amino acid analyser) or tandem mass spectrometry (MS/MS). Gas liquid chromatography with mass detection is used for organic acid analysis in all laboratories that perform UMP. Table 1 outlines the technologies used for amino and organic acid UMP in Australia.

Table 1 Technologies used for UMP in Australia

State	Amino acid analysis	Organic acid analysis (semi-quantitative)
Queensland	HPLC (quantitative)	GC/MS
New South Wales[a]	HVE (semi-quantitative)	GC/MS
Victoria[b]	MS/MS (quantitative)	GC/MS
South Australia[c]	HPLC (quantitative)	GC/MS
Western Australia	TLC (semi-quantitative)	GC/MS

Source: Advisory panel
Abbreviations: HPLC, high performance liquid chromatography; GC/MS, gas liquid chromatography-mass spectrometry; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography
[a]Testing for patients from the Australian Capital Territory is performed in New South Wales
[b]Testing for patients from Tasmania is performed in Victoria
[c]Testing for patients from the Northern Territory is performed in South Australia

Methods for amino acids analysis

Thin layer chromatography

Thin layer chromatography (TLC) is a solid-liquid chromatographic technique that can be used to determine the compounds present in a sample, such as urine. Small volumes of each sample are applied, spaced evenly in a line 10 mm from one edge of a thin layer plate. Standard and/or quality control samples are usually also included on the plate. After drying, the plate is placed vertically into a tank containing a small amount of solvent mixture, and the tank is then sealed. The solvent migrates up the plate by capillary action dissolving the sample's components that are then carried with the solvent. The rate that components move up the plate is dependent on their affinity with the stationary phase (the thin layer) compared with the mobile phase (solvent). This concept is referred to as partitioning. Polarised substances are not easily dissolved by the solvent and are carried up the plate more slowly compared with substances that are dissolved easily and which therefore move faster in the solvent medium. The plate is removed and dried thoroughly when the solvent nears the top of the plate.

Separated compounds may not have innate colours. Some compounds are visible under ultraviolet light. Most analyses require a further chemical reaction to render the separated compounds visible. In this case, a detection reagent is applied, usually by spraying. A compound in a sample can be identified by its reaction to the detection reagent (including the colour formed), its position compared with known compounds in controls, or standards chromatographed in parallel on the same plate, the retention factor, or a combination of these outcomes. The distance travelled by the compound is divided by the distance travelled by the solvent (the solvent front) to calculate the retention factor. The retention factor is reproducible for a given compound under identical conditions. Chromatography may be performed in two directions; in this case, it is referred to as 2-dimensional chromatography. Amino acids in physiological samples are difficult to separate completely in any one solvent system. Almost complete separation can be achieved when combined with chromatography in a different solvent at right angles to the first. The solvent pairs are selected based on their combined abilities to separate all amino acids. Only one sample can be assayed on each plate in 2-dimensional chromatography.

Stationary phases commonly used for thin layer chromatography include silica, alumina and cellulose, which are coated onto a backing of aluminium, plastic or glass to provide physical support.

Amino acids analysis normally uses cellulose for the stationary phase partnered with ninhydrin as the detection reagent. Other reagents may be used in sequence to characterise specific amino acids.

Samples may require pre-treatment to render them suitable for chromatography. Urine samples are de-salted using a column and are concentrated before being applied to the TLC plate.

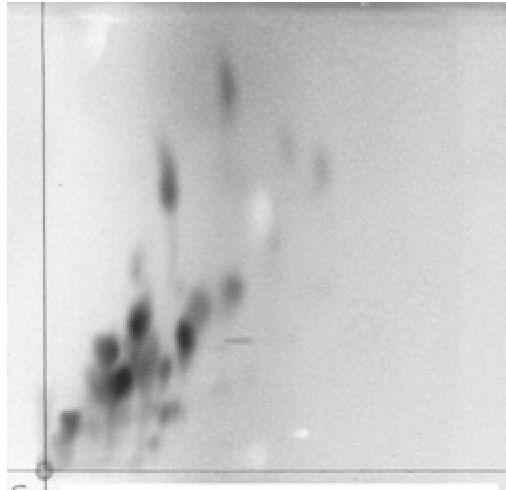

Figure 1 Example of thin layer chromatography of urine amino acids, stained with ninhydrin
Source: Courtesy of Princess Margaret Hospital, Perth WA. Published with permission

High voltage electrophoresis

High voltage electrophoresis (HVE) is a separation technique for charged compounds that is based on different movement rates in solution when an electric field, usually over 1000 volts, is applied. This technique is used for amino acids, sugars, indoles, purines and pyrimidines.

An electrode apparatus consists of a high voltage power supply, electrodes, buffer, and a support for the buffer, usually filter or chromatography paper. The supporting medium is moistened with a buffer solution to enable it to conduct the electric current. There are two compartments each containing the buffer solution. The positive pole of the electric field (anode) feeds into one compartment, and the negative pole (cathode) is connected to the other compartment. For safety reasons, one electrode is usually at ground and the other is biased positively or negatively. An interlock system prevents access without disconnecting the power supply.

The samples to be tested are evenly spaced in a line across the support medium. When the sample applications are dry, the support is moistened evenly with the buffer, and blotted, before being positioned in the tank. Each end of the support is immersed in a buffer tank and the electrodes are connected. When an electric field is applied, electrovalent ions separate and migrate according to charge. Positively charged ions migrate to the cathode (negative) and negatively-charged ions are attracted to the anode. Compounds separate principally on the basis of their charge. Uncharged covalent molecules may show movement as an effect of electro-osmosis. After electrophoresis is complete, the support medium is removed and dried. Compounds are viewed under ultraviolet light after the support medium is sprayed with a detection reagent.

Formic and acetic acids at pH 2 (amino acids are positively charged at this level) are commonly used as buffers for amino acids analysis. Samples are applied at the anode end of the support; amino acids migrate toward the cathode. The detection reagent is usually ninhydrin, although others may be used in sequence to detect specific amino acids.

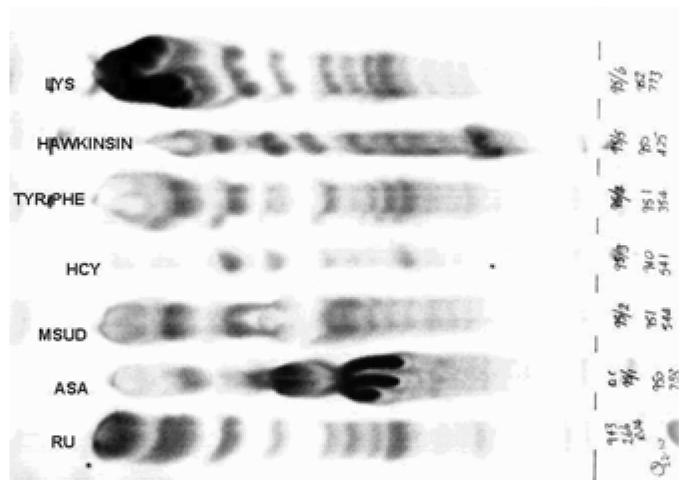

Figure 2 Example of high voltage electrophoresis of urine amino acids stained with ninhydrin
Source: Courtesy of New South Wales Biochemical Genetics Service. Published with permission

High performance liquid chromatography

High performance liquid chromatography (HPLC) is used to separate compounds on the basis of their chemical characteristics, such as polarity, molecular size and degree of charge. The factors that influence separation of compounds are complex and usually more than one factor influences separation. It is a technique that is especially useful for analysing biological samples. There are many forms of HPLC—the most common is reverse phase HPLC—but ion-exchange chromatography in a column packed with a polystyrene-divinylbenzene resin is used for amino acids analysis of physiological samples. This is the form of HPLC used by all laboratories in Australia that conduct urinary metabolic profiling (UMP).

HPLC separates amino acids according to electrical charge in a pH gradient.
HPLC components include an automatic sampler, buffer pump, column, column oven, spectrophotometer, and a post-column detection system to enable the separated amino acids to be detected after exposure to a detection reagent, usually ninhydrin.
The detection system consists of a facility to store ninhydrin under an inert gas at a controlled pressure, a constant speed reagent pump, and a post-column reactor operating at between 110°C and 130°C. A computer system controls HPLC and collects data for subsequent review. Amino acids analysers can either be purpose-built or modified from existing HPLC technologies.

Amino acids separation is achieved by a cation exchange process, referred to as ion-exchange HPLC. The column is packed with a resin that is negatively charged; the amino acids are introduced at a low pH level where they are all positively charged. The pH level is then gradually increased according to a pre-determined course (pH gradient) and with increasing concentration of counter ions. These changes result in reaching the isoionic point (neutral charge) of each amino acid where the ionic attraction to the resin is lost and the amino acid elutes from the column. The counter-ion for complex amino acids analysis is usually lithium; this ion replaces the amino acids on the resin as they elute from the column.

The pH gradient is formed either by switching between a number of buffers of different and increasing pH (step gradient) or by changing the proportions of two or three buffers to create a continuous pH gradient. The pH gradient typically begins at about pH 2 and increases to pH 10 to allow all amino acids to elute.

The eluting amino acids react with ninhydrin when heated to form mostly red to violet derivatives detectable by spectrophotometry. The colour intensity can be interpreted to accurately quantify each amino acid compared with calibration standards assayed under identical conditions. Identification of each amino acid is based on the time elapsed (elution time) between sample injection and appearance in the chromatogram.

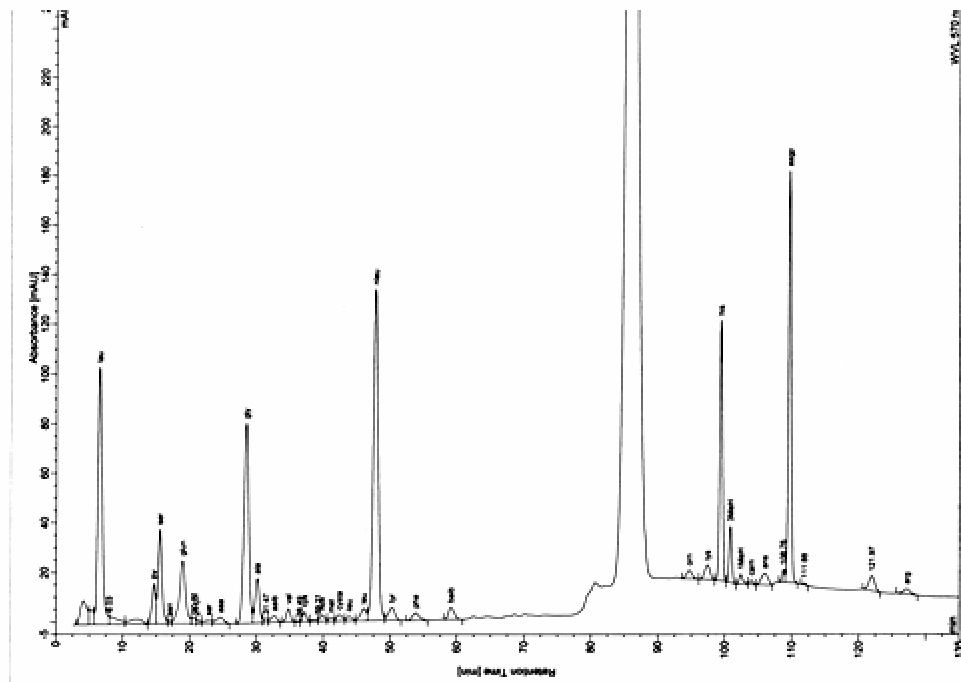

Figure 3 HPLC output example: Urine amino acids by ion-exchange HPLC using lithium buffers and detection with ninhydrin

Source: Courtesy of Genetic Medicine, Adelaide Women's and Children's Hospital. Published with permission

Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) is an analytical technique used to detect and analyse molecules such as acylcarnitines, amino acids and fatty acids. MS/MS can differentiate and measure levels of these compounds in blood and urine and is used to screen for over 30 different rare inherited metabolic disorders. Mass spectrometry has high sensitivity, speed, and good specificity for detecting compounds with molecular weights up to 1000 (McLafferty 1981).

MS/MS can separate and quantify ions based on their mass-to-charge (m/z) ratios (Charrow et al 2000). It is most often performed using a triple quadrupole mass spectrometer comprising an ionisation source, three mass filters connected in sequence and a conventional electron or photomultiplier detector (Carpenter 2002).

Samples exposed to MS/MS separate and move. Lighter molecular weight chemicals move and separate faster from the sample than heavier chemicals. Ions produced in the ionisation source are passed into the first quadrupole for transmission to the second quadrupole—the collision cell. In the collision cell, ions undergo collision-induced dissociation (CID); ions collide with molecules of an inert collision gas such as argon (Carpenter 2002). The fragments produced are transmitted to the final quadrupole where they are selected for transmission to the detector. These pass over a special filter that enables computer-based collection and collation of data indicating types and amounts of chemicals present in the sample. Tandem mass spectrometry (MS/MS) can achieve specificities and sensitivities equivalent to radio-immunoassay and gas chromatography/mass spectrometry (GC/MS) (McLafferty 1981). Analysis is rapid—results are obtained in about 2–3 minutes per sample (Carpenter 2002).

MS/MS can be used to screen for amino acid abnormalities excreted in urine as part of urinary metabolic profiling (UMP). MS/MS equipment carries high capital costs and the technology is unable to separate and accurately quantify all amino acids.

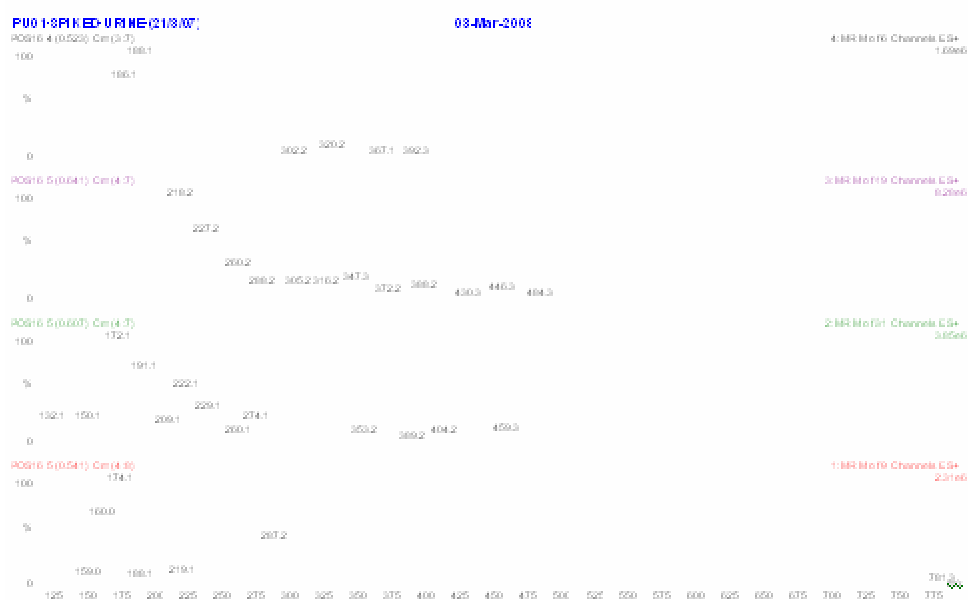

Figure 4 **Example output of tandem mass spectrometry urine amino acids screen (positive ion mode)**

Source: Courtesy of Victorian Biochemical Genetics Service. Published with permission

Note: Urine is analysed in negative and positive ion modes. Specific marker metabolites are targeted. Internal standards are applied to enable quantification. Each peak represents a separate metabolite or internal standard

Organic acids analysis methods

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC/MS) is a chromatographic technique that uses gas as the mobile phase (carrier) and a silicon based oil as the stationary phase. The stationary phase coats the inside of a fused silica capillary tube in a layer of predetermined and uniform thickness. The sample is injected into the column under

controlled carrier gas flow and temperature conditions. The sample is heated and vaporised as it enters the column. The compounds separate according to their respective affinities for the stationary and mobile phases as the sample travels through the column. The column may be heated further during the chromatography phase (temperature gradient) to enhance separation, especially for complex compounds that include components with a wide range of boiling points.

Sample compounds emerge from the column and enter a mass detector where they are bombarded by a stream of electrons. The electron beam fragments each compound based on the mass-to-charge (m/z) ratios of these fragments. When aggregated, the fragments form a virtual fingerprint of a compound. The mass detector measures these fragments by inducing them to pass through a quadrupole mass filter to an ion detector. The quadrupole becomes a mass filter by applying varying alternating frequencies. The frequencies' settings dictate the m/z ratio of fragments impacting on the detector at a given time. Computer-based data analysis determines and measures m/z ratios and abundance of the fragments. These data can be compared with a database (library) of similar compounds for identification purposes. The time taken for a compound to emerge from the column (the retention time) can also be used to assist in identification.

GC/MS has wide applications in pharmaceutical, food and forensics settings because it can accurately detect volatile organic compounds that occur in very small quantities. It is one of the most common methods used in the biomedical sciences to identify drugs and metabolites in blood and urine, including the presence of markers for inborn errors of metabolism associated with organic acids metabolism (Duez et al 1996, Hites 1997, Kuhara 2001).

Organic acid samples for analysis by GC/MS require considerable preparation. The method used by Australian laboratories performing UMP requires that organic acids are extracted from acidified, salt saturated urine using ethylacetate. Diethylether may also be used in addition. The solvent mixture is then dried under an inert gas and the organic acids in the residue exposed to a derivatising agent to form volatile derivatives amenable to GC/MS. Derivatisation to form trimethylsiliyl derivatives is common practice. This is achieved using *bis*-trimethylsilyltrifluoroacetamide, often containing 1 per cent trimethylchlorosilane, which acts as a catalyst. Methoxime or ethoxime derivatisation may be used before extraction to stabilise α-ketoacids.

GC/MS is a widely used, highly sensitive and specific method to identify and measure organic acids in urine. The technology is reproducible and enables global exchange of data among scientists.

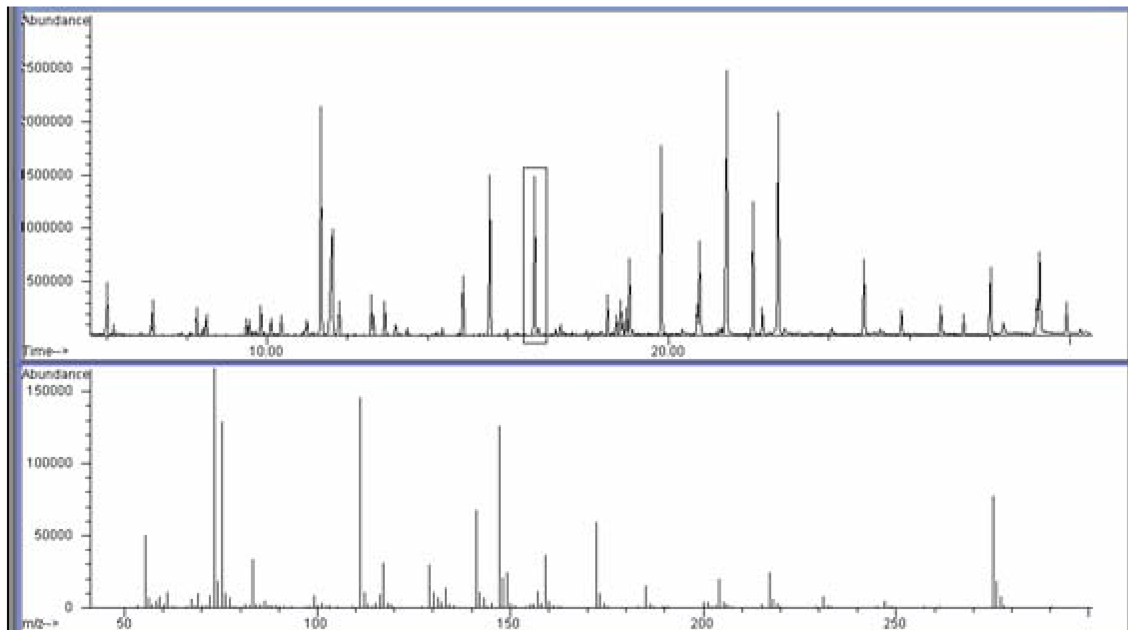

Figure 5 GC/MS output example: trimethysilyl derivatives of organic acids upper frame: total ion chromatogram; lower frame: fragmentation peak of adipic acid (indicated by boxed element in upper frame)

Source: Courtesy of Genetic Medicine, Adelaide Women's and Children's Hospital. Published with permission

Intended purpose

The intended purpose of UMP is to detect genetic metabolic disorders among:

- asymptomatic newborns with positive screening results
- people with clinical presentations suggestive of genetic metabolic disorders
- at-risk family members of patients with specific genetic metabolic disorders.

Clinical need and burden of disease

Inborn errors of metabolism (IEM) are a range of conditions typically caused by a deficiency in one or more metabolic enzymes. IEM is a collective term that refers to over 500 known conditions (Winter and Buist 1998), in the same way that the term 'heart disease' can refer to a range of heart conditions, or 'diabetes' can refer to either type 1 or type 2 diabetes mellitus. IEMs can be broadly classified as errors of carbohydrate, amino acid, organic acid or fatty acid metabolism or transport (Saudubray et al 2006).

Most IEMs arise from defects in single genes that code for specific enzymes involved in converting one substance to another, or that are involved in transporting proteins (Saudubray et al 2006, Martins 1999). The reduced activity of a particular enzyme in a pathway can lead to accumulation of intermediate metabolites that cannot be broken down. This can result in toxic effects and contribute to deficiencies in substances produced later in particular metabolic pathways. In some cases, substances that cannot be metabolised normally may be broken down via alternative pathways, leading to accumulation of metabolites of those alternative pathways (Martins 1999). IEMs are primarily inherited autosomal recessive conditions, although some are X-linked (passed from the mother who carries the gene for the condition). Other conditions result from mutations of the mitochondrial DNA (Martins 1999). Much less often, IEMs can feature autosomal dominant patterns of inheritance (Kahler and Fahey 2003).

IEMs can be evident at birth or present later in life (Sedel et al 2007a, Sedel et al 2007b). If a parent has one copy of an abnormal gene that codes for metabolism of a specific amino or organic acid, and one copy of a corresponding dominant normal gene, the parent is a carrier, but is not considered to be positive for the condition. Children whose parents are both carriers of an abnormal gene may inherit two copies of the abnormal gene. In this case, the child will have the condition. Until these children are diagnosed, most parents are unaware that they are carriers of the abnormal gene. For this reason, there is often no prior documented family history of the disease. IEMs can sometimes occur spontaneously—neither parent is a carrier of the abnormal gene, but the child's genetic material mutates spontaneously, resulting in an inborn error of metabolism.

Diagnoses are generally made from screening test results at birth or when symptoms occur. These occurrences alert families about underlying genetic predispositions for the condition. When diagnoses are confirmed, patients' first degree relatives may also be tested for the condition to determine carrier status. The results have important implications for the future health of family members and for family planning decisions (Winter and Buist 1998, King et al 2005, Applegarth and Toone 2005).

Although each IEM is rare, they collectively affect a significant number of people. Estimates place the cumulative global incidence rate at between one in 5000 and one in 1000 live births (Raghuveer et al 2006, Martins 1999, Winter and Buist 1998).
If untreated, IEMs can have serious effects on short- and long-term health outcomes; and negatively impact growth, neurological development, movement, vision, skin, organ size and function, and behaviour (Raghuveer et al 2006). Common symptoms of IEMs in infancy and childhood include lethargy, vomiting, seizures, organomegaly, hypo- or hypertonia, failure to thrive, and mental and physical retardation (Raghuveer et al 2006, Saudubray et al 2006, Kahler and Fahey 2003). Symptoms of IEMs among adolescents and adults are varied and may include peripheral neuropathy, behavioural and psychiatric

disturbances (Sedel et al 2007a, Sedel et al 2007b). Untreated IEMs can be fatal, and have been implicated as a possible cause of sudden infant death syndrome (Loughrey et al 2005). Diagnosing specific IEMs can be challenging, not only because the conditions are relatively rare, but also for the reason that many symptoms are not unique to these conditions (Winter and Buist 1998, Martins 1999).

The nine most common IEMs, excluding phenylketonuria, (based on the expert opinions of advisory panel members) are included in this assessment. These disorders predominantly fall under the broad concept of inborn errors of intermediary metabolism that cause acute or progressive symptoms, resulting from the accumulation of toxic compounds. The nine disorders are:

1. cystinuria
2. homocystinuria (cystathionine beta-synthase [CβS] deficiency)
3. ornithine transcarbamylase (OTC) deficiency
4. non-ketotic hyperglycinaemia (NKH)
5. maple syrup urine disease (MSUD)
6. glutaric aciduria type I (GA1)
7. methylmalonic aciduria (MMA)
8. lactic acidoses (LA)
9. medium-chain acyl-CoA dehydrogenase deficiency (MCADD).

Each of these conditions is discussed in detail in the following sections. A more complete list of IEMs is presented in Appendix H. Although phenylketonuria is among the most common types of IEM, it was not considered in this assessment because it is usually not diagnosed by UMP.

Cystinuria

Cystinuria is an autosomal recessive condition caused by a defect in amino acid transport. Mutations in at least two of the transport proteins located in the luminal brush border membrane of the renal proximal tubule result in a dysfunction of cystine reabsorption that can also affect reabsorption of ornithine, lysine and arginine. Under normal circumstances, almost the entire filtered load of these amino acids is reabsorbed by the proximal tubule. This mechanism is impaired or absent in patients with cystinuria, which causes the load of these amino acids to become concentrated. When the concentrated load reaches the acidic environment of the distal nephron, cystine's solubility in acid is exceeded and it begins to crystallise, forming characteristic hexagonal calculi (Font-Llitjós et al 2005, Goodyer 2004, Rogers et al 2007, Townsend et al 2004, Goldfarb 2005).

Cystinuria can be classified into three types—A, B, and AB—based on the type of mutation found in affected individuals. Type A is characterised by two mutations in the SLC3A1 gene. In type B, two mutations occur in gene SLC7A9. In type AB, one mutation occurs in each of these genes (Font-Llitjós et al 2005).

Morbidity and mortality

Cystinuria is characterised by the presence of yellow-coloured cystine calculi and excessive levels of cystine, ornithine, lysine and arginine in the urine, although some patients remain asymptomatic (Rogers et al 2007). Other symptoms include abdominal and groin pain, urinary tract infections, nausea and vomiting, loss of appetite, and failure to thrive. The formation of large calculi that block the urinary tract can precede haematuria, pyuria, infection, and fever. Patients with cystinuria may also develop renal insufficiency or renal failure (Rogers et al 2007, Goldfarb 2005). Mortality as a direct result of the condition is rare, but it is typically attributable to end-stage renal disease (Goldfarb 2005).

Incidence rate

The prevalence and incidence of cystinuria varies widely among populations.
In Australia, newborn screening programs have estimated frequency at between one in 17,000 and one in 4000 people (Shekarriz et al 2002, Wilcken et al 1980). However, data that informed these estimates date from the 1970s and 1980s (Smith and Wilcken 1984, Wilcken et al 1980, Smith et al 1979, Turner and Smith 1973). Data from the NSW cystinuria register, reported between 1980 and 1989, indicates that the incidence may be as low as one in 40,000 (Wilcken, personal communication, December 2007).
The reliability of this estimate is obscured because many people remain asymptomatic into adulthood. Asymptomatic patients would not have been reported to the registry, thereby avoiding inclusion in incidence data.

Current treatment

The main aim of medical management is to prevent or delay formation of recurrent calculi. Without treatment, symptoms recur throughout life. The basis of management is hyperhydration, urine alkalinisation, dietary changes, and pharmacotherapy to reduce levels of free cystine in the urine (Dello, Strologo and Rizzoni 2006, Knoll et al 2005, Rogers et al 2007). Medications used to treat cystinuria are typically associated with low efficacy and high frequency of adverse events. This can lead to poor patient compliance, further hampering the effectiveness of management strategies. As a result of recurrent calculi formation and repeated interventions, many patients develop renal insufficiency, which must be monitored and treated appropriately. Transplantation may be considered for patients who progress to end-stage renal disease (Rogers et al 2007). Regular follow-up and optimal pharmacotherapy can significantly increase intervals between episodes (Knoll et al 2005).

Homocystinuria

Homocystinuria is an autosomal recessive disorder affecting metabolism of the amino acid, homocysteine. Homocysteine is formed from methionine in a series of enzyme-mediated steps, and can be re-methylated to methionine in a single step.
In the presence of excess methionine, homocysteine is metabolised to cysteine via the trans-sulphuration pathway. This occurs in two steps: the rate-limiting step is catalysed by the enzyme cystathionine beta-synthase (CβS). B group vitamins, particularly pyridoxine, also play a role in homocysteine metabolism. Homocystinuria is most commonly caused by a deficiency of CβS, resulting in markedly elevated blood and urine levels of homocystine and methionine and decreased levels of cystathionine and cysteine (Gaustadnes et al 2002, Bjørke Monsen and Ueland 2003, Pan et al 2007).

Homocystinuria is caused by a mutation on the CβS gene located on chromosome 21. There are currently over 92 known CβS gene mutations; two mutations are responsible for most occurrences of homocystinuria (Yap 2003).

Morbidity and mortality

Homocystinuria affects the eyes, skeleton, cardiovascular system, and the central nervous system. Symptoms include ectopia lentis, myopia and glaucoma; thromboembolic events, which are a major cause of death among patients with the condition; mental retardation, behavioural difficulties, psychiatric disorders and convulsions; and skeletal abnormalities such as osteoporosis and lengthening of the limbs and fingers similar to Marfan syndrome (Pandor et al 2004, Refsum et al 2004, Gaustadnes et al 2002). Homocystinuria is a progressive disease. Symptoms do not generally manifest at birth and patients may not be diagnosed until they begin to show clinical signs of the disease later in infancy or childhood, by which time irreversible damage may have occurred (Pandor et al 2004).

Incidence rate

The reported incidence of homocystinuria at birth ranges from one in 355,000 to one in 6400. This wide variation may be due to differences in genetic predisposition, as well as differences in screening and detection methods. Some of the highest incidence rates have been reported in Ireland and Scandinavia (Gaustadnes et al 2002, Refsum et al 2004, Yap 2003). In Australia, data from the 1970s indicates incidence in NSW at one in 58,000 (Wilcken and Turner 1978). The NSW homocystinuria register records an incidence of one in 63,000 people (Wilcken, personal communication, December 2007). In a 1997 cohort study of patients with homocystinuria in NSW, 43 individuals with the condition were identified from a population of six million people (Wilcken and Wilcken 1997).

Current treatment

Treatment involves supplementation with B group vitamins, including folate, pyridoxine and cobalamin, together with a low methionine diet. Patients are classified according to whether their biochemical abnormalities are responsive to treatment with pyridoxine. Approximately half of all people with homocystinuria respond to this treatment and consequently have milder or more slowly developing disease than people with unresponsive types. People with unresponsive disease are typically treated with the methyl donor medication betaine (Yap et al 2001, Pandor et al 2004, Wilcken and Wilcken 1997). Overall, treatment, especially early treatment, is successful in preventing the most serious consequences of the disorder (Yap et al 2001).

Ornithine transcarbamylase deficiency

Ornithine transcarbamylase (OTC) deficiency is a defect of the urea cycle, which is responsible for the metabolism of ammonia to urea during amino acid metabolism. The condition results from a deficiency of the mitochondrial enzyme ornithine transcarbamylase, which catalyses conversion of ornithine and carbamyl phosphate to citrulline, resulting in hyperammonaemia (Summar and Tuchman 2001, Gyato et al 2004). Excess carbamyl phosphate accumulates in OTC and is metabolised via an alternative pathway to orotic acid, which can be detected at high levels in blood and urine (Gyato et al 2004). The condition is a partially dominant X-linked trait, so occurs more frequently and severely in males, although females can also be affected.

Around 85 per cent of women remain asymptomatic; the remaining 15 per cent have symptoms and complications common to the disorder that range in type and severity (Summar and Tuchman 2001, Gyato et al 2004). OTC deficiency may present at any time from early infancy to adulthood (Summar and Tuchman 2001, Gordon 2003, Wilcken 2004). This is due to the severity of the underlying condition; some people retain residual enzyme activity and others have none at all (Summar and Tuchman 2001).

Morbidity and mortality

Infants with early-onset OTC deficiency, who are almost exclusively males, experience feeding difficulties, lethargy, vomiting, hyperventilation, hypotonia, seizures, and in severe cases, may become comatose within the first week of life. Hyperammonaemia can be fatal if not detected and reversed. The risk of neurocognitive delays, visual problems and cerebral palsy is high, particularly if infants are rescued from coma (Summar and Tuchman 2001, Gropman and Batshaw 2004). People with late-onset OTC deficiency present with loss of appetite, migraines, vomiting, ataxia, lethargy and behavioural abnormalities; less commonly, they may experience sleep problems, delusions and hallucinations and can become comatose (Summar and Tuchman 2001, Gordon 2003).

Incidence rate

OTC deficiency is the most common of the urea acid cycle disorders, with an estimated incidence of one in 14,000 in the USA (Gyato et al 2004, Gropman and Batshaw 2004). In NSW, 32 occurrences of OTC deficiency were detected between 1974 and 2003, representing 57 per cent of all urea cycle disorders diagnosed in the state during this period (Wilcken 2004).

Current treatment

Treatment aims to maintain low plasma levels of ammonia and glutamine. This is achieved through a reduced protein diet with supplementation of individual amino acids and micronutrients as required. Anthropometric, clinical and biochemical status should be monitored regularly. Ammonia-scavenging drugs are administered to lower plasma ammonium levels. Liver transplantation may be considered in severe cases (Ahrens et al 2001, Gordon 2003, Berry and Steiner 2001).

Pre-natal diagnosis and family planning

OTC deficiency is an X-linked (passed on from the mother, who carries the gene for the condition) disorder and accurate diagnosis is necessary because of the potential for wide-ranging impact beyond the first degree relatives of the person diagnosed with the condition (Winter and Buist 1998, King et al 2005, Applegarth and Toone 2005).

Non-ketotic hyperglycinaemia

Non-ketotic hyperglycinaemia (NKH), also known as glycine encephalopathy, is a disorder of amino acid metabolism. It is caused by defects in the glycine cleavage enzyme complex in the mitochondrion, which is responsible for catabolising glycine to methylene tetrahydrofolate and carbon dioxide. The result is elevation of glycine concentration in the brain, cerebrospinal fluid (CSF), plasma and urine. Glycine is a neurotransmitter, acting as an inhibitor in the brainstem and spinal cord and in an excitatory manner in the cerebral cortex (Hoover-Fong et al 2004). NKH has an autosomal recessive inheritance profile (Applegarth and Toone 2005). There have been two prevalent gene mutations identified, one in the Finnish population and another in the Israeli-Arab population (Flusser et al 2005).

Morbidity and mortality

There are two types of NKH—classical and atypical. Patients with the classical form of NKH present shortly after birth with symptoms that include lethargy, feeding problems, apnoea requiring ventilation, and movement disorders. Mortality is high, and survivors generally develop seizures and neurocognitive delays. Atypical forms of the disease have a varied phenotypical presentation, dependent on the age of onset. Those with atypical forms usually grow and develop normally until later in infancy or early childhood, although some occurrences of NKH that develop during the first month of life are atypical in aetiology. More commonly, atypical NKH begins to develop sometime after one month of age, although often not until six months of age or later. At this time, patients begin to develop clinical signs and symptoms of the disease similar to infants with classical NKH, but symptoms may be less severe. Patients may also experience delirium, choreoathetosis, behavioural problems and intermittent febrile illnesses. Patients with later onset (after the age of two years) can develop lower limb spasticity, movement disorders, optic atrophy, peripheral neuropathy and neurological degeneration. Late-onset symptoms tend to be milder and patients often have a normal lifespan (Dinopoulos et al 2005, Hoover-Fong et al 2004).

Incidence rate

The prevalence of NKH was studied in British Columbia, Canada, by reviewing birth and medical records for the province between 1969 and 1996. There were 18 diagnoses reported from a total birth cohort of 1,142,912, equating to a prevalence rate of one in 63,495 (Applegarth et al 2000). In the USA, incidence has been reported at one in 250,000 live births, but this figure is higher in Finland and Israel (Kure et al 1999). The prevalence is also high among the Maori population of New Zealand. Between 2002 and 2007, eight diagnoses of NKH were detected by neonatal screening, six of whom were Maori infants (Wilson et al 2007). Recent Australian data places the national incidence rate at one in 81,500 (Tan et al 2007).

Current treatment

Treatment aims to reduce glycine levels in body fluids. This is achieved by a low protein diet in conjunction with medications that reduce glycine concentration and antagonise over-stimulation of N-methyl-D-aspartate (NMDA) receptors. Treatment is not curative and there is a lack of consistency among individual responses to various treatment

options (Dinopoulos et al 2005, Applegarth and Toone 2005). Treatment is ineffective for early-onset (classical) NKH.

Maple syrup urine disease

Maple syrup urine disease (MSUD) is named for the characteristic sweet-smelling urine observed in some patients. MSUD is an autosomal recessive disorder of amino acid metabolism caused by decreased activity of the α-ketoacid dehydrogenase complex in the mitochondrion. This enzyme complex is involved in the metabolism of the three branched-chain amino acids: valine, leucine, and isoleucine. Under normal circumstances, branched-chain amino acids are first metabolised to α-ketoacids then decarboxylated (with thiamine pyrophosphate acting as a coenzyme) to form acetyl-CoA and succinyl-CoA, which feed into the Krebs cycle. In MSUD, there is a decrease in the activity of one of the enzymes in the enzyme complex, branched-chain 2-ketoacid dehydrogenase (BCKD), at the second step. This leads to an accumulation of α-ketoacids and branched-chain amino acids (Mitsubichi et al 2005, Pandor et al 2004, Chuang et al 2006). Higher than normal levels of valine, leucine, isoleucine and ketoacids can be detected in the urine of people with MSUD (Pan et al 2007).

More than 60 mutations and four types of the condition have been identified in patients with MSUD. The types are categorised based on the gene in which the mutation is found, which translate to different clinical phenotypes related to the severity and frequency of symptoms, and the degree of residual BCKD activity in affected individuals. Classical MSUD manifests in the first days of life and the symptoms tend to be severe. In the intermediate and intermittent forms of the disease, enzyme activity is higher and symptoms are milder with later onset. Patients with the intermittent form of the condition may present with symptoms only at times of metabolic stress, but they may also manifest as impaired development. The fourth type, thiamine-responsive MSUD, is the least common and least severe form of the condition, and responds to dietary supplementation with thiamine (Pandor et al 2004, Chuang et al 2006).

Morbidity and mortality

People with the classical form of the disease usually appear to be free of the condition at birth. Within the first days of life, infants develop feeding problems, vomiting, and lethargy associated with ketoacidosis. If left untreated, affected infants progress rapidly to weight loss, seizures, disordered breathing and neurological symptoms. Severe ketoacidosis can result in coma and death. Survivors typically have neurological delays, blindness and cerebral palsy (Mitsubichi et al 2005, Pandor et al 2004). Patients with intermediate MSUD do not experience the same acute episode of ketoacidosis, but present with failure to thrive, mild systemic acidosis and neurological deficits. In intermittent MSUD, attacks are triggered by infections or other acute illnesses. People with this form of MSUD experience recurrent ataxia, lethargy and sometimes elevated levels of branched-chain amino acids and ketoacids; most are not intellectually impaired. Patients with the thiamine-responsive form of MSUD present with hyperaminoacidaemia similar to those with the intermediate form of the condition, but which can be successfully treated with dietary thiamine supplementation (Mitsubichi et al 2005).

Incidence rate

The incidence of MSUD varies among regions and populations. The worldwide incidence of MSUD is estimated to be one in 185,000 births. In Europe and Japan it is lower, at one in 290,000 and one in 560,000 births, respectively. In stark contrast, the incidence in the Mennonite population of North America is between one in 358 and one in 176 births (Mitsubichi et al 2005, Puffenberger 2003). In Australia, the National Health and Medical Research Council funded a national study between 1994 and 2002 to assess *inter alia* the incidence of IEMs, which found that the incidence of MSUD is around one in 280,000 live births (Wilcken, personal communication, December 2007).

Current treatment

Treatment during acute symptomatic episodes involves rapidly reducing the accumulation of ketoacids and branched-chain amino acids, and correcting other metabolic and clinical problems. Administration of a diet free from branched-chain amino acids that meets all other protein, energy and nutrient requirements is the cornerstone of treatment. Hyponatraemia and cerebral oedema are common complications that are managed by administering mannitol and diuretics. When acute attacks have been resolved, the goal of therapy shifts to prevention of further episodes. Patients are maintained on a diet low in valine, leucine and isoleucine; the degree of restriction is tailored for the patient based on the amount of residual enzyme activity present. Liver transplantation is an option. Thiamine supplementation is indicated for the subgroup of patients with thiamine-responsive MSUD (Mitsubichi et al 2005, Chuang et al 2006).

Glutaric aciduria type I

Glutaric aciduria type I (GA1) is a recessive autosomal disorder of organic acid metabolism resulting from a deficiency of the mitochondrial enzyme glutaryl CoA dehydrogenase (GCDH). GCDH is involved at two steps in the metabolism of the amino acids lysine, hydroxylysine and tryptophan. In people with GA1 both steps are affected, causing the accumulation of glutaric, 3-hydroxyglutaric and glutaconic acids in the body, which are then excreted in the urine. Because carnitine is used in the metabolism of glutaric acid, the condition also results in secondary carnitine depletion as the body attempts to reduce the level of glutaric acid (Hoffman and Zschocke 1999, Gordon 2006). Elevated levels of glutarylcarnitine, formed from glutaric acid and carnitine, may be detected in the urine, even sometimes in cases of secondary carnitine depletion (Kölker et al 2007).

The genetic basis of GA1 is a mutation in a single gene that is usually heterogeneous, though some homogeneous mutations have been seen in certain populations of people who are genetically similar. To date more than 70 mutations have been identified in this gene, with a strong correlation between genetic mutation and level of metabolites excreted in the urine. However, siblings with the same mutations may have varying levels of symptom severity, suggesting that environmental factors also play a role in how the disease presents phenotypically (Hoffman and Zschocke 1999, Hedlund et al 2006).

Morbidity and mortality

Most people with GA1 are macrocephalic when they are born, or become so shortly after birth. Infants may remain asymptomatic or develop mild neurological impairments in the first months of life. Typically, the disease presents sometime between three and 36 months of age. This is usually precipitated by a febrile illness, immunisation or surgery that triggers severe encephalopathy accompanied by metabolic acidosis, resulting in necrosis of the striatum. Patients may develop concurrent dyskinesias, stroke-like symptoms, seizures, and experience lapses in consciousness. Children with GA1 are also prone to subdural haemorrhages from minor head injuries sustained when learning to walk. In some cases, GA1 may not present until adulthood. In these rare instances, symptoms can include headaches, vision problems and leukoencephalopathy (Kölker et al 2007, Gordon 2006, Hedlund et al 2006, Hoffman and Zschocke 1999). Long-term outcomes have not been researched extensively, although there is good evidence indicating that detection of GA1 during early life when the infant is asymptomatic can significantly reduce development of symptoms and result in a better long-term prognosis (Kölker et al 2007).

Incidence rate

Since it was first described in the mid-1970s, the global incidence of GA1 has been estimated at around 400 people. GA1 is over-represented in certain populations including the Amish and Oji-Cree peoples in North America, and nomadic peoples originating from Ireland ('Irish Travellers') (Kölker et al 2007). The overall mean frequency of the condition is estimated to be around one in 106,900 births, based on figures from screening programs in various countries, including Australia. Reviews of screening data from NSW have found that the prevalence of GA1 is lower than the global rate at between one in 181,000 and one in 137,000 (Lindner et al 2004).More recent data indicates that the birth incidence of GA1 in NSW is 1:90,000 (Wilcken et al, in press).

Current treatment

Maintenance treatment of GA1 involves a diet low in lysine and tryptophan along with dietary supplementation of carnitine; additional riboflavin and other micronutrients may also be beneficial. It is important that carers and treating health professionals anticipate the likelihood of a GA1-related crisis during periods of illness, immunisation and surgery, and that emergency treatment be carried out early and aggressively. Movement disorders may be treated pharmacologically. Assessment by a neurologist is recommended, and additional assistance may be sought from dietitians, speech therapists, physiotherapists and other allied health professionals in managing the condition (Kölker et al 2007, Gordon 2006, Hedlund et al 2006, Hoffman and Zschocke 1999).

Methylmalonic aciduria

Methylmalonic aciduria (MMA) includes several similar disorders of organic acid metabolism, all autosomal recessive disorders. Although there are numerous causes of the condition, the most common is a defect in the conversion of methylmalonyl-CoA to succinyl-CoA. This reaction is catalysed by the enzyme methylmalonyl-CoA mutase. MMA is most commonly the result of a defect in the methylmalonyl-CoA mutase apoenzyme, or in the processing of hydroxocobalamin to deoxyadenosyl cobalamin. This leads to the accumulation of intermediate metabolites proximal to the block in metabolism (Pandor et al 2004, Dionisi-Vici et al 2006). Methylmalonic acid can be detected at elevated levels in the urine; levels of trimethylamine N-oxide (betaine) and diethylamine are also increased in the urine of patients with MMA (Pan et al 2007).

Morbidity and mortality

MMA generally presents in the hours or days following birth. Symptoms of this form of the condition include feeding problems, vomiting and weight loss, progressing rapidly to neurological impairments such as hypotonia, seizures, respiratory distress, hypothermia and coma. If untreated, most affected infants die within a few days; those who survive generally have significant neurological damage (Deodato et al 2006, Dionisi-Vici et al 2006). Late-onset presentation can also occur, with clinical signs first detected during the early years of childhood. In late-onset MMA, the condition manifests as acute encephalopathy and intermittent or chronic problems that vary in severity. These may include ataxia, feeding and behavioural problems, failure to thrive, recurrent vomiting and selective refusal of protein-rich foods (Dionisi-Vici et al 2006). In addition to neurological deficits, potential complications include renal dysfunction, cardiomyopathy and acute attacks of pancreatitis (Deodato et al 2006). Early detection and management of the disease is imperative to ensure good long-term prognoses. Screening programs have been shown to improve survival and prevent or delay the onset of clinical symptoms (de Baulny et al 2005), although treatment response remains poor (de Baulny et al 2005, Pandor et al 2004). Screening programmes also ensure a diagnosis in those cases that might otherwise be thought to have died of neonatal sepsis.

Incidence rate

The National Health and Medical Research Council recently funded a study that included defining the national incidence of IEMs The study found that between 1994 and 2002 the incidence of MMA was 13 in 2,000,000 equating to a rate of around one in 154,000 (Wilcken, personal communication, December 2007).

Current treatment

Treatment of acute episodes of the condition centres on normalising plasma amino acid levels, correcting acid-base imbalances, removing toxic substances and treating presented signs and symptoms. High-energy, protein-free nutrition is pivotal; protein is reintroduced gradually when toxic substances are cleared. Maintenance therapy involves a low-protein, high-energy diet with specific B group vitamins and carnitine supplementation. The high prevalence of feeding problems among children with the condition means that enteral nutrition may be needed to supplement oral intake (de Baulny et al 2005). Other treatment includes liver transplantation, which has been successful in a few cases.

Lactic acidosis

Lactic acidosis is caused by the accumulation of lactate in body tissues (Stacpoole et al 2006) and occurs when tissue levels of lactic acid become excessive due to an underlying disturbance in lactic acid metabolism. Lactic acidosis may be secondary to multi-system organ failure or a congenital DNA mutation (Stacpoole 1997a). Elevated levels of lactic acid can be detected in the urine (Stacpoole et al 1997b).

The congenital lactic acidoses are a group of conditions associated with cellular energy deficiency. They are most often caused by disorders of the mitochondrial respiratory chain due to mutations in either nuclear or mitochondrial DNA, and result in a lack of adenosine triphosphate (ATP). Other causes are related to problems in pyruvate metabolism including deficiencies in the pyruvate dehydrogenase complex (PDC) or pyruvate carboxylase. PDC is involved in glucose metabolism; it catalyses the conversion of pyruvate to acetyl-CoA, which feeds into the Krebs cycle (Stacpoole 1997a).
When pyruvate cannot be catabolised to acetyl-CoA, it is converted to lactate and alanine in the cytoplasm.

Morbidity and mortality

Phenotypically, the presentation of these disorders is extremely diverse, even among people with the same genetic mutation. The condition is often expressed in highly oxidative tissues such as cardiac, neural, muscle, renal, ocular and aural structures and symptoms include skeletal deficits, cardiomyopathy, hypotonia, renal disorders, psychomotor delays, deafness, and neurological deterioration. Multi-system organ failure can be fatal (Stickler et al 2006, Stacpoole 2006).

Incidence rate

Congenital lactic acidosis prevalence data are scarce; however, it appears to be more common in certain populations; incidence is higher among indigenous Canadians than the non-indigenous population (Haworth et al 1991). In Australia, based on figures collected between 1987 and 1996, the minimum birth prevalence of all respiratory chain defects in three states (New South Wales, Victoria, South Australia) was one in 20,000 births (Skladal et al 2003). A disproportionate number of children diagnosed with respiratory chain disorders (21%) were of Lebanese descent (Skladal et al 2003).

Current treatment

A number of dietary interventions, such as supplementation with carnitine and pyruvate, aimed at stimulating glucose oxidation have been used to treat congenital lactic acidosis, with varying degrees of success. Pharmacological treatment with dichloroacetate has shown some promising results (Stacpoole 1997, Stacpoole et al 2006), but overall, these conditions are not curable.

Medium-chain acyl-CoA dehydrogenase deficiency

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an inherited autosomal recessive abnormality of fatty acid beta-oxidation. Fatty acids are normally released from adipose tissue and enter the circulation to be used for energy during exercise or fasting. They are converted into acyl-CoA esters in the cell cytoplasm before being oxidised by acyl-CoA dehydrogenases in the mitochondria in the first step of beta-oxidation.
In MCADD, medium-chain acyl-CoA dehydrogenase is lacking in the mitochondria, resulting in the release of incompletely oxidised acyl-CoA intermediates that enter the circulation, particularly as octanoylcarnitine (Tran et al 2006). MCADD can be diagnosed by testing blood for the presence of octanoylcarnitine and urine for excess levels of hexanoylglycine (Wilcken et al 2007). Other metabolites detectable at elevated levels in the urine of patients with MCADD during times of metabolic stress include medium-chain dicarboxylic acids, and suberylglycine; ketones may be absent or present at reduced levels (Pasquali et al 2006).

Morbidity and mortality

Hypoglycaemia and acute encephalopathy are common clinical symptoms of MCADD, both of which can be fatal. Patients with MCADD are generally asymptomatic until their first acute episode of the disease, which typically occurs during the first two years of life. Around a quarter of patients will die, and a third of survivors will experience irreversible neurological damage (Tran et al 2006). The most frequent cause of MCADD is a single base-change mutation; A to G at position 985, resulting in a lysine to glutamate substitution. Most people with the condition are homozygous for this change (Rhead 2006), although mutation profiles differ between children detected during screening programs and those diagnosed clinically. The type of mutation dictates disease severity (Andresen et al 2001, Haas et al 2007).

Incidence rate

As with other genetically inherited IEMs, the incidence of MCADD varies among ethnic and geographic groups. Rhead (2006) surveyed national, regional and state-based screening programs in northern Europe, USA and Australia, in which 8.25 million babies were screened, and found an overall incidence of one in 14,600. The incidence varied from one in 51,000 in Japan to one in 6600 in Bavaria, Germany. This study recorded the incidence in Australia as one in 15,400 newborns; NSW incidence was slightly lower at one in 19,200. These data should be interpreted with caution because of a lack of standardisation in screening methods in the regions surveyed. An Australian cohort study (Wilcken et al 2007) reviewed the number of diagnoses among unscreened children under four years of age between 1994 and 1998 (before the introduction of screening programs), and screened and unscreened children under four years of age between 1998 and 2002. Results showed an incidence of 2.3 per 100,000 people among the total unscreened population and an incidence of 5.2 per 100,000 in the screened group.

Current treatment

Patients with MCADD benefit from avoiding fasting. Management consists of dietary therapy monitoring by a metabolic disease clinic, together with rapid intervention during acute episodes (Tran et al 2006). Mortality and serious morbidity after diagnosis is extremely rare (Wilson et al 1999).

Eligible population

Estimates of the number of eligible patients in each of the three populations identified for this assessment are required to determine the total number of patients who may be eligible for UMP tests. Australian epidemiological data are the best source to elicit this information. In this assessment, epidemiological data were not available for each of the three populations considered; these data are presented where they were available. To address these gaps, data were obtained from the actual number of tests conducted over two 12-month periods: January to December 2007; and financial year 2006–2007 (advisory panel).

Data were obtained from laboratories performing UMP in each state[1]. All laboratories are attached to major teaching hospitals. The use of UMP tests in Australia is estimated to be approximately 11,150 patients annually. Most tests (approximately 98%) are performed to investigate individuals with a clinical presentation suggestive of metabolic disorder. The total patient population is not expected to increase significantly over the next five years (expert opinion, advisory panel).

The number of patients considered to be eligible for UMP testing is presented separately for each of the three patient populations—asymptomatic newborns with screening results suggestive of metabolic disorders, people with clinical presentations that are suggestive of metabolic disorders, and at-risk family members of people with specific metabolic conditions.

Asymptomatic newborns with screening results suggestive of metabolic disorders

The New South Wales Newborn Screening Program is funded by the NSW and ACT Departments of Health and uses tandem mass spectrometry (MS/MS) to test for rare amino and organic acidopathies and fatty acid oxidation defects. Tests are performed on a few drops of newborn's blood taken from the heel, two or three days after birth. Because MS/MS can be applied to detect a wide range of compounds, this technology can simultaneously screen for a range of disorders (Wilcken et al 2007). About 90 of over 90,000 babies who are tested annually in NSW require urgent assessment and treatment. Similar programs are conducted in South Australia and Victoria that also include the neonatal populations of Tasmania and the Northern Territory (Wilcken et al 2003). In most cases, early diagnosis and treatment for infants with inborn errors of metabolism enables affected people to lead healthier lives.

Screening tests identify infants who may have one of these disorders but do not diagnose disorders. Infants whose screening test results are abnormal require additional assessment to confirm the presence or absence of the disorder. Because MS/MS is a sensitive technique, there can be many reasons for an abnormal (false positive) test result to occur in a healthy baby. Therefore, every infant whose screening test result is abnormal, regardless of the absence of symptoms, requires additional testing.

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[1] The actual number of tests performed over a 12 month period was available for 5 of the 6 laboratories performing UMP in each state (source, advisory panel). The number of tests performed in the remaining laboratory was estimated.

The annual number of births in Australia has been increasing steadily since 2002. There were 265,949 births registered in Australia in 2006[2] (ABS 2007). This birth rate was exceeded only in 1971 (276,400 births) (ABS 2007). The Australian Bureau of Statistics (ABS 2005) has released population projections for Australia between 2004 and 2010[3]. The fertility rate in 2004 was 1.76. Assuming that the fertility rate increases to 1.85 in 2011, the projected number of births that year is 276,803. If it is assumed that the fertility rate would then decrease from its 2004 rate to 1.63 in 2011, the projected number of births in 2011 would be 235,968 (ABS 2005).

Approximately 0.2 per cent of newborns have positive screening test results that require further sampling (expert opinion, advisory panel). Further testing would include UMP. Approximately 180 newborns with positive screening results are estimated to require further investigation with UMP each year (expert opinion, advisory panel).

Individuals with clinical presentations suggestive of genetic metabolic disorders

This patient population comprises individuals who present with symptoms and/or test results that suggest they have an inborn error of metabolism. Epidemiological data were not available to estimate the size of the eligible population. According to data indicating the number of UMP tests performed in Australia for two 12 month periods (January to December 2007; financial year 2006–2007; expert opinion advisory panel), approximately 10,940 tests would be performed annually to investigate people who have clinical presentations suggestive of genetic metabolic disorders.

At-risk family members of patients with a specific genetic metabolic disorders

This patient population comprises individuals who are first-degree family members of patients known to have a specific inborn error of metabolism. Epidemiological data were not available to estimate the size of the eligible population. Approximately 30 UMP tests are required each year to investigate at-risk family members of patients with specific genetic metabolic disorders (expert opinion, advisory panel).

Existing procedures

Alternative tests, considered to be comparators if UMP is unavailable, are presented in Appendix I.

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[2] At the time of writing (May 2008) complete birth data for 2007 were not available.

[3] Population projections were not available for 2008–2010; therefore, population projections for 2011 are presented.

Comparator

Definition of comparators was challenging, given that UMP is currently considered to be the standard of care to conduct further investigations for these patients, and as the most effective way to diagnose a range of inborn errors of metabolism (IEMs). Mutation analysis, functional studies, and assays performed using plasma, cerebrospinal fluid or solid organ samples have a role in diagnosing a small number of conditions. These tests could have a role where diagnosis of IEM is suspected, based on either newborn screening results or classical clinical presentation, but are limited in terms of timely availability and turn-around. They would have a very limited role in investigating patients who present with non-specific symptoms.

Mutation analysis

Gene mutations can disrupt the functioning and ability of proteins to perform normal chemical reactions that can cause metabolic errors. Clinical detection of these conditions can be challenging. Effects from mutated genes can present throughout life, from birth to old age, but may never manifest clinically or present variability in the pathophysiology of the condition (Yamaguchi et al 2006). Molecular methods to screen for mutations include Southern blotting, single-strand conformational polymorphism, fluorescence DNA melting curve analysis, and/or DNA sequencing of exons and exon-intron boundaries (Yamaguchi et al 2006).

Medium-chain acyl-CoA dehydrogenase deficiency

Andresen et al (1995) reported that a patient who was a member of a Danish family affected by medium-chain acyl-CoA dehydrogenase deficiency (MCADD) presented as being homozygous for the G985A mutation. This point mutation results in glutamic acid being substituted for lysine at amino acid position 304 of the mature protein (Kelly 1990). Subsequently, 11 of 12 patients presenting with MCADD were also found to be homozygous for this mutation (Andresen et al 1995). Among 50 verified MCADD patients, 80 per cent were homozygous for the mutation, 18 per cent were heterozygous and 2 per cent did not present with the mutation. As a result, the G985A assay is now used in routine diagnosis (Andresen et al 1995). Patients with symptomatic MCADD may not carry even one copy of this mutation. Carriage of only one copy does not distinguish between MCADD and being a healthy carrier (c. 1:70 of the Australian population).

Lactic acidosis

Blood and tissue samples from 1725 patients suspected of manifesting mitochondrial disorders were analysed between 1990 and 2001 (Marotta et al 2004). Clinical features varied considerably among patients. Of the 1184 people who were screened for the gene mutation responsible for mitochondrial encephalopathy lactic acidosis and stroke (MELAS A3243G), 6.17 per cent were positive for mitochondrial DNA mutation (Marotta et al 2004). Of these, 26.7 per cent exhibited elevated lactate levels.
Several people who were tested were asymptomatic when the mutation was detected (Marotta et al 2006).

Ornithine transcarbamylase deficiency

Mutation of the ornithine transcarbamylase (OTC) gene causes blockage in the urea cycle from the incorrect formation and structure of ornithine transcarbamylase enzyme. This results in ammonia accumulating at toxic levels which causes neurological problems (Yamaguchi et al 2006). Over 300 OTC gene mutations have been identified. About 80 per cent of patients with enzymatically proven OTC deficiency are found to have mutations in this gene (Yamaguchi et al 2006).

Glutaric aciduria type I

Zschocke et al (1999) used gel electrophoresis to identify mutations on both alleles of the glutaryl-CoA dehydrogenase gene among 48 patients with confirmed glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I [GA1]). No mutations in this gene were found among patients whose enzyme studies were normal.

Plasma acylcarnitines

Analysis of plasma acylcarnitines can be used in the diagnosis of conditions such as medium-chain acyl-CoA dehydrogenase deficiency (MCADD) and glutaric aciduria type I (GA1) (Chace et al 1997, van Hove et al 1993, Tortorelli et al 2005, Hedlund et al 2006). Accumulation of medium-chain acylcarnitines, such as octanoylcarnitine, is evident in MCADD regardless of the underlying mutation, symptomatic state or treatment. Increased levels of plasma glutarylcarnitine are indicative of GA1. Plasma glutarylcarnitine levels can be normal among patients with GA1 without elevation of urine glutaric acid. These rare patients can be missed by tandem mass spectrometry (MS/MS) screening of neonates (Hedlund 2006). Plasma acylcarnitine analyses are performed using electrospray ionisation MS/MS (Tortorelli 2005). Criteria used in the diagnosis of MCADD include elevated C-8 acylcarnitine concentration (> 0.3 μM), a ratio of C8/C10 acylcarnitines > 5, and a lack of elevated species of chain length > C10 (van Hove et al 1993).

Enzyme liver biopsy of non-ketotic hyperglycinaemia

Non-ketotic hyperglycinaemia (NKH) can be assessed by analysing enzyme activity in liver biopsies. Glycine enzyme activity assay examines the rate of conversion of glycine to serine in liver homogenates (de Groot et al 1970). Glycine cleavage systems that are blocked, absent or not functioning lead to glycine accumulation (Tada et al 1974). NKH can also be investigated by analysing plasma or cerebrospinal fluid (CSF) concentration of glycine (Hayasaka et al 1983). Overall, glycine cleavage system activities in the liver and brain were found to be extremely limited in patients with typical NKH (Hayasaka et al 1983).

Reverse phase high performance liquid chromatography

Urinary amino acids can be assayed by reverse phase high performance chromatography. Carducci et al (1996) reported using this method to determine primary and secondary amino acids in cystine and homocystine in urine samples. The RPHPLC procedure applied by Carducci et al (1996) involved precolumn derivatisation to detect amino acids in biological samples. Compounds adhere to reverse phase high performance liquid

chromatography columns in high aqueous mobile phase and are eluted from columns in the high organic mobile phase. Carducci et al (1996) reported that this method achieved shorter analysis times and increased sensitivity. Complex amino acid mixtures, such as in urine, were identified to aid diagnosis of hereditary and acquired disorders of amino acid metabolism, with good precision and sensitivity, minimal manual sample handling, and used a more versatile apparatus than ion-exchange analysers (Carducci et al 1996). The RPHPLC technique satisfactorily separated 38 amino acids in 92 minutes. It successfully identified a patient with cystinuria and phenylketonuria (Carducci et al 1996). RHPLC is not used routinely in Australia for detection of IEMs. Use of this technology would require samples to be sent overseas.

Amino acid analysis

Changes in amino acid profiles are sensitive indicators of a wide variety of physiological and pathological conditions (Slocum et al 1991). Analytical methods are based on chromatographic separation of amino acids present in samples. Samples are precipitated and the clear, ultra-filtrate is centrifuged. The protein-free ultra filtrate is adjusted to about pH 2.2 to facilitate absorption by the ion-exchange resin (Slocum et al 1991). Amino acids are then eluted from the column according to their dissociation characteristics. Elution is aided by a series of lithium buffers with increasing pH and ionic strength levels (Slocum et al 1991).

Enzymology and enzyme analysis of muscle or liver samples

Glutaric aciduria type I

Assays for glutaryl-CoA dehydrogenase can be performed in amniotic fluid cells and cultured fibroblasts for pre- and post-natal diagnosis of glutaric aciduria type I (GA1) (Christensen et al 1978, Christensen et al 1983).

Methylmalonic aciduria

Methylmalonyl CoA mutase or adenosylcobalamin metabolism activity may be impaired in methylmalonic aciduria (MMA). MMA can be detected by analysing cultured skin fibroblasts (Chalmers et al 1991).

Lactic acidosis

Current practice requires measurement of respiratory chain enzymes in muscle and liver biopsy tissue to achieve maximum sensitivity. Biopsy under general anaesthetic is usually required. Pyruvate dehydrogenase complex (PDC) enzyme analysis can be performed on skin fibroblasts.

Marketing status of the technology

Technologies that are currently used in Australia for urinary metabolic profiling (UMP) of amino acids and organic acids are listed in Table 2.

Table 2 Technologies used for urinary metabolic profiling of amino and organic acids in Australia

State	Amino acids	Organic acids
Victoria[a]	Waters Quattro LC Tandem Mass Spectrometer (MS/MS)	Agilent 6890 with 5973 Mass Detector (GC/MS)
South Australia[b]	Dionex Summit HPLC with Pickering PCX5200 Derivatisation unit (HPLC)	Agilent 6890 with 5973 Mass Detector (GC/MS)
Western Australia	Thin layer chromatography (TLC) No instruments used	Agilent 6890 with 5973 Mass Detector (GC/MS)
New South Wales[c]	Hoefer PS3000 with Multiphor 2 (HVE)	Agilent 6890 with 5973 Mass Detector (GC/MS)
Queensland (Mater Hospital)	Biochrom 30 (HPLC)	Agilent 6890 with 5973 Mass Detector (GC/MS)
Queensland (Royal Brisbane Hospital)	Waters Alliance HPLC with Pickering PCX5200 Derivatisation unit (HPLC)	Agilent 6890 with 5973 Mass Detector (GC/MS)

Abbreviations: GC/MS, gas chromatography mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography
[a]Testing for patients from Tasmania is performed in Victoria
[b]Testing for patients from the Northern Territory is performed in South Australia
[c]Testing for patients from the Australian Capital Territory is performed in New South Wales

Current reimbursement arrangement

The Medicare Benefits Schedule (MBS) in Australia does not currently fund urinary metabolic profiling (UMP) for analysis of amino and organic acids. UMP is currently funded individually by states.

MBS item number 66756 may cover particular aspects of UMP in some instances, but only if a quantitative method is used, and levels of 10 or more amino acids are being tested (MBS 2007). MBS item 66756 permits up to four tests to be conducted in a 12 month period, suggesting that subsequent tests are applied for monitoring purposes. This MBS item is therefore not considered to cover qualitative or semi-quantitative methods tests for fewer than 10 amino acids, or tests for organic acids (expert opinion, advisory panel). This item may cover the costs of UMP testing in laboratories in some states, but others charge significantly more than the scheduled fee.

Other MBS items may cover other particular aspects of UMP testing. MBS item number 66752 covers single tests for quantitation of cysteine, homocysteine and cystine, among others (MBS 2007).

Approach to assessment

Research questions and clinical pathways

Suspected metabolic disorders in newborns

The PPICO criteria (target population, prior tests, index test, comparator, outcomes) for assessment of urinary metabolic profiling (UMP) to detect genetic metabolic disorders following newborn screening are indicated in Table 3.

Table 3 PPICO criteria for the assessment of urinary metabolic profiling for detection of genetic metabolic disorders following newborn screening

Population	Prior tests	Intervention/test	Comparator	Reference standard	Outcomes
Asymptomatic newborns with positive screening test results	Routine newborn screening tests[a]	UMP[b]: by HPLC, GC/MS, TLC, HVE, MS/MS	UMP not available: (See Appendix I for alternative tests)	Clinical follow-up; molecular genetic analyses; enzyme analyses	Change in clinical outcomes[c] Change in clinical management[d] Diagnostic accuracy[e] Safety outcomes[f]

Abbreviations: GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] MS/MS result indicative of possible (genetic) metabolic disorder; manual chemical fluorescence test for galactose metabolites (to detect galactosaemias): this test is not performed in Victoria

[b] HPLC, quantitative for amino acids; gas chromatography/mass spectrometry (GC/MS, semi-quantitative for organic acids); HVE, qualitative for amino acids; MS/MS, quantitative for amino acids; TLC, qualitative for amino acids

[c] Survival (overall survival); morbidity (disease progression)

[d] Treatment varies depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)—avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase (OTC) deficiency—low protein diet, complex medications, liver transplantation for severe presentations; non-ketotic hyperglycinaemia (NKH)—no effective treatment for most patients—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some instances, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDC deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of management for GA1, OTC deficiency, MMA, and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis

[e] Sensitivity and specificity estimates, positive and negative likelihood ratios, summary diagnostic measures (eg diagnostic odds ratio, summary receiver operating characteristics)

[f] Safety outcomes were not considered to be applicable because UMP is non-invasive and requires only urine samples from patients

The research question for this indication, based on these criteria, was as follows.

To what extent is urinary metabolic profiling (UMP):

- safe
- effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and
- cost-effective

in the assessment of genetic metabolic disorders in asymptomatic newborns with a positive screening result in comparison to alternative tests when UMP is not available?

The clinical pathway for UMP for suspected metabolic disorder in newborns is shown in Figure 6. This flowchart displays the clinical management pathway to the point of patient diagnosis.

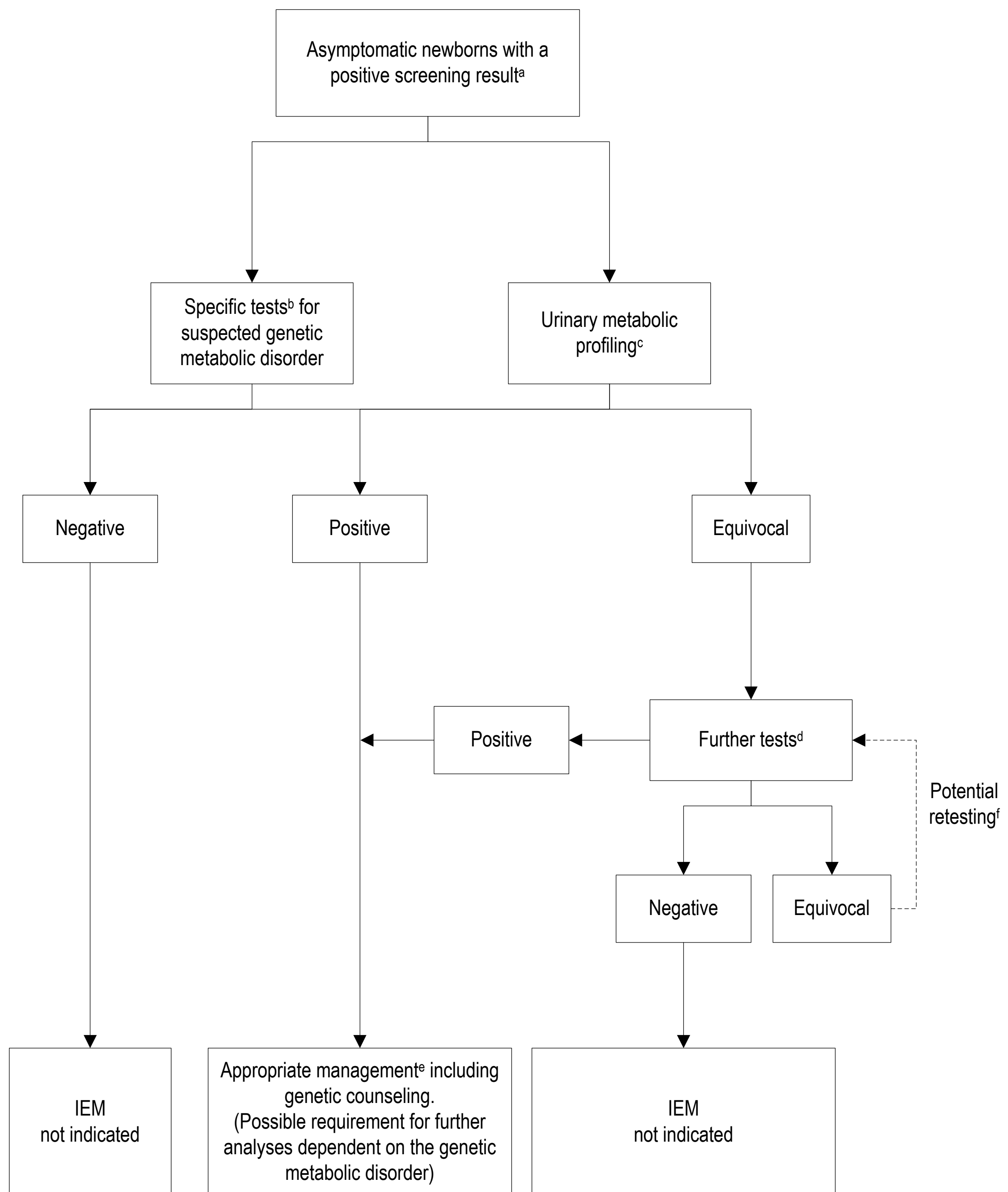

Figure 6 Clinical pathway for urinary metabolic profiling of suspected metabolic disorders in newborns

[a] Tandem mass spectrometry screening result indicative of genetic metabolic disorder, manual chemical fluorescence test for galactose metabolites (to detect galactosaemias); this test is not performed in Victoria
[b] See Appendix I
[c] High performance liquid chromatography (HPLC, quantitative for amino acids); gas chromatography/mass spectrometry (GC/MS, semi-quantitative for organic acids); high voltage electrophoresis (HVE, qualitative for amino acids); tandem mass spectrometry (MS/MS, quantitative for amino acids); thin layer chromatography (TLC, qualitative for amino acids).
[d] See Appendix J
[e] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)—avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase (OTC) deficiency—low protein diet, complex medications, liver transplantation for severe presentations; non-ketotic hyperglycinaemia (NKH)—no effective treatment for most patients—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some instances, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDC deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GA1; OTC deficiency; MMA; and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis
[f] Re-testing may be required for example where testing has failed or the results are inconclusive

Clinical presentations suggestive of genetic metabolic disorders

The PPICO criteria (target population, prior tests, index test, comparator, outcomes) for UMP assessment of people with clinical presentations suggestive of genetic metabolic disorders are indicated in Table 4.

Table 4 PPICO criteria to assess urinary metabolic profiling for clinical presentations suggestive of genetic metabolic disorders

Population	Prior tests	Intervention/test	Comparator	Reference standard	Outcomes
Individuals with clinical presentation[a] and/or test results suggestive of genetic metabolic disorder	Tests[b] to investigate potential reasons for non-specific symptoms	UMP[c]: by HPLC, GC/MS, TLC, HVE, MS/MS	UMP not available (see Appendix I for alternative tests)	Clinical follow-up, molecular genetic analyses, enzyme analyses	Change in clinical outcomes[d] Change in clinical management[e] Diagnostic accuracy[f] Safety outcomes[g]

Abbreviations: GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] Possible symptoms include: encephalopathy, seizures, hypotonia, dystonia, spasticity, vomiting, failure to thrive, jaundice and other signs of liver failure, cardiomyopathy, rhabdomyolysis, renal calculi; skeletal abnormalities, dislocation of the ocular lenses, abnormal hair. This list is not exhaustive because of the wide range of possible symptoms that can be associated with genetic metabolic disorders. Examples of specific symptoms highly suggestive of metabolic disorders in acute presentations include clinically unexplained encephalopathy, coma and increased respirations, with metabolic acidosis

[b] Tests used vary depending on particular symptoms, but routine investigations include: blood tests for electrolytes, glucose, ammonia, lactate, acid base, urea, creatinine, calcium, magnesium, phosphorus, creatine kinase, lead; liver function tests; full blood count; thyroid function tests, imaging abdominal ultrasound; magnetic resonance imaging of the head , skeletal survey; electroencephalogram for patients with seizures. This list is not exhaustive because of the wide range of possible symptoms that can be encountered in clinical presentations suggestive of genetic metabolic disorder

[c] HPLC, quantitative for amino acids; GC/MS, semi-quantitative for organic acids; HVE, qualitative for amino acids; MS/MS, quantitative for amino acids); TLC, qualitative for amino acids

[d] Survival (overall survival), morbidity (disease progression)

[e] Treatments vary depending on the specific genetic metabolic disorder: cystinuria hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)—avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase (OTC) deficiency—low protein diet, complex medications, liver transplantation for severe cases; non-ketotic hyperglycinaemia (NKH)—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some instances, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDC deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GA1; OTC deficiency; MMA; and MSUD. Genetic counselling is also a component of cystinuria, homocystinuria, and lactic acidosis management

[f] Sensitivity and specificity estimates; positive and negative likelihood ratios; summary diagnostic measures (eg diagnostic odds ratio, summary receiver operating characteristics)

[g] Safety outcomes were not considered to be applicable because UMP is non-invasive and requires urine samples only from patients

The research question for this indication, based on these criteria, was as follows.

To what extent is urinary metabolic profiling (UMP):

- safe
- effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and
- cost-effective

in the assessment of genetic metabolic disorders in individuals with a clinical presentation and/or test results suggestive of genetic metabolic disorders in comparison to alternative tests when UMP is not available?

The clinical pathway for UMP of individuals with clinical presentation and/or test results suggestive of genetic metabolic disorder is shown in Figure 7. This flowchart illustrates the clinical management pathway to the point of patient diagnosis.

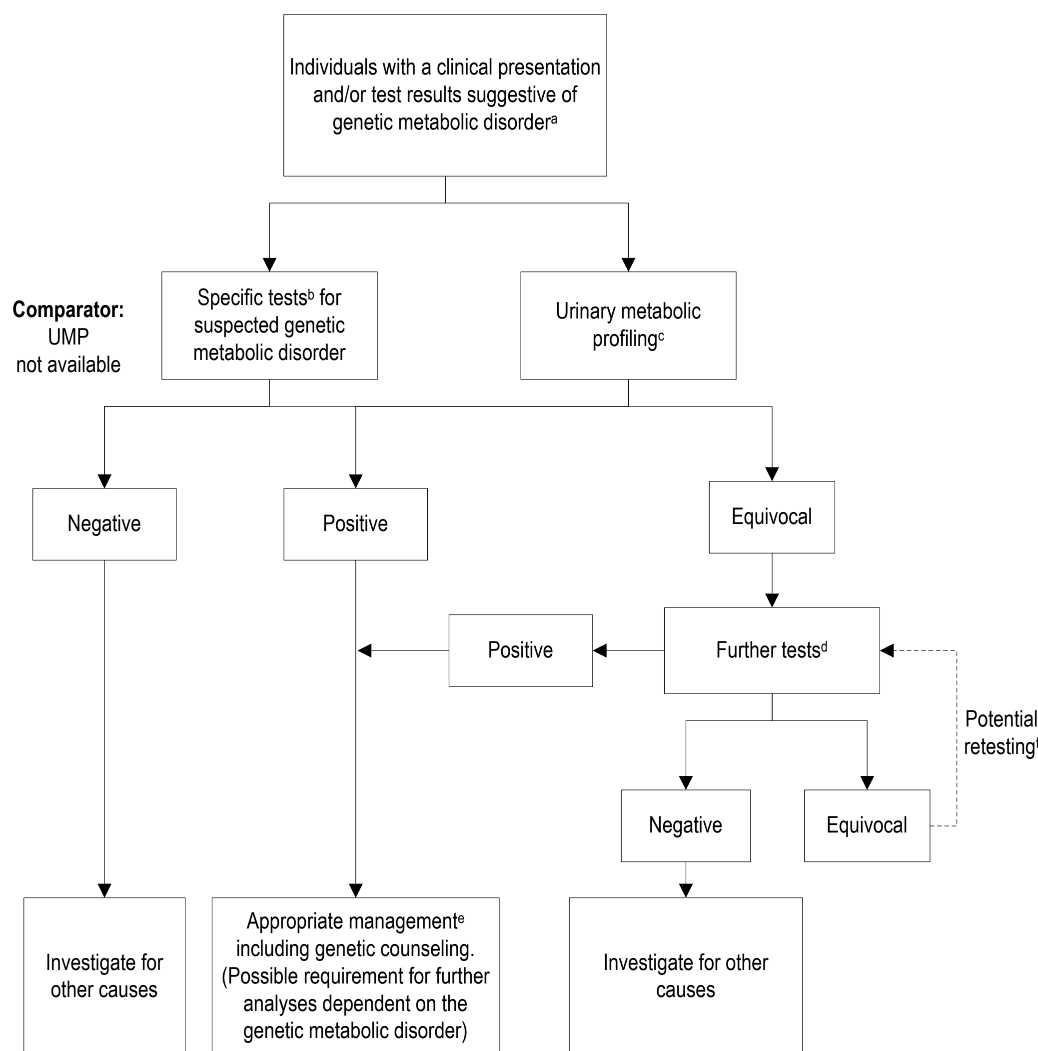

Figure 7 Clinical pathway for urinary metabolic profiling for people with clinical presentations and/or test results suggestive of genetic metabolic disorders

[a] Possible symptoms include encephalopathy, seizures, hypotonia, dystonia, spasticity, vomiting, failure to thrive, jaundice and other signs of liver failure, cardiomyopathy, rhabdomyolysis and renal calculi, skeletal abnormalities, dislocation of the lenses, abnormal hair. This list is not exhaustive because of the wide range of possible symptoms that can be associated with genetic metabolic disorders. Examples of specific symptoms highly suggestive of metabolic disorders in acute presentations include clinically unexplained encephalopathy, coma and increased respirations, with metabolic acidosis. Tests used will vary depending on the particular symptoms, but routine investigations include blood tests for electrolytes, glucose, ammonia, lactate, acid base, urea, creatinine, calcium, magnesium, phosphorus, creatine kinase, lead; liver function tests; full blood count; thyroid function tests, imaging abdominal ultrasound; magnetic resonance imaging of the head , skeletal survey; electroencephalogram for patients with seizures. This is not an exhaustive list because of the wide range of possible symptoms that may be encountered in clinical presentations suggestive of genetic metabolic disorders

[b] See Appendix I

[c] High performance liquid chromatography (HPLC, quantitative for amino acids); gas chromatography-mass spectrometry(GC/MS, semi-quantitative for organic acids); high voltage electrophoresis (HVE, qualitative for amino acids); tandem mass chromatography (MS/MS, quantitative for amino acids); thin layer chromatography (TLC, qualitative for amino acids)

[d] See Appendix J

[e] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)— avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase (OTC) deficiency—low protein diet, complex medications, liver transplantation for severe cases; non-ketotic hyperglycinaemia (NKH)—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some cases, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDC deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GA1; OTC deficiency; MMA; and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis

[f] Re-testing may be required for example where testing has failed or the results are inconclusive

At-risk family members of patients with specific genetic metabolic diseases

The PPICO criteria (target population, prior tests, index test, comparator, outcomes) to conduct UMP assessment of at-risk family members of patients with specific genetic metabolic diseases are provided in Table 5.

Table 5 PPICO criteria to conduct urinary metabolic profiling for at-risk family members of patients with specific genetic metabolic diseases

Population	Prior tests	Intervention/ test	Comparator	Reference standard	Outcomes
At-risk family members of a patient with a specific genetic metabolic disease	No prior tests	UMP[a]: by HPLC, GC/MS, TLC, HVE, MS/MS	UMP not available: See Appendix I for alternative tests	Clinical follow-up, molecular genetic analyses, enzyme analyses	Change in clinical outcomes[b] Change in clinical management[c] Diagnostic accuracy[d] Safety outcomes[e]

Abbreviations: GC/MS, gas chromatography -mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] HPLC, quantitative for amino acids; GC/MS, semi-quantitative for organic acids; HVE, qualitative for amino acids; MS/MS, quantitative for amino acids; TLC, qualitative for amino and organic acids

[b] Survival (overall survival); morbidity (disease progression)

[c] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)—avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase deficiency—low protein diet, complex medications, liver transplantation for severe cases; non-ketotic hyperglycinaemia (NKH)—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some cases, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDC deficiency, vitamin cocktails possibly useful· Genetic counselling for pre-natal diagnosis is a component of the management of GA1; OTC deficiency; MMA; and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis

[d] Sensitivity and specificity estimates; positive and negative likelihood ratios; summary diagnostic measures (eg diagnostic odds ratio, summary receiver operating characteristics)

[e] Safety outcomes were not considered to be applicable because UMP is non-invasive that requires urine samples only from patients

The research question for this indication, based on these criteria, was as follows.

To what extent is urinary metabolic profiling (UMP):

- safe,
- effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and
- cost-effective

in the assessment of genetic metabolic disorder among at-risk family members of patients with specific genetic metabolic diseases, in comparison with alternative tests when UMP is not available?

The clinical pathway to conduct UMP for at-risk family members of patients with specific genetic metabolic disorders is shown in Figure 8. This flowchart indicates the clinical management pathway to the point of patient diagnosis.

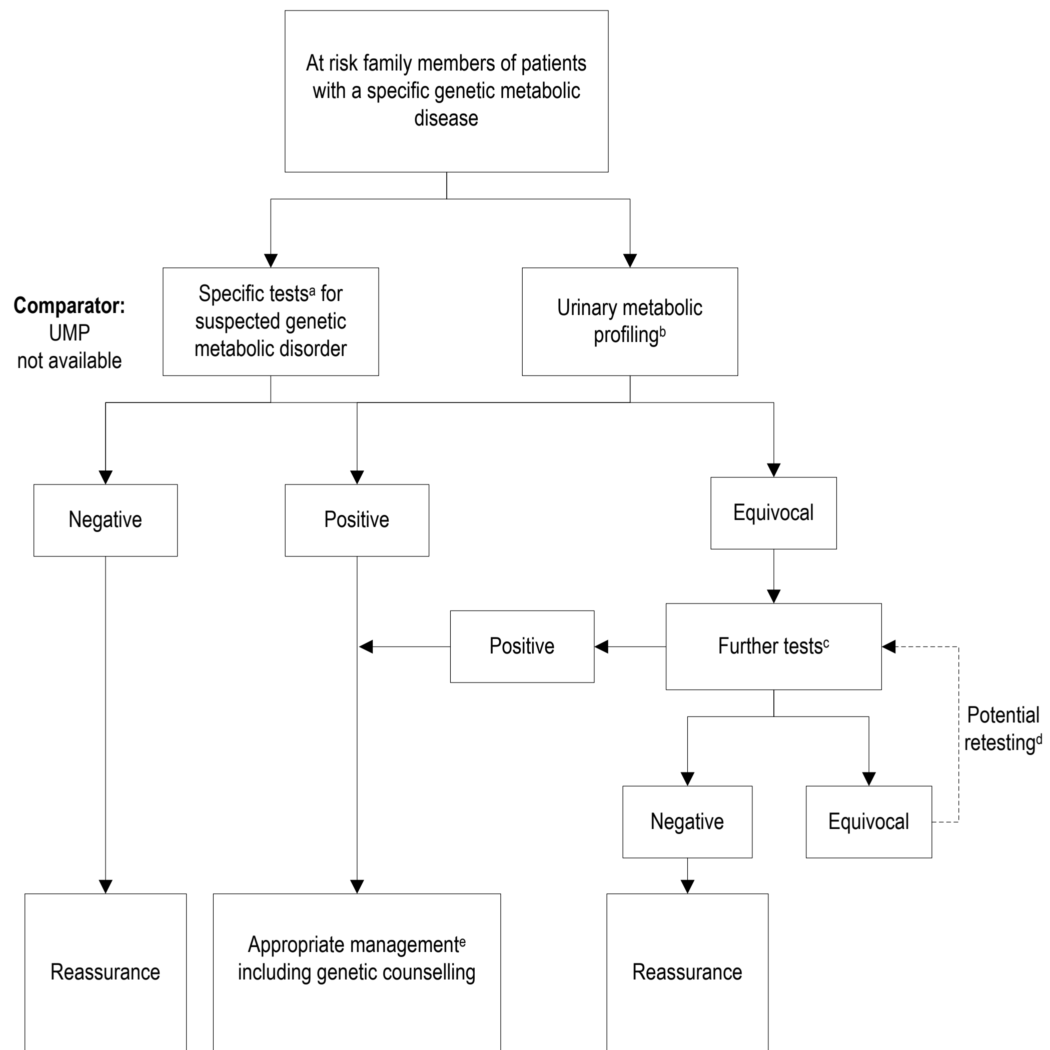

Figure 8 Clinical pathway to conduct urinary metabolic profiling for at-risk family members of patients with specific genetic metabolic disorders

[a] See Appendix I

[b] High performance liquid chromatography (HPLC, quantitative for amino acids); gas chromatography/mass spectrometry (GC/MS, semi-quantitative for organic acids); high voltage electrophoresis (HVE, qualitative for amino acids); tandem mass spectrometry (MS/MS, quantitative for amino acids); thin layer chromatography (TLC, qualitative for amino acids)

[c] See Appendix J

[d] Re-testing may be required for example where testing has failed or the results are inconclusive

[e] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)— avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase (OTC) deficiency—low protein diet, complex medications, liver transplantation for severe cases; non-ketotic hyperglycinaemia (NKH)—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some cases, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDC deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GA1; OTC deficiency; MMA; and MSUD. Genetic counselling is also a component of cystinuria, homocystinuria and lactic acidosis management

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Assessment framework

Types of evidence

A systematic review of the literature was undertaken to identify relevant studies that examined the value of UMP for assessment of suspected metabolic disorders in newborns, individuals with clinical presentations and/or test results suggestive of genetic metabolic disorders, and at-risk family members of patients with specific genetic metabolic disorders. Direct evidence regarding the impact of UMP on health outcomes was sought. The literature search was not limited by outcomes or comparators. In the absence of studies providing direct evidence, indirect evidence indicating the impact of UMP on clinical management and diagnostic accuracy was assessed.

Review of the literature

The medical literature was searched to identify all relevant studies and reviews published before late 2007.

Search strategy

Primary databases

Searches were conducted in the primary databases indicated in Table 6.

Table 6 Electronic databases searched during the review of UMP for assessment of suspected metabolic disorders

Database	Date searched
Medline and EMBASE[a]	25 October 2007
PreMedline[b]	18 December 2007
Cochrane Library	1 November 2007

[a] Search performed using the EMBASE.com interface
[b] Search performed using the PubMed interface on 1 November 2007 and repeated when checking identified an inaccuracy in search logic
Search terms were limited to metabolic disorders and metabolites

The search terms included the following (as determined from PPICO criteria):

- thin layer chromatography, high voltage electrophoresis, amino acid analysis, tandem mass spectrometry, gas chromatography, and high performance liquid chromatography

- cystinuria, medium-chain acyl-CoA dehydrogenase deficiency, aciduria, glutaric aciduria, disorders of amino acid and protein metabolism, methylmalonic acid, homocystinuria, maple syrup urine disease, lactic acidosis, ornithine carbamoyltransferase and hyperglycinemia

- cystine, arginine, ornithine, acylcarnitines, acylglycine, glutaric acid, homocystine, methionine, methylmalonic acid, and orotic acid.

Complete details of the literature searches performed using the primary databases are presented in Appendix F.

Secondary databases

A review of databases maintained by health technology assessment (HTA) agencies was undertaken to identify existing UMP reports. The list of secondary databases searched is presented in Appendix F.

Additional searches were conducted to source quality of life, epidemiological and economic information, as required.

Citation lists

The citation lists of included studies were searched to identify any additional studies.

Selection criteria

Selection criteria (Table 7, Table 8 and Table 9) were applied to the citations identified in the literature search results. Studies that did not meet specified inclusion criteria were excluded from further analysis.

Table 7 Selection criteria for identifying relevant studies assessing urinary metabolic profiling for diagnosis of metabolic disorders in asymptomatic newborns following routine screening tests

Characteristic	Inclusion criteria	Exclusion criteria
Publication type	Clinical studies included	Non-systematic reviews, letters, editorials, animal, in-vitro and laboratory studies will be excluded Case studies
Patient	Asymptomatic newborns with a positive screening result[a]	Wrong population
Intervention/test	UMP[b] by HPLC, GC/MS, TLC, HVE, MS/MS	Wrong technology Wrong usage Not UMP
Comparators	Clinical follow-up, molecular genetic analyses, enzyme analyses[c]	
Reference standard[c]	Alternative tests when UMP is considered not available: see Appendix I	Inadequate reporting of reference standard
Outcome	Change in clinical outcomes Change in clinical management Diagnostic accuracy	Wrong outcome Inadequate data reporting
Language	Non-English language articles will be excluded unless they are deemed necessary to the review. Translation of such articles will significantly increase the timeframe of the review	

Abbreviations: GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography; UMP, urinary metabolic profiling
[a] MS/MS result indicative of possible (genetic) metabolic disorder; manual chemical fluorescence test for galactose metabolites (to detect galactosaemias): this test is not performed in Victoria
[b] HPLC, quantitative for amino acids; GC/MS, semi-quantitative for organic acids; HVE, qualitative for amino acids; MS/MS, quantitative for amino acids; TLC, qualitative for amino acids
[c] Only applicable to diagnostic test studies

Table 8 Selection criteria to identify relevant studies that assessed the value of urinary metabolic profiling of individuals with clinical presentations and/or test results suggestive of genetic metabolic disorder

Characteristic	Inclusion criteria	Exclusion criteria
Publication type	Clinical studies included	Non-systematic reviews, letters, editorials, animal, *in vitro* and laboratory studies will be excluded
Patient	Individuals with a clinical presentation[a] and/or test[b] results suggestive of genetic metabolic disorder	Wrong population
Intervention/test	UMP[c] by HPLC, GC/MS, TLC, HVE, MS/MS	Wrong technology Wrong usage Not UMP
Comparators	Clinical follow-up, molecular genetic analyses, enzyme analyses	
Reference standard[d]	Alternative tests when UMP is considered not available (see Appendix I)	Inadequate reporting of reference standard
Outcome	Change in clinical outcomes Change in clinical management Diagnostic accuracy	Wrong outcome Inadequate data reporting
Language	Non-English language articles will be excluded unless they are deemed necessary to the review. Translation of non-English articles significantly increases review timeframes	

Abbreviations: GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] Possible symptoms include encephalopathy, seizures, hypotonia, dystonia, spasticity, vomiting, failure to thrive, jaundice and other signs of liver failure, cardiomyopathy, rhabdomyolysis and renal calculi, skeletal abnormalities, dislocation of lenses, abnormal hair. This list is not exhaustive because of the wide range of possible symptoms that may be caused by genetic metabolic disorders. Examples of specific symptoms highly suggestive of a metabolic disorder in an acute presentation include clinically unexplained encephalopathy; coma and increased respirations, with metabolic acidosis

[b] Tests used will vary depending on the particular symptoms, but routine investigations include: blood tests for electrolytes, glucose, ammonia, lactate, acid base, urea, creatinine, calcium, magnesium, phosphorus, creatine kinase, lead; liver function tests; full blood count; thyroid function tests, imaging abdominal ultrasound; magnetic resonance imaging of the head , skeletal survey; electroencephalogram for patients with seizures. This is not an exhaustive list because of the wide range of possible symptoms that may be encountered in clinical presentations suggestive of genetic metabolic disorder

[c] HPLC, quantitative for amino acids; GC/MS, semi-quantitative for organic acids; HVE, qualitative for amino acids; MS/MS, quantitative for amino acids; TLC, qualitative for amino acids.

[d] Applicable only to diagnostic test studies

Table 9 Selection criteria for studies of urinary metabolic profiling at-risk family members of patients with a specific genetic metabolic disorder

Characteristic	Inclusion criteria	Exclusion criteria
Publication type	Clinical studies included	Non-systematic reviews, letters, editorials, animal, *in vitro* and laboratory studies will be excluded Case studies
Patient	At-risk family members of patients with a specific genetic metabolic disorder[a]	Wrong population
Intervention/test	UMP[b] by HPLC, GC/MS, TLC, HVE, MS/MS	Wrong technology Wrong usage Not UMP
Comparators	Clinical follow-up, molecular genetic analyses, enzyme analyses	
Reference standard[c]	Alternative tests when UMP is considered not available (see Appendix I)	Inadequate reporting of reference standard
Outcome	Change in clinical outcomes Change in clinical management Diagnostic accuracy	Wrong outcome Inadequate data reporting
Language	Non-English language articles will be excluded unless they are deemed necessary to the review. Translation of non-English articles significantly increases review timeframes	

Abbreviations: GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] After advisory panel consultation it was decided that it would be more appropriate to focus this assessment on the most common genetic metabolic disorders because many are extremely rare. The most common genetic metabolic disorders considered were cystinuria, medium-chain acyl-CoA dehydrogenase deficiency (MCADD), glutaric aciduria type I (GA1), ornithine transcarbamylase(OTC) deficiency, non-ketotic hyperglycinaemia (NKH), homocystinuria (CβS deficiency) ,methylmalonic aciduria (MMA), maple syrup urine disease (MSUD), and lactic acidosis

[b] HPLC, quantitative for amino acids, GC/MS, semi-quantitative for organic acids, HVE, qualitative for amino acids, MS/MS, quantitative for amino acids, TLC, qualitative for amino acids.

[c] Applicable only to diagnostic test studies

Search results

The QUOROM (quality of reporting of meta-analyses) flowchart (Figure 9) summarises reasons for exclusion of studies. A total of 1434 non-duplicate references were identified: 113 were reviewed for evidence of the clinical effectiveness of UMP.

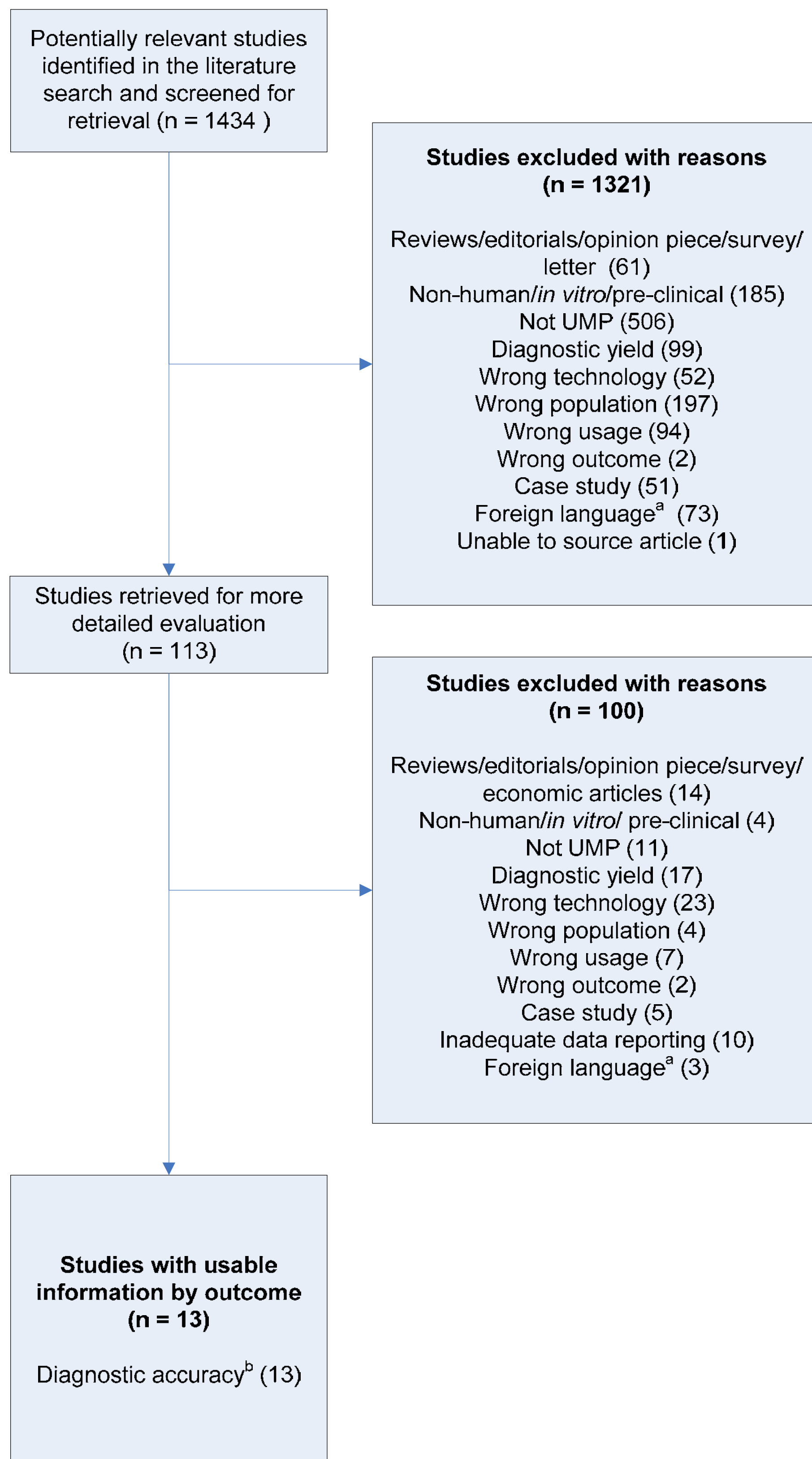

Figure 9 **QUOROM flowchart used to identify and select studies for the literature review of urinary metabolic profiling**

[a] These studies were not reviewed due to time limitations
[b] One identified study was a subset of another study
Adapted from Moher et al (1999)

Study appraisal

Direct evidence indicating the value of UMP relative to current clinical practice, when used in the relevant patient group, is required to justify public funding. Evidence should ideally be in the form of studies reporting effects on patient-centred health outcomes. Alternatively, evidence of greater diagnostic accuracy than the comparator, along with linked evidence indicating changes in management and confirmation that treatment will affect health outcomes is required.

Where an additional diagnostic test is to be used in the clinical pathway, evidence of an effect on management change is a key component of the evidence base. The most appropriate design for investigation of the effects on management change is a pre-test/post-test case series study. Where a pre-test management plan is not reported, the outcomes of a study do not truly represent change in patient management and outcomes are likely to be biased.

The ideal design for a study of the comparative accuracy of diagnostic tests is one in which each test is performed in a population with a defined clinical presentation, in a consecutive series. The study should be an independent, blinded comparison with a valid reference standard (NHMRC 2005).

Assessment of eligible studies

Evidence retrieved from the literature searches were assessed according to the NHMRC dimensions of evidence (Table 10) where applicable. There are three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified for a particular diagnostic test. The other two domains require expert clinical input as part of the determination process.

An aspect of the strength of the evidence domain is the level of evidence of the study, which was assigned using the NHMRC levels of evidence outlined in Table 11.
The quality and applicability of the included studies was assessed according to pre-specified criteria (Appendix C).

Table 10 Dimensions of evidence

Type of evidence	Definition
Strength of the evidence	
Level	The study design used, as an indicator of the degree to which bias has been eliminated by design [a]
Quality	The methods used by investigators to minimise bias within a study design
Statistical precision	The *p* value, or alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect
Size of effect	The distance of the study estimate from the null value and the inclusion of only clinically important effects in the confidence interval
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used

Source: NHMRC (2005)
[a] See Table 11

Table 11 Designations of levels of evidence according to type of research question

Level	Intervention [b]	Diagnosis[e]
I[a]	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[f] among consecutive patients with a defined clinical presentation [g]
III-1	A pseudo-randomised controlled trial (ie alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard [f] among non-consecutive patients with a defined clinical presentation [g]
III-2	A comparative study with concurrent controls: Non-randomised, experimental trial [c] Cohort study Case-control study Interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for level II and III-1 evidence
III-3	A comparative study without concurrent controls: Historical control study Two or more single arm study [d] Interrupted time series without a parallel control group	Diagnostic case-control study [g]
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard) [h]

Source: NHMRC (2005)

[a] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence

[b] Definitions of these study designs are provided on pages 7–8 How to use the evidence: assessment and application of scientific evidence (NHMRC 2000b)

[c] This also includes controlled before-and-after (pre-test/post-test) studies, as well as indirect comparisons (ie. utilise A vs. B and B vs. C, to determine A vs. C)

[d] Comparing single arm studies ie case series from two studies

[e] The dimensions of evidence apply only to studies of diagnostic accuracy. To assess the effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes. See: Medical Services Advisory Committee. 2005. *Guidelines for the assessment of diagnostic technologies*. Canberra: Commonwealth of Australia. Available at: www.msac.gov.au

[f] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study. See Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. 2003. 'The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews'. *BMC Medical Research Methodology* 3: 25

[g] Well-designed population based case-control studies (eg. population based screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease are compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease and conditions mimicking the disease are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias because the spectrum of study participants will not be representative of patients seen in practice

[h] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternative when there is no reliable reference standard

Note 1: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms are rare and cannot feasibly be captured within randomised controlled trials; physical harms and psychological harms may need to be addressed by different study designs; harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results

Note 2: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question eg level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence

The quality of studies of diagnostic accuracy was ranked using the composite grading system described in the assessment of studies of diagnostic accuracy guidelines (Table 12). In accordance with MSAC guidelines, studies of diagnostic accuracy are described according to the extent that they achieve the component factors of study validity.

Table 12 **Grading system for the appraisal of studies evaluating diagnostic tests**

Validity criteria	Description	Grading system
Appropriate comparison	Did the study evaluate a direct comparison of the index test strategy versus the comparator test strategy?	C1 direct comparison CX other comparison
Applicable population	Did the study evaluate the index test in a population that is representative of the subject characteristics (age and sex) and clinical setting (disease prevalence, disease severity, referral filter and sequence of tests) for the clinical indication of interest?	P1 applicable P2 limited P3 different population
Quality of study	Was the study designed to avoid bias?	
	High quality = no potential for bias based on pre-defined key quality criteria	Q1: high quality
	Fair quality = some potential for bias in areas other than those pre-specified as key criteria	Q2: fair quality
	Poor quality = poor reference standard and/or potential for bias based on key pre-specified criteria	Q3: poor quality

Source: Medical Services Advisory Committee. 2005. *Guidelines for the assessment of diagnostic technologies*. Canberra: Commonwealth of Australia

Data analysis

Data extraction

A single reviewer extracted relevant information using a standardised data extraction form designed specifically for this assessment. Any uncertainties were resolved by discussion with another reviewer.

Measurement of test accuracy

Evaluating the accuracy of diagnostic tests involves comparing a new test with its comparators and a reference standard—the best available proxy for the true condition status. The new diagnostic test and its comparators can be independently compared with the reference standard to determine measures of test accuracy such as sensitivity, specificity and predictive values.

	Reference standard	
Index test	Disease positive	Disease negative
	True positive	False positive
	False negative	True negative

Figure 10 **Data used to calculate measures of test accuracy**

- True positive = number of patients who test positive and are classified positive by the reference standard
- False negative = number of patients who test negative and are classified positive by the reference standard

- False positive = number of patients who test positive and are classified negative by the reference standard
- True negative = number of patients who test negative and are classified negative by the reference standard.

Sensitivity is defined as the proportion of all patients with a specified condition whose results are determined to be positive according to the index test. Sensitivity is calculated by dividing the true positive (TP) value by the sum of the true positive and the false negative (FN):

$$\frac{TP}{TP+FN}$$

Specificity is the proportion of all patients, who do not have the specified condition, whose results are negative according to the index test. Specificity is calculated by dividing the true negative (TN) by the sum of the true negative and false positive (FP):

$$\frac{TN}{TN+FP}$$

The *positive predictive value* (PPV) is the proportion of patients with a positive index test result who have the specified condition. The equation to calculate PPV is:

$$\frac{TP}{TP+FP}$$

The *negative predictive value* (NPV) of a test is the proportion of patients with a negative index test result who do not have the specified condition. The equation to calculate NPV is:

$$\frac{TN}{TN+FN}$$

Expert advice

An advisory panel with expertise in general practice, medical genetics, biochemistry and consumer affairs, was established to evaluate the evidence and provide advice to MSAC from the clinical perspective. In selecting members for advisory panels, MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations and consumer bodies for nominees. Membership of the advisory panel is provided in Appendix B.

Assessment of the body of evidence

The overall body of evidence and individual studies were assessed. An evidence level from A (excellent) to D (poor) was assigned after considering each of the components outlined in the body of evidence matrix presented in Table 13.

Table 13 Body of evidence assessment matrix

Component	A Excellent	B Good	C Satisfactory	D Poor
Volume of evidence	Several level I or II studies with low risk of bias	One or two level II studies with low risk of bias or a systematic review/multiple level III studies with low risk of bias	Level III studies with low risk of bias, or level I or II studies with moderate risk of bias	Level IV studies, or level I to III studies with high risk of bias
Consistency	All studies consistent	Most studies consistent and inconsistency may be explained	Some inconsistency reflecting genuine uncertainty around clinical question	Evidence is inconsistent
Clinical impact	Very large	Substantial	Moderate	Slight or restricted
Generalisability	Population/s studies in body of evidence are the same as the target population	Population/s studies in the body of evidence are similar to the target population	Population/s studied in body of evidence different to target population but it is clinically sensible to apply this evidence to the target population	Population/s studied in body of evidence different to target population and hard to judge whether it is sensible to generalise to target population
Applicability	Directly applicable to Australian healthcare context	Applicable to Australian healthcare context with few caveats	Probably applicable to Australian healthcare context with some caveats	Not applicable to Australian healthcare context

Source: National Health and Medical Research Council. 2005. *NHMRC additional levels of evidence and grades for recommendations for developers of guidelines: Pilot Program 2005–2006.* Canberra: NHMRC. Available from: www.nhmrc.gov.au/consult/docfeedback.htm

Results of assessment

Summary

A linked evidence approach was applied to evaluate the diagnostic effectiveness of urinary metabolic profiling (UMP) and its impact on patient management and treatment. The UMP test procedure is not considered to have any safety issues for patients.

There were 13 studies identified for inclusion in the analysis (Bonafé et al 2000, Bonham Carter et al 1991, Carpenter et al 2001, Costa et al 2000, Giugliani et al 1987, Kuhara et al 2001, Korman et al 2007, Landaas and Jakobs 1977, Paik et al 2005, Pitt 1993, Schor et al 2002, Tsai et al 1980, Waddell et al 2006). Studies by Carpenter et al (2001) and Waddell et al (2006) enabled consideration of the role of UMP in the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency (MCADD) in screened newborns. Korman et al (2007), whose study investigated UMP for detection of glutaric aciduria type I (GA1), enabled comparison of UMP to be made with a pre-specified comparator test. These studies were considered to provide significant evidence to support use of UMP for diagnosing inherited metabolic disorders in newborns whose screening results are positive (Carpenter et al 2001, Waddell et al 2006) and symptomatic patients (Korman et al 2007). Results provided limited evidence because the studies were not designed as diagnostic test investigations or were direct comparisons with other tests. For the purpose of assessing the diagnostic performance of UMP, Korman et al (2007) was subject to selection bias because patient inclusion was based on the results of the reference standard.

The other identified studies enabled comparions to be made between UMP results from patients with previously diagnosed inborn errors of metabolism (IEMs) with UMP results from healthy controls (Landaas and Jakobs 1977, Tsai et al 1980, Giugliani et al 1987, Bonham Carter et al 1991, Pitt 1993, Bonafé et al 2000, Costa et al 2000, Kuhara et al 2001, Schor et al 2002, Paik et al 2005). Overall, results indicated that UMP can be considered a reliable form of diagnosis for the IEMs in focus, although the reliability of evidence was limited by study design. Inherent spectrum bias, introduced by including patients with known IEMs, and deficiencies in blinding samples to researchers, were common problems among many of these studies. Overall, evidence quality was poor.

In addition to the main body of studies identified for this assessment, data were sourced from a quality assurance/educational program conducted by the Australasian Society for Inborn Errors of Metabolism. This program involved blinded reference samples of urine from patients with known IEMs being sent to all six Australian laboratories currently undertaking UMP testing. Of the six laboratories, five correctly diagnosed all disorders, including reaching negative diagnoses for samples from patients who were free of IEMs. Only one laboratory obtained a single false negative result (for ornithine transcarbamylase deficiency). These findings appear to support the use of UMP for detection of IEMs. Evidence from this program was limited by the small number of samples for each IEM tested by the laboratories. Details about patients who provided samples, technologies used by the laboratories, and test positivity criteria were not reported. The case-control study design precluded determination of how well UMP would perform in IEM diagnosis where conditions were suspected but not been pre-diagnosed. Based on these results it was not possible to categorically conclude if UMP is a highly specific and sensitive test to diagnose the IEMs examined in this assessment.

Is it safe?

Urinary metabolic profiling (UMP) is a non-invasive test conducted on urine samples. The UMP test procedure is not considered to present safety issues for patients.

Is it effective?

Linked evidence

A linked evidence approach was undertaken to evaluate the diagnostic effectiveness of UMP and its impact on patient management and treatment.

A linked evidence approach was necessary because direct evidence indicating the impact of UMP on health outcomes was not available.

Diagnostic accuracy

Linked evidence indicating the diagnostic effectiveness of UMP was sought for three patient populations:

- asymptomatic newborns with positive screening results
- patients with clinical presentations suggestive of genetic metabolic disorders
- at-risk family members of patients with specific genetic metabolic disorders.

Studies by Carpenter et al (2001), Waddell et al (2006) and Korman et al (2007) enabled comparisons to be made between UMP performance and a comparator test. Carpenter et al (2001) and Waddell et al (2006) reported data that enabled assessment of UMP used to investigate suspected metabolic disorders in newborns. Carpenter et al (2001) reported on a subset of the data set reported by Waddell et al (2006). The study by Carpenter et al (2001) is considered to better represent routine newborn screening and subsequent UMP than described Waddell et al (2006) (expert opinion, advisory panel). The main focus of the study by Waddell et al (2006) was investigation of genotype-phenotype correlations. Korman et al (2007) reported UMP use to investigate suspected metabolic disorders in symptomatic patients.

The literature search also identified 10 non-comparative studies that evaluated technologies used for UMP among patients with known inborn errors of metabolism (IEMs) and control subjects (Landaas and Jakobs 1977, Tsai et al 1980, Giugliani et al 1987, Bonham Carter et al 1991, Pitt 1993, Bonafé et al 2000, Costa et al 2000, Kuhara et al 2001, Schor et al 2002, Paik et al 2005).

Landaas and Jakobs (1977) conducted UMP using gas chromatography/mass spectrometry (GC/MS) for patients with lactic acidosis and ketoacidosis. Tsai et al (1980) measured urine amino acid levels using high performance liquid chromatography (HPLC). Giugliani et al (1987) applied thin layer chromatography (TLC) to analyse amino acid levels in patients with cystinuria. Bonham Carter et al (1991) conducted UMP using GC/MS for patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD).

Pitt (1993) analysed acylglycines in the urine of patients with MCADD using GC/MS. Bonafé et al (2000) used tandem mass spectrometry (MS/MS) to analyse urinary acylglycines in patients with proven MCADD, methylmalonic aciduria (MMA) and glutaric aciduria type I (GA1). Costa et al (2000) used GC/MS for patients with MCADD. Kuhara et al (2001) applied GC/MS to conduct UMP for patients with homocystinuria. Schor et al (2002) performed UMP using GC/MS for patients with GA1. GC/MS analysis was used by Paik et al (2005) to profile urinary amino and organic acids. Characteristics of all included studies are presented in Table 14.

Inadequate data reporting in these 10 non-comparative studies meant that it was not possible to determine how patients were identified initially. It is possible that patients selected for inclusion in these studies were initially identified following newborn screening, by clinical presentation, or were at-risk family members. The results of these studies are regarded as providing evidence to indicate the effectiveness of UMP in all three patient populations considered in this assessment.

Table 14 Characteristics of included studies

Author (year) Country	Study design	Patient characteristics (n)	Test characteristics	Quality and applicability
Bonafé et al (2000) Argentina, Italy, Switzerland	Case-control Blinding, NR[a]	**Cases**: Urine samples were collected from patients with known MCADD (n = 5 patients, 15 samples; age range, NR; gender, NR) Details of tests used to diagnose MCADD, NR **Controls**: Details, NR (n = 54 samples, number of patients, NR; age range = < 1 week–15 years; gender, NR). Details of control selection, NR	**Urine sample**: Sampling time, NR; samples were derivatised to acylglycine methylesters, acylglycine butylesters or acetylated acylglycine butylesters **Instrumentation**: ESI-MS/MS was carried out on a Perkin-Elmer SCIEX API 365 LC/MS/MS system **Internal standard**: 50 µL of internal standard contained 150 ng d3-acetylglycine, 20 ng d3-propionylglycine, 30 ng d7-butyrylglycine, 30 ng d9-valerylglycine and 20 ng d3-heanoylglycine in methanol; all internal standards were 2H-labelled in the acyl group **Creatinine measurement**: Creatinine concentrations were measured by the Jafe-modified kinetic method by a Beckman Synchron CX5 analyser **Reference standard**: NR	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported **Applicability**: Applicable
Bonham Carter et al (1991) UK	Case-control Blinding, NR[a]	**Cases**: Urine samples were collected from all members of one family in which a child was known to have MCADD (n = 9 family members, 3 with MCADD whose ages ranged from 6 months to 14 years; age range of those without MCADD was 2 to 46 years) Details of tests used to diagnose MCADD, NR **Controls**: n = 29; aged 5 months to 56 years; details of control selection, NR	**Urine sample**: Sampling time, NR; urine was acidified to pH 1–2 with 0.1 mL of 2 M HCl, saturated with NaCl and extracted with ethyl acetate **Instrumentation**: HP capillary GC interfaced to HP MS (model 5988A). The GC was fitted with a BP1 aluminium or polyimide clad fused silica column (12 or 25 m x 0.22 mm ID) **Internal standard**: Known amounts of the acyl $^{13}C_2$-glycines (100–500 ng) **Creatinine measurement**: All samples were analysed for creatinine using a creatinine kit based on complex formation with picric acid **Reference standard**: NR	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported **Applicability**: Applicable

Author (year) Country	Study design	Patient characteristics (n)	Test characteristics	Quality and applicability
Carpenter et al (2000) Australia	Prospective cohort Consecutive recruitment Blinding, NR[a] April 1998 to March 2001	Newborns with initial octanoylcarnitine levels ≥1 µmol/L on newborn screening by MS/MS who had further analysis of urinary organic acids for investigation of suspected MCADD Median age at screening was 3 d; >99% of newborns sampled before day 6 (n = 17[b] out of 275,653 consecutive neonates undergoing newborn screening)	**Urine sample:** Sampling time, centrifugation and/or filtration and pH adjustment, details NR; organic acids extracted with ethyl acetate and converted to trimethylesters **Instrumentation:** Hewlett Packard 5890 GC and 5971 MSD; reference compounds, NR **Internal standard:** NR **Creatinine measurement:** NR **Additional tests:** Mutation analysis, test details NR Plasma carnitine analysis; test details, NR	NA
Costa et al (2000) Portugal & Netherlands	Case-control Blinding, NR[a]	**Cases:** Patients with proven MCADD (n = 10) Details of tests used to diagnose MCADD, NR **Controls:** Children unaffected by IEM (age range 2 days to 12 years; n = 19) Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling time NR; pH adjusted to 1–2 with 2 N HCl and saturation with NaCl; details of sample filtration or centrifugation, NR **Instrumentation:** GC/MS—610 ATI-Unicam GC coupled to an Automass series 1 ATI-Unicam MS (Cambridge); reference compounds, details, NR **Internal standard:** [$^{2}H_{7}$]BG, [$^{2}H_{7}$]IBG, [$^{13}C_{2}$]2MBG, [$^{2}H_{3}$]IVG, [$^{13}C_{2}$]HG, [2-^{13}C,^{15}N]SG and [2-^{13}C,^{15}N]PPG **Creatinine measurement:** Details, NR **Reference standard:** Tests used to diagnose MCAD; details, NR	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported **Applicability**: Applicable
Giugliani et al (1987) Brazil	Case-control Blinding, NR[a]	**Cases:** Cystinuria heterozygotes (age range, NR; n = 32) Tested positive on prior HPLC **Controls:** Normal phenotype, (age range NR, n = 23) Unclear as to whether controls were type I or non-cystinuric patients Prior testing by HPLC, unclear	**Urine sample:** Sampling time random, details NR; 6 mol/L HCL acidification; details of sample filtration or centrifugation, NR **Instrumentation:** TLC; details, NR; reference compounds, details, NR **Internal standard:** NR **Creatinine measurement:** Details, NR **Reference standard:** Cases—prior HPLC, with automatic amino acid analyser; test details, NR	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Potential spectrum bias Inadequate reference standard **Applicability**: Applicable

Author (year) Country	Study design	Patient characteristics (n)	Test characteristics	Quality and applicability
Korman et al (2007) Israel	Direction unclear Consecutive recruitment, unclear Blinding, NR[a] 5 year period ending mid-2004	Patients diagnosed with GA1 Investigated in response to: acute encephalopathy (n = 3); neurological symptoms (n = 6); macrocephaly and history of mother's pregnancy termination due to anencephaly and congenital abnormalities (n = 1); macrocephaly and suspicion of GA1 (n = 1); and close family relative of known GA1 patient (n = 1) (age range 7 days to 30 years) (n = 12)	**Urine sample:** Ethylacetate/diethyl extraction and trimethylsilyl derivatisation; sample timing, NR **Instrumentation** GC/MS; details of system and running methods NR **Internal standard:** Details, NR **Creatinine measurement:** Details, NR **Reference standard:** Mutational analysis of GCDH gene on genomic DNA from peripheral blood; 11 exons and flanking intronic regions of GCDH gene analysed by PCR, further details, NR **Comparator test:** Blood spot analysed for acylcarnitine by MS/MS, further details NR	NHMRC III-2 C1, Q3, P1 **Quality***:* Poor Selection bias No blinding Review bias **Applicability**: Applicable
Kuhara et al (2000) Japan	Case-control Blinding, NR[a]	**Cases**: Symptomatic homocystinuria patients (age range, 4 to 20 years; n = 4) Prior tests for case selection: 2 unclear, 1 diagnosed enzymatically (details, NR), 1 diagnosed enzymatically and using UMP(details, NR) **Controls**: Normal based on neonatal screening tests (age range, NR; n = NR) Testing used during neonatal screening, unclear	**Urine sample**: Sampling time, NR; samples were treated with urease at 37°C for 10 min to remove excess urea, deproteinised with ethanol, centrifuged to remove precipitate and trimethylsilylated by adding a BSFTA and TMCS mixture then heating at 80°C for 30 min **Instrumentation**: GC/MS using bench-top HP GC-MSD (HP6890/MSD 5973). Separation performed on a fused-silica DB-5 (30 m x 0.25 mm ID) with a 0.25 μm film thickness of 5% phenylmethylsilicone **Internal standard**: Known amounts of stable, isotope-labelled creatinine, methionine, cystine, homocystine, uracil, orotate and methylmalonate **Creatinine measurement**: Using d3-creatinine as an internal standard, the value of endogenous creatinine and creatine were determined and the evaluation of metabolite levels relative to creatinine was reported **Reference standard**: Details, NR	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Potential spectrum bias Inadequate reference standard **Applicability**: Applicable

Author (year) Country	Study design	Patient characteristics (n)	Test characteristics	Quality and applicability
Landaas & Jakobs (1977) Norway & Germany	Case-control Blinding, NR[a]	**Cases:** Patients acutely ill with lactic acidosis or combined lactic and ketoacidosis (age range 1 to 27 years; n = 5) Details of tests used to diagnose patients, NR **Controls:** Healthy members of study laboratory staff (age range 21 to 30 years; n = 9) Details of tests to confirm absence of IEM, NR	**Urine sample:** 24 hour collections not made due to rapid change in clinical state of patients; adjusted to pH 1 by 6 M HCl; details of sample filtration or centrifugation, NR **Instrumentation:** GC/MS—Varian 1440 gas chromatograph with Varian 112 MS; Varian 2740 CG with double focusing MS (Varian MAT, type 311 A); reference compounds, details NR **Internal standard:** Malonic acid **Creatinine measurement:** Details, NR **Reference standard:** Details of tests used to diagnose cases and confirm absence of IEM in controls, NR	NHMRC III-3 CX, Q3, P1 **Quality***:* Poor Potential spectrum bias Inadequate reporting: details of tests used to select cases and controls not reported **Applicability***:* Applicable
Paik et al (2005) South Korea	Case-control Blinding, NR[a]	**Cases:** Patient with MSUD (age unclear; n = 1), MMA (age unclear; n = 1) Details of tests used to diagnose patients, NR **Controls:** Normal individuals (age range, 0.25–1.25 years, n = 3) Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling time, NR; samples (equivalent to 0.25 mg creatinine) adjusted to pH ≥12 after internal addition of standard; samples subjected to sequential EOC/MO/TBDMS reactions; details of sample filtration or centrifugation, NR **Instrumentation:** GC/MS—Agilent 6890 GC interfaced to Agilent 5973 mass selective detector reference compounds[c] **Internal standard:** Details, NR **Creatinine measurement:** Details, NR **Reference standard:** Details of tests used to diagnose cases and confirm absence of IEM in controls, NR	NHMRC III-3 CX, Q3, P1 **Quality***:* Poor Details of tests used to diagnose cases and confirm absence of IEM in control patients not reported One case for each IEM **Applicability***:* Applicable

Author (year) Country	Study design	Patient characteristics (n)	Test characteristics	Quality and applicability
Pitt (1993) Australia	Case-control Blinding, NR[a]	**Cases:** MCADD subjects (age range, NR; n = 8) Diagnoses confirmed by DNA mutation analysis and/or a positive phenylpropionate loading test; further details of both tests, NR **Controls:** Subjects typically with mild developmental delay (similar age to MCADD subjects; age range NR; n = 15) Metabolic screens normal; test details, NR	**Urine sample:** Sampling time random, details NR. Details centrifugation and/or filtration, NR, Urine acidified with 6 mol/L HCl; organic acids converted to TMS derivatives **Instrumentation:** GC/MS, instrument details NR. Reference compounds: IG, HG, 4HMG, 7HOG, 8HOG, SG **Internal standard:** details NR **Creatinine measurement:** Details, NR **Reference standard:** Cases—diagnoses confirmed by DNA mutation analysis and/or a positive phenylpropionate loading test; further details of both tests, NR	NHMRC III-3 CX, Q2, P1 **Quality**: Medium Spectrum bias **Applicability**: Applicable
Schor et al (2002) Netherlands	Case-control Blinding, NR[a]	**Case:** Patient with GA1 (age, NR) GCDH deficiency confirmed enzymatically; test details, NR **Controls:** Children unaffected by IEM (age range, NR; n = 12) Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling time, NR; adjusted to pH <1 by 6 M HCl; saturated with NaCl and centrifuged—supernatant applied to OASIS SPE column, 3-HGA eluate converted to triflouroacetyl-di-PFB-3-HGA **Instrumentation:** GC/MS—Hewlett Packard 5890 series GC, Hewlett Packard 5989B MS; reference compounds, details, NR **Internal standard:** $^{2}H_{4}$-3-HGA **Creatinine measurement:** Details, NR **Reference standard:** Cases—GCDH deficiency confirmed enzymatically; test details, NR Controls—details of tests to confirm absence of IEM, NR	NHMRC III-3 CX, Q3, P2 **Quality**: Poor Single case included No pre-specified criteria for test positives No blinding **Applicability**: Applicable

Author (year) Country	Study design	Patient characteristics (n)	Test characteristics	Quality and applicability
Tsai et al (1980) USA	Case-control Blinding, NR[a]	**Cases:** Recessive cystinuria (aged from 4 months to 3 years; exact age, NR; n = 1); NKH patients (aged 4 months to 3 years; exact age, NR; n = 2) Details of tests used to diagnose patients, NR **Controls:** Healthy and hospitalised newborns (newborn–3 weeks, n = 38) and infants (4 months to 3 years, n = 68); excluded individuals with diseases known to cause changes in urine amino acid excretion Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling untimed, details NR; adjusted to pH 2.3 with 5 mol/L HCl; sample aliquots centrifuged and filtered (0.45 µm Millipore filter) **Instrumentation:** HPLC—121 M fully automated analyser (Beckman Instruments) with a single 2.8 x 205 mm column of Beckman AA-10 ion-exchange resin; reference compounds: standard calibration mixtures of 40 amino acids, glutamine and homocystine added **Internal standard:** details, NR **Creatinine measurement:** Determined by Jaffé reaction with an IL 919 analyser (Instrumentation Laboratory); further details, NR **Reference standard:** Details of tests used to diagnose patients and confirm absence of IEM in controls, NR	NHMRC III-3 CX, Q3, P1 **Quality**: Poor No prespecified criteria for test positives No blinding **Applicability**: Applicable
Waddell et al (2006) Australia	Prospective, unclear Consecutive recruitment, unclear Blinding, NR April 1998 to October 2004	Newborns with initial octanoylcarnitine levels ≥1 µmol/L on newborn screening by MS/MS who had further analysis of urinary organic acids for investigation of suspected MCADD Screening performed between 48 and 72 hours of age (n = 36 out of 592,785 neonates undergoing newborn screening)	**Urine sample:** Sampling time and preparation NR **Instrumentation:** GC/MS; further details NR **Internal standard:** NR **Creatinine measurement:** NR **Additional tests:** Mutation analysis by PCR and sequencing; further details, NR Plasma acylcarnitine analysis by MS/MS; further details, NR	NA

Abbreviations: BG, butyrylglycine; DNA, deoxyribonucleic acid; EOC/MO/TBDMS, ethoxycarbonyl/methoxime/*tert*-butyldimethylsiyl; GA1, glutaric aciduria type I; GC, gas chromatograph; GCDH, glutaryl CoA dehydrogenase; GC/MS, gas chromatography–mass spectrometry; HG, hexanoylglycine; HGA, hydroxyglutaric acid; HP, Hewlett Packard; NKH, non-ketotic hyperglycinaemia; HMG, methylhexanoylglycine; HOG, hydroxyoctanoylglycine HPLC, high performance liquid chromatography; IEM, inborn error of metabolism; IBG, isobutyrylglycine; IG, isocaproylglycine; IVG, isovalerylglycine; MBG, methylbutyrylglycine; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MMA, methylmalonic aciduria; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MSUD, maple syrup urine disease; NA, not applicable; NR, not reported; PCR, polymerase chain reaction; PFB, pentafluourobenzyl; PPG phenylpropionylglycine; SG, suberylglycine; SPE, solid phase extraction; TLC, thin layer chromatography; TMS, trimethysilyl

[a] Either details of blinding between tests used to select cases or controls, or operators were blinded about whether samples were from cases or controls

[b] Includes unpublished data for 5 newborns (B Wilcken, personal communication, June 2008)

[c] Reference values determined from ethoxycarbonyl/methoxime/*tert*-butyldimethsilyl derivatives of 186 amino, carboxylic and ketoacid standards

Suspected metabolic disorders in newborns

The study by Carpenter et al (2001) was considered to provide evidence for use of UMP to investigate suspected metabolic disorders in newborns. Carpenter et al (2001) reported an evaluation of the New South Wales Newborn Screening Programme between 1998 and 2001. This program screens all newborns in NSW and the Australian Capital Territory (ACT) and has used tandem mass spectrometry (MS/MS) since April 1998. This prospective study evaluated screening by MS/MS for medium-chain acyl-CoA dehydrogenase deficiency (MCADD). Although this study represents a subset of the newborns screened in the study by Waddell et al (2006), it is considered to be a more appropriate representation of routine screening and subsequent follow-up by UMP and other tests.

The studies by Carpenter et al (2001) and Waddell et al (2006) were not designed as diagnostic test studies. Furthermore, the results of UMP and other tests are considered together when investigating a possible diagnosis of MCADD in screened newborns. Therefore, these studies do not enable the diagnostic performance of UMP alone for assessing the presence or absence of MCADD to be estimated. Because the diagnosis of MCADD in clinical practice does not apply UMP in isolation, studies by Carpenter et al (2001) and Waddell et al (2006) are presented to demonstrate the use of UMP in clinical practice.

Carpenter et al (2001) studied 275,653 consecutive babies born in NSW and the ACT between April 1998 and March 2001 who underwent routine newborn screening. This represented more than 99 per cent of all babies born during that period. Octanoylcarnitine levels were > 1 μmol/L in the initial dried blood spot samples from 23 newborns. Of these 23 newborns, 11 did not test positive for the A985G mutation (see page 25) and octanoylcarnitine had normalised on repeat sampling in six of these 11. One newborn had blood octanoylcarnitine of 1 μmol/L on retesting, but MCADD was excluded on the basis of results of testing plasma and urinary acylglycines. The remaining four newborns died from a variety of causes before a second blood sample was analysed. Further investigations eliminated MCADD as the cause of death.

Acylcarnitines were persistently elevated in 12 newborns who were also found to carry at least one copy of the A985G mutation. Further investigations of these 12 babies included urinary organic acid analysis and/or quantitation of plasma carnitine and acylcarnitine.

Results from the further investigations conducted for the 12 newborns who had persistently elevated acylcarnitine levels are presented (Table 15). All 12 babies met one or more criteria assigned to confirm MCADD: homozygous for A985G mutation; elevated hexanoglycine and suberglycine in urine; and increased hexanoylcarnitine, octanoylcarnitine, or decanoylcarnitine in plasma.

The results from the further investigations conducted for the 12 newborns indicate that urinary acylglycines and plasma acylcarnitine levels were elevated in nine patients (patients 1 and 3–10) (Table 15). Mutational analysis indicated that patients were either homozygous (patients 2 and 5–7) or heterozygous (patients 1, 3, 4 and 8–12) for A985G substitution known to cause MCADD.

Urinary acylglycine analysis was not performed for patient 2, but the results of mutational and plasma acylcarnitine analyses and additional tests (results not reported) confirmed MCADD. Patient 11 recorded a trace increase in urinary acylglycine, but mutational

analysis and plasma acylcarnitine measurement confirmed MCADD (Table 15). Urinary acylglycines were not detected and plasma acylcarnitine measurement was not performed; mutational analysis and additional tests (results not reported) confirmed MCADD for patient 12.

These results show that where patients had clearly defined MCADD (A985G homozygotes), analysis by UMP and plasma carnitine measurement consolidated the diagnosis. Of the 12 newborns with persistently elevated acylcarnitines (patients 1–12), eight were heterozygotes for the A985G mutation (patients 1, 3, 4 and 8–12). Based on mutational analysis results alone, these patients could mistakenly be classified as carriers of MCADD (see page 25). Results further indicate that UMP and plasma carnitine analysis were able to establish that these patients had MCADD and were not carriers (except patient 12) (Table 15).

Table 15 Assessment of MCADD in screened newborns (Carpenter et al 2001)

Author (year) Country	Patient	Urinary acylglycines[a]		Plasma carnitines (μmol/L)[b]			A985G mutational analysis[c]	Quality and applicability
		Hexanoyl	Suberyl	C6 (<0.1)	C8 (<0.3)	C10:1 (<0.3)		
Carpenter et al (2001) Australia	1	+++	+++	0.6	2.0	0.3	+/-	NHMRC III-2
	2	NP	NP	1.3	5.6	0.5	+/+	C1, Q3, P1
	3	+++	+++	1.0	3.8	0.6	+/-	
	4	+++	+++	0.9	3.8	0.3	+/-	**Quality**: Poor
	5	+++	+++	0.5	2.6	0.3	+/+	No blinding
	6	+++	+++	1.3	8.4	2.5	+/+	
	7	+++	+++	1.1	5.7	0.7	+/+	Review bias
	8	++	++	0.2	1.1	0.4	+/-	Selection bias
	9	+	++	1.1	3.1	0.4	+/-	
	10	+	+	0.2	0.7	0.2	+/-	**Applicability**: Applicable
	11	TR, ND	TR, ND	0.7	1.9	1.0	+/-	
	12	ND	ND	NP	NP	NP	+/-	
Unpublished data[d]	13	ND	ND	0.09	0.29	0.21	-/-	
	14	ND	ND	NP	normal	NP	-/-	
	15	ND	ND	0.1	0.3	0.2	-/-	
	16	ND	ND	NP	normal	NP	-/-	
	17	ND	ND	NP	0.13	NP	-/-	

Abbreviations: C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanolycarnitine; ND, not detected; NP, not performed; TR, trace increase
[a] +++, grossly elevated; ++, moderately elevated; +, slightly elevated
[b]Cut off values in parentheses
[c]+/+, homozygous for A985G; +/- heterozygous for A985G; -/- A985G mutation absent from both alleles
[d]B Wilcken, personal communication, June 2008
Note: Patient numbering is arbitrary and does not represent order of entry into the study
Source: Carpenter et al (2001). 'Evaluation of newborn screening for medium-chain acyl-CoA dehydrogenase deficiency in 275 000 babies'. *Arch Dis Child Fetal Neonat* 85: F105–F109. Reprinted with permission of BMJ Publishing Group Ltd

Additional unpublished data are presented (Table 15) for five of 11 patients who did not test positive for the A985G mutation after initial newborn screening (Wilcken, personal communication, June 2008). Urinary acylglycines were not detected in any of the five patients, although plasma carnitine levels were at threshold values in one newborn (patient 15) and just below threshold in another (patient 13). Limited results indicated that plasma carnitine levels were not elevated in the other three newborns (patients 14, 16 and 17). The A985G mutation was not detected on either allele. These results indicate that UMP can be used to confirm the results of A985G mutational analysis (patients 13–17) and plasma carnitine analysis (patients 14, 16 and 17). Furthermore, where the results of plasma carnitine analysis were somewhat ambiguous (patients 13 and 15), UMP results indicated that MCADD was absent.

Although this study, together with unpublished data, does not enable assessment of the clinical effectiveness of UMP alone, it demonstrates test use in clinical practice. Carpenter et al (2001) provide important evidence of UMP use in the diagnosis of inherited metabolic disorders in newborns with positive screening results. This prospective study evaluated screening for MCADD in 275,000 consecutive newborns, which represented more than 99 per cent of all newborns in NSW and the ACT between 1998 and 2001. Patients who required further investigation were identified on the basis of screening results indicating MCADD. These newborns underwent urinary acylglycine analysis, which together with the results of mutational analysis and plasma acylcarnitine analysis, enabled conclusive diagnosis of MCADD to be made. Unpublished data showed that UMP together with plasma carnitine analysis ruled out a diagnosis of MCADD in several newborns who initially tested positive on screening.

The study by Waddell et al (2006) is considered to provide further evidence supporting the use of UMP to investigate suspected MCADD in newborns. Although the objective of this study was to explore the relationship between the phenotype and genotype of patients with MCADD, the reported data also demonstrate the clinical use of UMP. Waddell et al (2006) reported data relating to 592,785 newborns undergoing routine screening by tandem mass spectrometry (MS/MS) in NSW between April 1998 and October 2004.

Of the newborns screened by MS/MS between April 1998 and October 2004, 60 babies were found to have octanoylcarnitine levels of 1 μmol/L or greater and were suspected to have MCADD. These babies were further investigated using UMP to detect organic acids, including acylglycines by GC/MS and plasma acylcarnitine analysis by MS/MS. Analysis to detect the common A985G mutation (see page 25) was performed on DNA extracted from the blood sample taken for newborn screening. Patients were considered to have MCADD if they had two previously described disease-causing mutations and/or increased urinary hexanoglycine. Urinary hexanoylglycine was semi-quantified and scored as slightly (approximately 5–10 μmol/mol creatinine), moderately (10–20) or grossly (> 20) increased as a function of organic acids analysis. The results of the analyses reported by Waddell et al (2006) are presented in Table 16.

MCADD was confirmed in 34 patients. Of these, 32 had increased levels of urinary hexanoylglycine excretion. Where performed or reported, plasma octanoylcarnitine levels were also elevated in these 32 patients. Trace increases in urinary hexanoylglycine excretion, with slight increase in plasma octanoylcarnitine levels, was identified in one patient (patient 35); and another had moderate increase (patient 36). UMP was not performed for two patients (Table 16).

Mutational analysis indicated that 17 of 34 patients were homozygous for the mutation c.985A>G; 17 were compound heterozygotes and 14 patients had one copy of the c.985A>G mutation and one copy of another mutation. The other three patients had mutations or genetic polymorphisms other than the A985G mutation. The two patients who had trace increases in urinary hexanoylglycine excretion were also compound heterozygotes. Patient 36 had one copy of the c.985A>G mutation together with a different mutation on the other allele, and patient 35 had different mutations on both alleles. The remaining 24 babies of the initial 60 who were found to have elevated octanoyl carnitine on initial screening underwent further testing and did not have MCADD (Wilcken, personal communication, June 2008).

In newborns among whom mutational analysis alone clearly indicated MCADD (A985G homozygotes, patients 1–17), both UMP and plasma carnitine analysis results also provided this indication. Investigation using UMP and plasma carnitine analysis also enabled diagnosis of MCADD in patients who were compound heterozygotes. This was so regardless of whether patients had one copy of the c.985A>G mutation and one copy of another mutation (patients 18–31 and 36), or had different mutations on both alleles (patient 35). Waddell et al (2006) also used the results of UMP and plasma carnitine analysis to diagnosis MCADD in a patient who had several genetic polymorphisms with unknown pathogenicity (patient 34).

Although the study by Waddell et al (2006) did not enable assessment of the clinical effectiveness of UMP alone, the use of the test in clinical practice was demonstrated. UMP results, together with plasma carnitine and mutational analyses, were used to assign diagnosis of MCADD in newborns suspected of the condition on the basis of screening test outcomes.

Table 16 Assessment of MCADD in screened newborns (Waddell et al 2006)

Author (year) Country	Patient	Urinary hexanoylglycine[a]	Plasma octanoylcarnitines (µmol/L)[b]	Mutational analysis[c]	Quality and applicability
Waddell et al (2006) Australia	1	+++	7.8	+/+	NHMRC III-2
	2	NP	NP	+/+	C1, Q3, P1
	3	+++	0.5	+/+	**Quality***:* Poor
	4	+++	6.5	+/+	Selection bias
	5	+++	NR	+/+	No blinding
	6	++	1.5	+/+	**Applicability***:* Applicable
	7	+++	7.6	+/+	
	8	+++	2.4	+/+	
	9	NP	5.6	+/+	
	10	++	4.5	+/+	
	11	+++	5.9	+/+	
	12	+++	8	+/+	
	13	++	4.1	+/+	
	14	+++	2.6	+/+	
	15	+++	NP	+/+	
	16	++	5	+/+	
	17	+++	2.7	+/+	
	18	+++	4.9	+/+	
	19	+++	2	+/+	
	20	+++	3.6	+/+	
	21	+++	3.1	+/+	
	22	+++	3.8	+/+	
	23	+++	3.8	+/+	
	24	++	1.1	+/+	
	25	+	3.3	+/+	
	26	++	1.4	+/+	
	27	+++	9.3	+/+	
	28	+	3.1	+/+	
	29	+	0.7	+/+	
	30	+	1	+/+	
	31	++	0.4	+/+	
	32	++	2.1	+/+	
	33	+++	2.9	+/+	
	34	+++	2.4	See footnote d	
	35	TR	0.3	+/+	
	36	TR,ND	1.9	+/+	

Abbreviations: MCADD, medium-chain acyl-coA dehydrogenase deficiency; ND, not detected; NP, not performed; NR, not reported

[a] +, slight increase; ++ moderate increase, +++ gross increase

[b] Normal value or cut-off for MCADD not reported. Note that Carpenter et al (2001) reported cut-off values for plasma carnitines of <0.1 µmol/L for C6 and <0.3 µmol/L for C8 and C10:1

[c] +/+: two MCAD mutations present, regardless of whether the same or different mutations are present on each allele: patients 1–17 homozygous for the A985G mutation; patients 18–31 & 36 had one copy of the A985G mutation and one copy of another mutation (details not reported here); patients 32,33 & 35 had different mutations on each allele

[d] Authors reported that patient 34 had several genetic polymorphisms, but none of these variations were reported as being pathogenetic

Source: Waddell et al (2006). 'Medium-chain acyl-CoA dehydrogenase deficiency: Genotype-biochemical phenotype correlations'. *Mol Genet Metab* 87:32–39. Reprinted with permission of Elsevier Limited

People with clinical presentations suggestive of genetic metabolic disorders

Korman et al (2007) present evidence that is considered to demonstrate the use of UMP for the investigation of glutaric aciduria type I (GA1) in symptomatic patients. UMP was performed by qualitative analysis of urinary organic acids by gas chromatography/mass spectrometry.

Korman et al (2007) studied 12 patients diagnosed with GA1 at a metabolic laboratory during a five-year period ending in mid-2004 in Israel. Most (11/12) were from Palestine. The 12 participants, from 11 families, included three (patients 2, 11 and 12) who were investigated for acute encephalopathy of unknown origin, and six (patients 1, 4–7 and 10) who underwent investigation for chronic or progressive neurological symptoms including dystonia, seizures, spasticity and developmental delay. The researchers noted that because of a tradition of intermarriage among close family members, the incidence of GA1 in the study population was higher than other populations.

The investigators reported that three patients (patients 3, 8 and 9) demonstrated presentations that were atypical of GA1. At routine examination, patient 3 was found to have macrocephaly; 12 months after initial treatment, GA1 was suspected by a paediatric neurologist. Patient 8 was evaluated during the first week of life because of macrocephaly. The mother of patient 8 had two previous terminations because of ultrasound-detected anencephaly and multiple congenital abnormalities. Patient 9 presented with his wife, who was his first cousin, when she was in her seventh week of pregnancy. Genetic counselling was sought because the couple were the maternal uncle and paternal aunt of patient 8, who had already been identified as a compound heterozygote for two GCDH mutations. Carrier testing for these mutations revealed that the mother was the carrier of one mutation and that the father was compound heterozygote for both mutations; metabolite testing subsequently confirmed that the father had GA1. Of all 12 patients, the diagnosis of GA1 was only considered in three patients (patients 3, 5 and 12) by clinicians referring samples to the laboratory for general metabolic screening.

Results of UMP, plasma carnitine analysis and mutational analysis from the study by Korman et al (2007) are presented (Table 17). Most patients were reported to have moderate to severe urinary excretion of glutaric acid (GA) and markedly increased excretion of 3-hydroxyglutarate (3-HGA). It was found that 3-HGA levels were elevated in the absence of ketonuria. Patients 1 and 12 had normal GA excretion (< 11 mmol/mol creatinine). Patient 1 had slightly elevated GA excretion while undergoing valproic acid therapy, which is known to elevate urinary GA level. Patients 1 and 12 had moderately elevated urinary 3-HGA excretion, but at a lesser degree than other patients. Plasma free carnitine deficiency was marked in patients 2 to 11; patients 1 and 12 had normal GA excretion; but plasma free carnitine levels were below normal levels in both these patients. Analysis of the GCDH gene found that all 12 patients had mutations in this gene. There were nine different mutations acknowledged, including four that were previously unidentified (details not reported). Patients 1–6, 10 and 11 were found to be homozygotes for various GCDH genotypes. Patients 7, 8, 9 and 12 were found to be compound heterozygotes.

In this assessment, plasma carnitine analysis is regarded as a comparator test if UMP is not available for the investigation of GA1, and mutational analysis is considered to be a suitable reference standard for UMP and comparator tests (see Appendix I). The diagnostic performance of UMP and plasma carnitine analysis can therefore be estimated

based on presented results (Table 17). In the expert opinion of the advisory panel for this assessment, 3-HGA is considered to be the marker for diagnosing GA1 in clinical practice. To derive diagnostic performance values for UMP, test results were considered to be positive where either GA and/or 3-HGA levels in urine were above normal ranges. That is, results are regarded as positive when GA levels are normal but 3-HGA levels are elevated or *vice versa*—an either-positive approach. Although 3-HGA is considered to be the marker for diagnosing GA1, the either-positive approach reflects the broader picture of GA1 diagnosis where GA levels would also be considered.

Table 17 Assessment of suspected glutaric aciduria type I

Author (year) Country	Patient (age at diagnosis)	Urinary metabolic profiling		Plasma free/total carnitines[c] (µmol/L)	GCDH mutational analysis[d]	Quality and applicability
		GA[a] (mmol/mol creatinine)	3-HGA[b] (mmol/mol creatinine)			
Korman et al (2007) Israel	1 (14 months)	6–50[e]	33	15.7/32.7	+/+	NHMRC III-2
	2 (4 months)	f	f	5.9/11.4	+/+	C1, Q3, P1
	3 (28 months)	6811	160	2.3/5.5	+/+	
	4 (14 months)	1199	61	5.8/10.3	+/+	**Quality**: Poor
	5 (10 months)	f	f	3.9/7.4	+/+	Selection bias
	6 (3 years)	13344	197	3.0/–	+/+	No blinding
	7 (1 year)	f	f	4.7/8.8	+/+	
	8 (7 days)	243	44	6.6/14.6	+/+	**Applicability**: Applicable
	9 (30 years)	728	49	4.7/7.9	+/+	
	10 (3 years)	1830	70	9.8/12.0	+/+	
	11(8 months)	f	f	4.0/13.1	+/+	
	12 (5 months)	6	15	18.1/30.5	+/+	

Abbreviations: GA, glutaric acid; GCDH, glutaryl CoA dehydrogenase; 3-HGA, 3-hydroxyglutarate

[a] Normal range = 0.5–11 mmol/mol creatinine

[b] Normal range = 0.9–4.5 mmol/mol creatinine

[c] Normal range: plasma free carnitine = 25–35 µmol/L; total carnitine = 35–45 µmol/L

[d] +/+ homozygous for mutation, whether the same or different mutation present on each allele . GCDH genotypes: Patient 1 = L283P/L283P; Patient 2 = G390R/G390R; Patients 3–6 = T4161I/ T4161I; Patient 7 = T4161/A293T; Patients 8 and 9 = A293T/T193_R194insH ; Patient 10 = T341P/T341P ; patient 11 = N392S/N392S ; Patient 12 = S119L/M405V

[e] GA elevated only on valproate therapy

[f] Markedly increased urinary excretion on qualitative organic acid analysis, but not quantified

Source: Korman et al (2007). 'Glutaric aciduria Type 1: Clinical, biochemical and molecular findings in patients from Israel'. *Eur J Paediatr Neurol* 11:81–89. Reprinted with permission of Elsevier Limited

Diagnostic performance is estimated from data where the results of all three tests (UMP, plasma carnitine analysis and mutational analysis) are available for each patient. Patients who test positive for GA1 by mutational analysis are considered reference standard test positives. To estimate the diagnostic performance of UMP and plasma carnitine analysis, the results of these tests were cross-referenced to mutational analysis results. This study did not include symptomatic patients who were negative by UMP or plasma carnitine analysis and also had mutational analysis. Therefore, it is not possible to estimate the sensitivity of UMP or plasma carnitine analysis because the false negative rate cannot be assessed. Nevertheless, it is possible to estimate the positive predictive value of these tests. The results of this analysis are presented in Table 18.

Table 18 Diagnostic performance for assessment of glutaric aciduria type I

Author (year) Country	Test for glutaric aciduria type 1		Quality and applicability
	Urinary metabolic profiling[a]	Plasma carnitine analysis[b]	
Korman et al (2007) Israel	PPV = 100%	PPV = 100%	NHMRC III-2 C1, Q3, P1 **Quality**: Poor: selection bias, no blinding **Applicability**: Applicable

Abbreviations: PPV, positive predictive value
[a] Using an either positive approach, all patients were test positive for GA1 by urinary metabolic profiling
[b] Levels of plasma free carnitine and total carnitine were decreased below normal levels in all patients, hence all patients were considered test positive plasma carnitine analysis

The results presented in Table 18 suggest that both analysis of urinary 3-hydroxyglutarate (HGA) excretion by UMP and analysis of plasma carnitine have a positive predictive value of 100 per cent, suggesting that all patients who test positive by either test have GA1. The positive predictive value of UMP was estimated using an either-positive approach. If diagnosis was based on analysis of urinary GA excretion alone, then patients 1 and 12 would have been considered to have negative test results because their GA excretion levels were normal. The either-positive approach in this analysis is considered to be representative of clinical practice: UMP test results would not be considered in isolation in this setting. It is also considered that 3-HGA is the more important marker for GA1 diagnosis.

The Korman et al (2007) study was not designed as a diagnostic test assessment. As a result, there are several biases that limit the conclusions that can be made. No patients were included whose symptoms and/or family history resulted in investigation for GA1, but who did not have a final diagnosis of GA1. This selection bias meant that it was not possible to determine how well UMP or plasma carnitine analysis can exclude suspected GA1: it was not possible to assess testing specificity. Sensitivity could not be estimated because there were no patients included who tested negative on UMP or plasma carnitine analysis, but were also investigated by the reference standard. This meant that the false negative rate could not be assessed. Although the positive predictive value for both UMP and plasma carnitine analysis was 100 per cent, this result should be interpreted with caution because positive predictive value is dependent on disease prevalence: a test performs less well as prevalence decreases. In addition, there was no blinding between UMP, the comparator test and the reference standard. However, because of the quantitative nature of most testing, it is less likely that test review bias (where test results are interpreted with knowledge of the reference standard) is present. Despite the limitations of this study, Korman et al (2007) demonstrated that UMP can be used in the diagnosis of GA1 where a metabolic disorder is suspected because of symptoms. It also provides limited evidence that UMP can be used to investigate possible metabolic disorders in individuals who are close relatives of patients known to have an IEM.

This study is considered to be applicable to Australian clinical practice. If regarded as a diagnostic test study, it is considered to present poor quality evidence because of selection bias and the absence of blinding. When classified by NHMRC levels of evidence for diagnostic test studies his study is considered to provide level III-2 evidence (NHMRC 2005).

Evaluation of UMP among confirmed patients and controls

The 10 other studies identified in the literature search investigated a variety of UMP technologies applied for people whose diagnoses were confirmed, and for control subjects (Bonafé et al 2000, Bonham Carter et al 1991, Costa et al 2000, Giugliani et al 1987, Kuhara et al 2001, Landaas and Jakobs 1977, Paik et al 2005, Pitt 1993, Schor et al 2002, Tsai et al 1980). Inadequate data reporting meant that it was not possible to determine how diagnosed people were identified initially. It is feasible that participants selected for these studies were initially identified following newborn screening, by clinical presentation, or were at-risk family members. The results of these studies are therefore presented as evidence to indicate the effectiveness of UMP in the three patient populations considered in this assessment.

Although these studies were not designed as diagnostic case-control studies, when appraised as such, several common features of design weakness become apparent. Information on how cases and controls were selected was frequently limited; case-control matching was seldom applied; and there was no blinding to the source of samples—whether they were cases or controls. Therefore, UMP test results could be interpreted with the knowledge of other test results used to select cases and controls. Criteria for test positivity were not pre-specified so it was not possible to determine if particular threshold levels of metabolites measured by quantitative UMP methods were diagnostic for a metabolic disorder. It was not possible to estimate measures of test performance where quantitative results were reported.

It should be noted that these studies were generally designed to determine whether different UMP technologies could detect and/or quantify metabolites known to be altered in metabolic disorders. The rarity of inherited metabolic disorders means that it is not practical to conduct diagnostic test assessment studies that do not have inherent bias: the relatively small numbers of people with these disorders means that it is not possible to randomly assign participants to study arms.

GC/MS for diagnosis of MCADD

Bonham Carter et al (1991), Costa et al (2000) and Pitt (1993) investigated gas chromatography/mass spectrometry (GC/MS) use in the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency (MCADD).

Bonham Carter et al (1991) compared urinary levels of acylglycines between patients with MCADD and controls (Table 19). Urine samples from patients with MCADD showed elevated levels of hexanoylglycine, octonoylglycine, 3-phenylpropionyl glycine and suberylglycine. This study also investigated UMP for the diagnosis of six family members of a patient with known MCADD. The six family members were tested using UMP and were found not to have raised metabolites and were not considered to have MCADD. Results are reported in Table 19. Intra-and inter-assay variability were also tested. Results indicated minimal variability for both.

Results from this study provide evidence that supports conduct of UMP using GC/MS to confirm diagnosis MCADD for people who have high indeces of suspicion of the condition being present, and of their close family members. Urinary excretion of several acylglycines was clearly abnormal compared with control subjects. The study investigated MCADD in nine family members among whom at least one child had previously been diagnosed. It was found that three family members had the condition. Although this

method included individuals who had not previously been diagnosed with MCADD, the study was limited because all patients were from the same family; this introduced spectrum bias.

This non-comparative study was applicable to Australian clinical practice, but the presence of spectrum bias meant that evidence quality was low. Details of tests used to diagnose patients and to exclude IEMs in controls were not reported. This study represents level III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

Costa et al (2000) applied GC/MS for urinary acylglycines analysis in patients with proven MCADD. These patients' urinary acylglycine profiles were compared with control subjects (Table 20). The urinary acylglycine profiles of patients with MCADD were characterised by a marked elevation of several acylglycine compounds compared with controls. Levels of butyrylglycine and branched chain conjugates were also elevated (Table 20). Analyses of intra- and inter-assay variability confirmed reproducibility status for this method of GC/MS (Table 20).

Pitt (1993) compared urinary acylglycine excretion in patients with MCADD with control subjects (Table 21). GC/MS was used in a quantitative assessment of acylglycine excretion. Results indicated that the urinary excretion of suberylglycine (SG), hexanoglycine (HG), isocaproylglycine (IG) and 4- methylhexanoylglycine (4-MHG) was elevated in MCADD patients (Table 21). These acylglycines were not detected in control subjects. Urinary excretion of SG, HG, IG and 4-MHG was lower in people with MCADD who were asymptomatic compared with patients who were experiencing an acute phase of the disorder. It was also reported that 8- and 7-hydroxyoctanoylglycine were detected in people with MCADD, but was usually excreted in significant amounts only when subjects were acutely ill with hypoglycaemia and had gross excretion of dicarboxylic acids (data not reported). Urinary excretion of these compounds was much lower or undetectable among asymptomatic subjects (data not reported). Intra- and inter-assay variabilities were not reported in this study.

The results of this study also suggest that measurement of urinary acylglycine excretion by GC/MS can be used in the diagnosis of patients with MCADD. However, similar to the studies conducted by Bonham Carter et al (1991) and Costa and colleagues (2000) this study is also limited by inherent spectrum bias introduced by the case-control design. It was not possible to determine whether the GC/MS methodology was able to distinguish MCADD patients from those with symptoms suggestive of metabolic disorder, or from patients with other metabolic disorders. Pitt (1993) studied patients whose diagnoses were confirmed by other tests: DNA mutation analysis and/or phenylpropionate loading, but test details were not reported. People in the control arm had normal metabolic screens, although test details were not reported. This study indicates that GC/MS analysis agreed with results of other tests. Although results from other tests informed selection of patients, this study cannot be considered to have reliably compared results from GC/MS with other tests because no information on test results used to select participants was presented.

This non-comparative study is considered applicable to Australian clinical practice. It is considered to present medium quality evidence: spectrum bias is inherent in the design, but tests used to select cases and controls were clearly reported. This study is classified as providing level III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

Table 19 UMP performed by gas chromatography/mass spectrometry in patients with MCADD: Bonham Carter et al (2000)

Author (year)	Study details		UMP results		Reproducibility		Quality and applicability
Country	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (relative SD)	
Bonham Carter et al (2000) UK	GC/MS[a]	MCADD	**Patients with MCADD**: HG: 14.09–56.56 OG: 0.28–1.24 3-PPG: 6.07–87.75 SG: 8.23–130.79(µg/mg creatinine, range) (n = 3: results for 3 family members who had MCADD) **Patients without MCADD**: HG: 0.38–1.13 OG: ND–0.02 3-PPG: 0.03–0.28 SG: 0.13–0.37 (µg/mg creatinine, range) (n = 6: results for 6 family members who did not have MCADD)	HG: 0.03–1.15 OG: ND–0.17 3-PPG: 0.01–0.09 SG: 0.02–1.53 (µg/mg creatinine, range) (n = 29)	HG: 0.9990, n = 10 OG: 0.9990, n = 10 3-PPG: 0.9981, n = 10, over 5 ng–1 µg SG: 0.9987, n = 8, over 20 ng–1 µg [b]	HG: 3.6%, n = 18 OG: 7.0%, n = 18 3-PPG: 7.3%, n = 18 SG: 7.9%, n = 10[b]	**Quality**: Poor NHMRC III-3 CX, Q3, P1 Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported **Applicability**: Applicable

Abbreviations: GC/MS, gas chromatography/mass spectrometry; HG, hexanoylglycine; HGA, hydroxyglutaric acid; IBG, isobutyrylglycine; IEM, inborn error of metabolism; MCAD, medium-chain acyl dehydrogenase; ND, not detected; OG, octanoyl glycine; PPG, phenylpropionylglycine; SD, standard deviation; SG, suberylglycine; UMP, urinary metabolic profiling

[a] GC/MS analysis employed negative chemical ionisation

[b] Intra-assay variability was investigated by inclusion of a water sample containing equal quantities of unlabelled and 13C2 acyl glycines with each batch of samples. For inter-assay variation, a blank sample of water containing the same quantities of acyl 13C2 glycines as the urine samples was submitted to the same analytical procedure when every batch was tested

Table 20 UMP performed by gas chromatography/mass spectrometry in patients with MCADD: Costa et al (2000)

Author (year)	Study details		UMP results		Reproducibility		Quality and applicability
Country	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Costa et al (2000) Portugal, Netherlands	GC/MS	MCADD	BG: 0.40 (0.14–0.91) IBG: 1.3 (0.078–3.6) 2-MBG: 0.81 (0.20–2.0) IVG: 2.4 (0.28–6.7) HG: 41 (5.5–122) PPG: 8.9 (0.10–28) SG: 87 (5.2–359) (mmol/mol creatinine, mean and range) (n = 10)	BG: 0.041 (0.007–0.12) IBG: 0.36 (0.003–1.5) 2-MBG: 0.20 (0.002–0.58) IVG: 0.37 (0.027–0.92) HG: 0.3 (0.014–0.83) PPG = 0.033 (<0.002–0.15) SG = 0.14 (0.024–0.52) (mmol/mol creatinine, mean and range) (n = 19)	Intra-assay variability: ranged from *V*=0.62% for 2MBG to *V*=5.62% for BG[c]	Inter-assay variability: ranged from *V* = 4.67% for 2MBG to *V* = 13.8% for BG[b]	**Quality** Poor NHMRC III-3 CX, Q3, P1 Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported: **Applicability**: Applicable

Abbreviations: BG, butyrylglycine; GC/MS, gas chromatography/mass spectrometry; HG, hexanoylglycine; IBG, isobutyrylglycine; IEM, inborn error of metabolism; IVG, isovalerylglycine; MBG, methylbutyrylglycine; MCAD, medium-chain acyl dehydrogenase; PPG, phenylpropionylglycine; SD, standard deviation; SG, suberylglycine; UMP, urinary metabolic profiling

[a] GC/MS analysis employed negative chemical ionisation

[b] Intra-assay variability was investigated by analysis of 5 identical urine samples in one sample preparation. For inter-assay variation, one urine sample was processed in five independent sample preparations on five different days; variability was assessed for IBG, BG, 2 MBG, IVG, HG, PPG and SG. Variability, *V* = sdx*100%

Table 21 UMP performed by gas chromatography/mass spectrometry in patients with MCADD: Pitt (1993)

Author (year)	Study details		UMP results		Reproducibility		Quality and applicability
Country	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Pitt (1993) Australia	GC/MS[a]	MCADD	Acute phase: SG: range 11–50 approx[b] HG: range 10–40 approx[b] IG: 3–6 approx[b] 4MHG: 2–4 approx[b] (μmol/mol creatinine) (n = 4) Asymptomatic: SG: ND–10 approx[b] HG: 1–9 approx[b] IG: 0.4–3 approx[b] 4MHG: 0.1–1.5 approx[b] (μmol/mol creatinine) (n = 6)[c]	SG: ND HG ND IG: 4-MHG 8HOG: ND 7HOG: ND (μmol/mol creatinine) (n = 15)	NR	NR	**Quality**: Medium NHMRC III-3 CX, Q2, P1 Spectrum bias **Applicability**: Applicable

Abbreviations: BG, butyrylglycine; EOC/MO/TBDMS, ethoxycarbonyl/methoxime/*tert*-butyldimethylsiyl; GC/MS, gas chromatography/mass spectrometry; HG, hexanoylglycine; HGA, hydroxyglutaric acid; IBG, isobutyrylglycine; IEM, inborn error of metabolism; IG, isocaproylglycine; IVG, isovalerylglycine; MBG, methylbutyrylglycine; MCAD, medium-chain acyl dehydrogenase; MHG, methylhexanoylglycine; ND, not detected; OG, octanoyl glycine; PPG, phenylpropionylglycine; Sd, standard deviation; SG, suberylglycine; UMP, urinary metabolic profiling

[a] Trimethylsilyl derivatives of urine organic acids and glycine conjugates identified qualitatively on GC/MS chromatograms

[b] Exact figures not reported, these data were derived from interpretation of acylglycine excretion data plotted on a logarithmic scale

[c] From a total of 8 cases results were presented for 4 patients during an acute phase and 6 patients when asymptomatic

GC/MS for diagnosis of maple syrup urine disease and methylmalonic aciduria

Paik et al (2005) compared the GC/MS retention index spectra of urine samples from one patient with maple syrup urine disease (MSUD) and another with methylmalonic aciduria (MMA) with reference values determined for a range of amino and carboxylic acids (Table 22). Urinary metabolite excretion was also investigated in control subjects, and was considered to be normal. It was found that 2-hydroxyvaleric acid was the most abundant metabolite, followed by leucine and valine in the patient with MSUDD. The level of methylmalonic acid was found to be extremely elevated in the patient with MMA compared with control retention index spectra. Intra- and inter-assay variabilities were not reported.

This qualitative analysis suggests that this GC/MS methodology can be used to identify altered patterns of urinary metabolite excretion in patients with MSUDD and MMA. However, the usefulness of data from this study was reduced because the analysis was limited to urine samples from one patient with each condition. Consequently, it was not known if the urinary metabolic profile would be similarly altered in other patients with these metabolic disorders. It is also unknown if this GC/MS method could be used to distinguish MSUDD and MMA patients from those who are similar symptomatically to patients with these conditions or different metabolic disorders.

This non-comparative study is considered applicable to Australian clinical practice, but presents poor quality evidence because only single cases were investigated. Furthermore, no details were reported for tests used to identify or confirm the absence of IEMs in controls. This study represents level III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

GC/MS for diagnosing glutaric aciduria type I

Schor et al (2002) described the use of gas chromatography/mass spectrometry (GC/MS) to quantify 3-hydroxyglutaric acid (3-HGA) in body fluids, including urinalysis of one patient with glutaric aciduria type I (GA1) confirmed by enzyme analysis. Levels of 3-HGA were also measured in control subjects' urine (Table 23). Compared with control subjects, urinary 3-HGA excretion was considerably elevated in the patient with GA1. This study (2002) also examined the reproducibility of GC/MS quantification of 3-HGA and reported that intra- and inter-assay variability was minimal (Table 23).

Schor and colleagues (2002) suggest that quantification of urinary 3-HGA by GC/MS could be used in the diagnosis of GA1. However, because results were presented from an individual, it is unknown whether these results would be similar to other patients with GA1.

This study is considered to be applicable to Australian clinical practice, but is regarded as poor quality because the analysis was based on one case, and specified criteria for test positives and operator blinding to the origin of urine samples were absent. According to the NHMRC levels of evidence for diagnostic test studies this study was classified as providing level III-3 evidence (NHMRC 2005).

Table 22 UMP performed by gas chromatography/mass spectrometry in patients with maple syrup urine disease and methylmalonic aciduria

Author (year)	Study details		UMP results		Reproducibility		Quality and applicability
Country	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Paik et al (2005) South Korea	GC/MS[a]	MSUD, MMA	MSUD patient—in retention index spectra 2-hydroxyvaleric acid was most abundant, followed by leucine and valine MMA patient—MMA extremely elevated compared with normal average retention index spectra. The second most prominent metabolite was glycine (n = 1 MMA; n = 1 MSUD)	Urinary excretion patterns normal (n = 3)	NR	NR	**Quality**: Poor NHMRC III-3 CX, Q3, P1 Details of tests used to diagnose cases and confirm absence of IEM in control patients not reported One case for each IEM **Applicability**: Applicable

Abbreviations: GC/MS, gas chromatography/mass spectrometry; IEM, inborn error of metabolism; MMA, Methylmalonic aciduria; MSUD, maple syrup urine disease; NR, not reported; SD, standard deviation; UMP, urinary metabolic profiling

[a] Simultaneous profiling of urinary amino and carboxylic acids was performed through retention index analysis. Reference values determined from ethoxycarbonyl/methoxime/*tert*-butyldimethsilyl derivatives of amino and carboxylic acid standards. GC profiles were transformed into patterns in bar graph form by plotting normalised peak area ratios of amino and carboxylic acids aGA1nst reference values as the identification numbers

Table 23 UMP performed by gas chromatography/mass spectrometry in a patient with glutaric aciduria type I

Author (year)	Study details		UMP results		Reproducibility		Quality and applicability
Country	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Schor et al (2002) Netherlands	GC/MS[a]	MMA	3-HGA = 262 (mmol/mol creatinine)	3-HGA range = 0.88–4.5 (mmol/mol creatinine)	3-HGA concentration: Intra assay[b] = 2.6 ±0.09 (n = 5) (µmol/L)	3-HGA concentration: Inter assay[c] = 2.6 ± 0.16)(µmol/L) (n = 8	**Quality**: Poor NHMRC III-3 CX, Q3, P2 Single case included No pre-specified criteria for test positives No blinding **Applicability**: Applicable

AbbrevAbbreviations: GA, glutaric aciduria; GC/MS, gas chromatography/mass spectrometry; 3-HGA, 3-hydroxyglutaric acid; IEM, inborn error of metabolism; MMA, methylmalonic aciduria; SD, standard deviation; UMP, urinary metabolic profiling

[a] Procedure comprised solid phase extraction of samples and conversion to triflouroacetyl-di-PFB derivatives before GC/MS

[b] Intra-assay variability assessed by serial analysis of five identical samples prepared at same time. Urine sample source not reported, based on 3-HGA levels most likely from controls

[c] Inter-assay variability established by processing and analysing one sample on at least six different days. Urine sample source not reported, based on 3-HGA levels most likely from controls

GC/MS for diagnosing homocystinuria

Kuhara et al (2000) examined the effectiveness of urinary metabolic profiling (UMP) using gas chromatography/mass spectrometry (GC/MS) for the diagnosis of homocystinuria (Table 24). Results of the analysis of urine samples from patients with homocystinuria were compared with results using the same method to analyse samples from controls. In comparison with control patients, elevated levels of homocystine (undetectable versus 4.35–26.36 mmol/mol creatinine) and methionine (3.00 versus 11.8–30.73) were observed in samples from patients with homocystinuria. Levels of other metabolites measured, including cystine, methylmalonate, uracil, orotate and cystathionine were not elevated in samples from patients with homocystinuria (Table 24). Intra-assay variability was determined by repeated GC/MS evaluation of a derivatised sample from urine of a patient with type I homocystinuria. Results were not reported. Details of testing for inter-assay variability were not reported in the study.

The results of this study appear to support the use of UMP by GC/MS for the diagnosis of homocystinuria. However, details of tests used to diagnose homocystinuria and confirm the absence of IEMs in control patients were not reported, introducing potential spectrum bias into the study. In addition, the results of tests undertaken to check reproducibility of results were not reported.

This study is considered to be applicable to Australian practice, but the potential spectrum bias and lack of reproducibility results mean that the quality of the study is poor. The study represents NHMRC III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

GC/MS for diagnosing lactic acidosis

Samples from patients with lactic acidosis, or combined lactic and ketoacidosis, were subjected to UMP testing by GC/MS in a study by Landaas and Jakobs (1977) (Table 25). This quantitative analysis found that these patients had elevated urinary excretion of 2-hydroxyisovalerate (2-HV), lactate and 3- hydroxybutyrate (3-HB). It was found that 2-HV was not detectable among control subjects, whereas lactate and 3-HB levels were considerably lower than levels in samples from patients with confirmed diagnoses. These results indicate that the measurement of urinary excretion of 2-HV, lactate and 3-HB by GC/MS can be applied to confirm diagnosis of lactic acidosis or combined lactic and ketoacidosis. This study did not clearly define whether patients had congenital or secondary lactic acidosis or combined lactic and ketoacidosis. Details of intra- and inter-assay variabilities were not reported.

Details of the approaches applied to select case and control subjects were not reported in this study, details of patients' symptoms in the case group were reported. If these symptoms are considered to be representative for patients seen in clinical practice, then spectrum bias may be reduced in this study.

This non-comparative study is considered applicable to Australian clinical practice, but poor quality, because of potential spectrum bias. This study presents level III-3 evidence according to NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

Table 24 UMP performed by gas chromatography/mass spectrometry in patients with homocystinuria

Author (year) Country	Study details		UMP results		Reproducibility		Quality and applicability
	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Kuhara et al (2000) Japan	GC/MS[a]	Homocystinuria	Homocystine: 4.35–26.36 Methionine: 11.8–30.73 Cystine: 0.97–14.78 Methylmalonate: 0.48–2.04 Uracil: 3.38–13.00 Orotate: 0.43–9.60 Cystathionine: 0.00–15.60 Creatinine: 7.13–21.66 (n = 4) (Results shown in mmol/mol creatinine, range)	Homocystine: ND Methionine: 3.00 Cystine: 7.70 Methylmalonate: 1.83 Uracil: 11.80 Orotate: 6.36 Cystathionine: 0–3.4 (n = 27) (Results shown in mmol/mol creatinine, mean or range)	Determined by repeated GC/MS evaluation of a derivatised sample from urine of a patient with type I homocystinuria; results, NR	Details, NR	**Quality**: Poor NHMRC III-3 CX, Q3, P1 Spectrum bias Inadequate data reporting: details of tests used to diagnose homocystinuria and confirm absence of IEM in controls not reported Results of reproducibility studies not reported **Applicability**: Applicable

Abbreviations: GC/MS, gas chromatography/mass spectrometry; ND, not detected; NR, not reported; SD, standard deviation
[a] GC/MS analysis employed negative chemical ionisation
[b] GC/MS for diagnosing lactic acidosis

Table 25 UMP using gas chromatography/mass spectrometry for patients with lactic acidosis or combined lactic and ketoacidosis

Author (year) Country	Study details		UMP results		Reproducibility		Quality and applicability
	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Landaas and Jakobs (1977) Norway, Germany	GC/MS[a]	Lactic acidosis or combined lactic and ketoacidosis	2HV: 44.2 (2–94) Lactate: 2032 (1040–4760) 3HB: 13623 (23–43000)[b] (mg/g creatinine, mean and range) (n = 5)	2HV: ND Lactate: 10–85 3HB: 3–20 (mg/g creatinine, range)[c] (n = 9)	NR	NR	**Quality**: Poor NHMRC III-3 CX, Q3, P1 Potential spectrum bias Inadequate reporting: details of tests used to select cases and controls not reported **Applicability**: Applicable

Abbreviations: GC/MS, gas chromatography/mass spectrometry; ND, not detected; NR, not reported; SD, standard deviation

Tandem mass spectrometry for diagnosing MCADD

Bonafé et al (2000) used tandem mass spectrometry (MS/MS) to analyse urinary acylglycines in patients with proven diagnoses of medium-chain acyl-CoA dehydrogenase deficiency (MCADD). MS/MS was also used to investigate patients with proven methylmalonic aciduria (MMA) and glutaric aciduria type I (GA1). MS/MS is not considered to be an appropriate technology for investigation of MMA and GA1 (expert opinion, advisory panel). Results from patients' with MMA or GA1 are therefore not presented.

Urinary acylglycine profiles of participants in the case and control groups were compared (Table 26). In comparison with controls, patients with MCADD demonstrated elevated urinary levels of C_7-G, 7-HOG or 8-HOG, PPG and SG: 3.8 (1.3–17.8), 1.1 (0–16.7), 2.6 (0.8–306.6) and 1.7 (0–11.7), respectively (mean [range]); none of these substances were detected in the urine of control patients. Reproducibility was not reported.

The results of this study provide evidence that supports use of MS/MS for UMP in the diagnosis of patients with MCADD. Urinary excretion of several acylglycines was clearly abnormal compared with control subjects. The study is limited by its examination of patients with known MCADD; and did not include a population of patients with suspected IEMs, so did not reflect clinical practice. This study was therefore considered to include spectrum bias.

This non-comparative study is considered to be applicable to Australian clinical practice, but provides poor quality evidence because of inherent spectrum bias. Details of tests used to diagnose patients and to rule out IEMs among participants in the control group were not reported. Urinary C_6-G results in samples from control group participants were not provided, which precluded comparison between patient groups. This study represents level III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

Thin layer chromatography for diagnosing cystinuria

Giugliani et al (1987) evaluated thin layer chromatography (TLC) for its suitability to detect heterozygous cystinuria. Participants in the case and control groups were selected on the basis of prior high performance liquid chromatography (HPLC) test results. Urine samples from case and control participants were analysed using TLC and results compared with HPLC test results (Table 27). Details of intra- and inter-assay variability were not reported.

In comparison with results from prior HPLC testing, 31 of 32 participants in the case group tested positive, and 20 of 23 control subjects tested negative (Table 27). If HPLC is considered to be the reference standard in this study, the sensitivity and specificity of TLC for the diagnosis of heterozygous cystinuria are 96.9 per cent and 87.0 per cent, respectively. Based on these results, this study suggests that TLC can be used in the diagnosis of cystinuria. However, inherent spectrum bias in the case-control study design has the potential to result in overestimation of the diagnostic performance of TLC.

This non-comparative study is considered to be applicable to Australian clinical practice, but offers poor quality evidence due to spectrum bias. This study presents level III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

Table 26 UMP using tandem mass spectrometry for patients with MCADD

Author (year) Country	Study details		UMP results		Reproducibility		Quality and applicability
	Test	**IEM**	**Cases**	**Controls**	**Intra-assay variability (correlation coefficients)**	**Inter-assay variability (SD)**	
Bonafé et al (2000) Switzerland, Argentina, Italy	MS/MS	MCAD	C_6-G: 31.4 (11.8–103.2) C_7-G: 3.8 (1.3–17.8) 7-HOG or 8-HOG: 1.1 (0–16.7) PPG: 2.6 (0.8–3.6.6) SG: 1.7 (0–11.7) (n = 15 samples from MCADD patients) (Results shown as median [range] in mmol/mol creatinine, per number of samples)	C_6-G: NR C_7-G: ND 7-HOG or 8-HOG: ND PPG: ND SG: ND (n = 54 samples) (Results shown as median [range] in mmol/mol creatinine, per number of samples)	NR	NR	**Quality**: Poor NHMRC III-3 CX, Q3, P1 Spectrum bias Inadequate data reporting: details of tests used to diagnose patients and rule out IEM in controls not reported; C_6-G results in controls not reported **Applicability**: Applicable

Abbreviations: C_6-G, hexanoylglycine; C_7-G, hepatnoylglycine; 7-HOG, 7-hydroxyoctanoylglycine; 8-HOG, 8-hydroxyoctanoylglycine; MCAD, medium-chain acyl dehydrogenase; MHG, 4-methylhexanoylglycine; MS/MS, tandem mass spectrometry; ND, not detected; NR, not reported; PPG, phenylpropionylglycine; SG, suberylglycine

Table 27 UMP using thin layer chromatography for cystinuria patients

Author (year)	Study details		UMP results		Reproducibility		Quality and applicability
Country	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Giugliani et al (1987) Brazil	TLC	Cystinuria (heterozygotes)	31 subjects tested positive[a] (n = 32)	20 subjects tested negative (n = 23)	NR	NR	**Quality**: Poor NHMRC III-3 CX, Q3, P1 Potential spectrum bias Inadequate reference standard **Applicability**: Applicable

Abbreviations: IEM, inborn error of metabolism; NR, not reported; TLC, thin layer chromatography; SD, standard deviation; UMP, urinary metabolic profiling
[a] Clearly defined spots corresponding to cystine and/or lysine considered positive

High performance liquid chromatography for diagnosing non-ketotic hyperglycinaemia

Tsai et al (1980) used high performance liquid chromatography (HPLC) to determine if urinary amino acid measurement can be used in the diagnosis of non-ketotic hyperglycinaemia (NKH). Results from the analysis of urine samples from two patients with NKH were reported (Table 28). Urinary glycine excretion was markedly elevated in patients with NKH compared with age-matched control subjects (Table 28). Details of intra- and inter-assay variability were not reported.

These results suggest that quantitative analysis of urinary amino acid levels by HPLC can be used in the diagnosis of NKH. However, results of this analysis were limited by the small population (n = 2). It is unknown whether these results would be similar to other patients with NKH.

This non-comparative, applicable study is considered to provide poor quality evidence. The analysis was limited to two patients; specified criteria for test positives were not used; and there was no operator blinding to the origin of urine samples. This study is classified as providing level III-3 evidence according to the NHMRC levels of evidence for diagnostic test studies (NHMRC 2005).

Table 28 UMP using high performance liquid chromatography for analysis of non-ketotic hyperglycinaemia

Author (year) Country	Study details		UMP results		Reproducibility		Quality and applicability
	Test	IEM	Cases	Controls	Intra-assay variability (correlation coefficients)	Inter-assay variability (SD)	
Tsai et al (1980) USA	HPLC	NKH	NKH[b]: Glycine 68450–72450 (µmol/g creatinine) (n = 2)	NKH comparison: Glycine 1013–26814 (7172 ± 5480) (µmol/g creatinine) (n = 38)	NR	NR	**Quality**: Poor NHMRC III-3 CX, Q3, P1 No pre-specified criteria for test positives No blinding **Applicability**: Applicable

Abbreviations: IEM, inborn error of metabolism, HPLC, high performance liquid chromatography; NKH, non-ketotic hyperglycinaemia; Sd, standard deviation; UMP, urinary metabolic profiling
[a] Urinary amino acid excretion measured twice, range presented
[b] Two patients had urinary amino acid excretion measured three times, range presented
[c] Control urinary amino acids for same age range as cases: cystinuria, 4 months–3 years (exact age NR); NKH, newborn–3 weeks of age; where reported, mean ± standard deviation are presented

Body of evidence

Individual rankings for components of the body of evidence are shown in Table 29. . The rarity of inherited metabolic disorders meant that it was not feasible to conduct diagnostic test assessment studies without inherent bias. The studies included in this assessment were not designed as diagnostic test studies. Therefore, an evidence-based approach could not be applied to an assessment of tests for diagnosing inherited metabolic disorders. Furthermore, because UMP is an established test, it is unlikely that any high quality evidence of its effectiveness would become available in the future. These issues require consideration when examining the presented body of evidence. The literature that made up the body of evidence that informed this assessment were not designed as diagnostic test studies, so application of MSAC criteria for their appraisal (MSAC 2005) resulted in only one study being regarded as providing medium quality evidence. The rest were regarded as poor quality studies. All studies were considered to be applicable to Australian clinical practice.

Table 29 Assessment of the body of evidence for UMP in diagnosis of inherited metabolic disorders

Component	Rank	Reason
Volume of evidence	D	One study was identified where the comparative diagnostic performance of UMP could be assessed. This study is regarded as level III-2 evidence
		Several studies were identified where the performance of different technologies used for UMP could be assessed in known cases of inherited metabolic disorder. These studies are regarded as level III-3 evidence
		Within the inherited metabolic disorders considered in this assessment, evidence was limited with regard to individual diseases
		No appropriate evidence was identified regarding the use of UMP for the diagnosis of ornithine transcarbamylase deficiency
		One study was regarded as medium quality. The remainder were considered to be of poor quality
Consistency	NA	The consistency of findings from included studies cannot be assessed due to limitations in the number of studies for each metabolic disorder, the number of included patients and inadequate reporting of patient characteristics
Clinical impact [a]	B	Derived diagnostic performance data indicate that UMP has a PPV of 100% for the diagnosis of GA1 in patients with clinical presentation suggestive of metabolic disorder
		The clinical impact of the results from the remainder of included studies cannot be assessed due to limitations in the number of studies for each metabolic disorder, the number of included patients and inadequate reporting of patient characteristics
Generalisability	A	The study populations in two studies corresponded to the research question regarding detection of metabolic disorder following newborn screening
		The population in one study corresponded to the research question regarding individuals with clinical presentation suggestive of genetic metabolic disorder
		In 10 studies, inadequate data reporting meant that it was not possible to determine how cases were initially identified. It is possible that the cases selected for these studies were initially identified following newborn screening, by clinical presentation or were at-risk family members. As a result the generalisability of evidence from these studies is unclear
Applicability	B	Despite variations between technologies used for UMP in included studies and those used in current Australian clinical practice, the evidence is considered applicable to the Australian health care setting
		In clinical practice the results of UMP are not considered in isolation from other tests when considering a diagnosis of a metabolic disorder. This may reduce the applicability of the results presented here

Abbreviations: GA1, glutaric aciduria type I; NA, not assessable; PPV, positive predictive value; UMP, urinary metabolic profiling
[a] Rank applies to studies where these components can be adequately assessed

ASIEM quality assurance/educational program 1992–2007

The Australasian Society for Inborn Errors of Metabolism (ASIEM), a special interest group of the Human Genetics Society of Australasia (HGSA), has conducted a quality assurance/educational program since 1992. The program assesses the performance of several laboratories that use UMP for the detection of IEMs. The scope of IEMs under the auspices of the program includes seven of the nine considered in this evaluation. Because the body of evidence identified for this assessment about UMP for the diagnosis of metabolic disorders was limited, data from the ASIEM quality assurance/educational program is presented as additional supportive evidence.

Reference samples of urine from people previously diagnosed with IEMS were sent to the six Australian laboratories that undertake UMP testing. The reference samples included two that were normal which were included to detect false positives. To test laboratories' abilities to interpret UMP results where there is interference from medication, two urine samples were also circulated from patients in whom no evidence for an IEM had been found. The patient's age, gender and symptoms were provided for each sample, which replicated information normally available to laboratories performing UMP. The samples were tested and results evaluated by the researchers as either correct (1) or incorrect (0) diagnoses. The scores achieved by each laboratory for each reference sample were summed to determine each laboratory's overall score.

Inclusion criteria required samples to:

- be of an adequate volume to allow distribution among the participating laboratories
- enable a definitive diagnosis to be made
- be challenging for laboratories; for example, cystinuria samples were not included because this is detected easily.

Although the underlying analytical principles of UMP testing remain largely unchanged, during the conduct of this program there have been advances in technology that are considered to have resulted in improved sensitivity and specificity in UMP testing.

Scores achieved by each of the six laboratories for the IEMs relevant to this assessment are presented in Table 30. Complete sample results for all evaluated IEMs are shown in Appendix G.

Table 30 **Results from the Australian Society for Inborn Errors of Metabolism quality assurance/educational program, 1992–2007**

		Laboratory					
Reference	**Disorder**	**1**	**2**	**3**	**4**	**5**	**6**
1992-01	Normal patient on valproate (mimics non-ketotic hyperglycinaemia)	1	1	1	1	1	1
1992-03	Normal (containing compounds which interfere with UMP)	1	1	1	1	1	1
1992-07	Medium-chain Acyl-CoA dehydrogenase deficiency	1	1	1	1	1	1
1995-01	Glutaric aciduria type1	1	1	1	1	1	1
1995-06	Non-ketotic hyperglycinaemia	1	1	1	1	1	1
1997-03	Ornithine transcarbamylase deficiency (heterozygote)	1	1	1	1	1	1
1998-07	Homocystinuria	1	1	1	1	1	1
1999-03	Maple syrup urine disease	1	1	1	1	1	1
1999-04	Glutaric aciduria type 1	1	1	1	1	1	1
2000-03	Non-ketotic hyperglycinaemia	1	1	1	1	1	1
2000-06	Homocystinuria	1	1	1	1	1	1
2000-07	Medium-chain acyl-CoA dehydrogenase deficiency	1	1	1	1	1	1
2000-10	Ornithine transcarbamylase deficiency	1	1	1	1	0[a]	1
2002-03	Normal	1	1	1	1	1	1
2002-12	Homocystinuria	1	1	1	1	1	1
2003-05	Glutaric aciduria type I	1	1	1	1	1	1
2004-01	Glutaric aciduria type I	1	1	1	1	1	1
2004-05	Methylmalonic aciduria	1	1	1	1	1	1
2005-02	Normal	1	1	1	1	1	1
2005-03	Homocystinuria	1	1	1	1	1	1
	Correct diagnoses (out of a possible 20)	**20**	**20**	**20**	**20**	**19**	**20**

Key: 1 = correct diagnosis; 0 = incorrect diagnosis
a There is no apparent explanation for this result

Of the six laboratories five correctly diagnosed all disorders including a negative diagnosis for each of the normal urine samples. A single incorrect detection of ornithine transcarbamylase (OTC) deficiency was obtained by one laboratory. Although it is unknown why detection of OTC deficiency was incorrect in this instance, expert opinion of the advisory panel indicated that because the timing of the collection of urine samples is an important factor for this test, OTC deficiency can be difficult to confirm.

It is encouraging that laboratories were able to correctly detect IEMs in almost all reference samples while blinded to the samples, and appears to support the use of UMP for the detection of IEMs. However, this study was limited by the small number of samples for each IEM tested by all six laboratories for the presence of metabolites indicative of each disorder. In addition, details about patients who provided samples and the technologies used by the laboratories are not provided, and no criteria for test positivity are available. Furthermore, the case-control study design precludes the determination of how well UMP would perform in the detection of IEMs where they are merely suspected and not pre-diagnosed. Based on these results alone, it is not possible to draw categorical conclusions about whether UMP is a highly specific and sensitive test for the diagnosis of the IEMs being examined for this assessment.

Patient management

No pre-test, post-test patient management studies were identified in the literature review. In practice, a definitive diagnosis from UMP test results would impact patient management (expert opinion, advisory panel). This has a number of implications for both the patient and family members. Resources are re-directed from searching for a diagnosis to focussing on disease management. Management may include nutritional changes, medication, surgical intervention, organ transplantation and other interventions that are focussed on the specific needs of each patient.

When a diagnosis is made by UMP, family studies are often indicated. Because these disorders are inherited, there are also important considerations for family planning.

Treatment effectiveness

Evidence for the effectiveness of treatment for inherited metabolic disorders is not presented because interventions for these disorders are considered to be well established.

What are the economic considerations?

Summary

The systematic literature review identified limited appropriate comparative clinical evidence associated with urinary metabolic profiling (UMP) technologies that are currently used in Australia. Insufficient evidence meant that it was not possible to conduct either cost-effectiveness or cost-utility analyses.

A budget impact analysis was conducted to estimate the potential total financial implications for the Medicare Benefits Schedule associated with introducing UMP technologies for detecting genetic metabolic disorders for three patient populations: asymptomatic newborns with a positive screening result, individuals with a clinical presentation suggestive of genetic metabolic disorder, and at-risk family members of patients with a specific genetic metabolic disorder.

The average unit cost of a UMP test was estimated at $131.50 and was based on information provided by four of six laboratories presently providing UMP testing in Australia.

According to the most recently available laboratory data, the utilisation of UMP tests in Australia was estimated to be approximately 11,150 patients annually. Based on the unit costs and total number of UMP tests performed, the potential financial implications associated with UMP tests is estimated to be $1,466,225 ($1,070,400 to $2,140,800) annually and is not expected to increase significantly over the next five years.

There are likely to be potential downstream cost savings associated with UMP testing by avoiding alternative investigations that would be required in the absence of UMP testing and allowing more timely diagnosis and management, however these are difficult to quantify.

Background and approach

Technologies associated with UMP are currently funded either by state governments or are paid for privately by patients. Further, the estimates of unit costs associated with UMP technologies vary among states. A review of the available literature revealed a lack of published economic analyses of UMP technologies.

UMP technologies used for both amino acid and organic acid analyses vary among Australian states. The systematic literature review revealed limited appropriate comparative clinical evidence associated with UMP technologies currently used in Australia (see *Results of assessment*). The evidence from the literature was insufficient to inform either cost-effectiveness or cost-utility analyses.

The limited body of comparative clinical evidence prompted conducting a budget impact analysis to estimate the total financial implications on the Medicare Benefits Schedule associated with introducing UMP technologies for detecting genetic metabolic disorders. The three populations considered in this analysis were:

- asymptomatic newborns with a positive screening result
- individuals with a clinical presentation suggestive of a genetic metabolic disorder
- at-risk family members of patients with a specific genetic metabolic disorder.

Unit costs

A lack of published cost estimates associated with UMP technologies in Australia meant that the total cost of UMP testing was estimated based on information provided by laboratories performing UMP in each state. This information was available from four of the six laboratories that presently provide UMP testing in Australia. All laboratories are attached to major teaching hospitals. The average unit cost of a UMP test was estimated at $131.50 and varied from $96 to $192. A number of factors could be the attributed to the variation in costs such as type of technology, volume of tests conducted, and overhead costs. This estimate was based on average unit cost estimates from Queensland, New South Wales, South Australia and Western Australia. It was assumed that New South Wales performed all UMP tests for people from the ACT, Victoria for the Tasmanian population, and South Australia for inhabitants of the Northern Territory. It was not possible to obtain a cost breakdown in terms of costs for variables such as consumables or staff time. Furthermore, the procedures for determining overheads vary among institutions, and in part, this is responsible for cost variations.

Patient population

Australian specific epidemiological data were not available for all patient populations eligible for UMP testing. Because UMP is not currently covered by public reimbursement systems, these estimates were not available from the Department of Health and Ageing. Data were therefore obtained from laboratories performing UMP in each state[4]. The same assumptions about state and territory relationships for the provision of UMP testing relating to costs were also applied to determine patient populations. The utilisation of UMP tests in Australia is estimated to be approximately 11,150 patients annually; most tests (approximately 98%) are performed to investigate individuals with clinical presentations suggestive of metabolic disorder. The total patient population is not expected to increase significantly over the next five years (expert opinion, advisory panel).

[4] The actual number of tests performed over a 12 month period was available for five of the six laboratories performing UMP in each state (source, advisory panel). The number of tests performed in the remaining laboratory was estimated.

Financial implications

Table 31 indicates the total potential financial implications associated with introducing UMP testing on the Medicare Benefits Schedule (MBS). These estimates are based on average unit costs from all states.

Table 31 Total potential financial implications for introducing UMP testing on the Medicare Benefits Schedule

Expected number of patients in Australia	Average unit cost	Potential total annual cost
11,150	$131.50	$1,466,225

Based on the average unit costs and the estimated number of UMP tests performed annually, the total cost associated with UMP tests is estimated to be $1,466,225 annually and is not expected to increase significantly over the next five years. Because the total number of eligible patients was estimated based on actual utilisation of UMP tests in Australia, rather than epidemiological data, sensitivity analysis based on population estimates was not possible. However, from a cost perspective, if the lowest (AUD$96) and highest (AUD$192) estimates for the cost of a UMP test are considered, the total financial implications vary from $1,070,400 to $2,140,800.

Potential cost savings associated with urinary metabolic profiling

Despite the absence of appropriate comparative evidence, it is important to consider the potential cost savings associated with UMP testing. In the absence of UMP testing, a number of other costly, and often invasive, laboratory tests would be required for amino acid and organic acid analyses (Appendix I). These tests often require extended analysis and interpretation time compared with UMP testing. For example, mutation analyses take about six weeks, and enzyme analyses can require up to six months for analysis. Mutational analysis for MCADD costs from $230 to $751, and enzyme analysis for GA1 costs $870 (Appendix I). In contrast, UMP testing requires only four to six hours of analysis and interpretation (expert opinion, advisory panel) and costs $131.50. In most circumstances, results from UMP testing are available within one to three days. Furthermore, UMP enables concurrent investigation for several inborn errors of metabolism (IEMs), and this is a major consideration.

In the absence of UMP, the number of tests required would vary depending on the symptoms being investigated. This makes estimating cost savings associated with UMP challenging. Depending on the particular IEM, a delay in implementing therapy could result in death or severe health consequences including mental and/or physical disability, with associated long-term management costs or death.

Other considerations

This section raises matters relating to urinary metabolic profiling (UMP) not addressed in the evidence evaluated to determine the clinical effectiveness and economic impact of the test. Information from the advisory panel and issues raised by the evaluators are presented for consideration.

Urinary metabolic profiling for excluding diagnoses of inborn errors of metabolism

The literature search identified studies that applied UMP for detecting specific inherited metabolic disorders. However, because none of the identified studies were designed as differential diagnosis studies, it was not possible to apply the available evidence to assess if UMP can exclude inborn errors of metabolism (IEMs) as part of a differential diagnosis.

In contrast, clinicians consider that the most valuable aspect of UMP, both from clinical and economic perspectives, is its use in excluding diagnoses of IEMs for patients who present with non-specific symptoms of unknown origin (expert opinion, advisory panel). Clinicians can request UMP testing without specifying a particular disorder. In this circumstance, a negative UMP result can inform differential diagnosis by excluding IEMs, indicating that other potential causes of symptoms should be investigated.

This assessment focussed on the nine most common IEMs. Although the IEMs presented in Appendix H are less common, they are nonetheless, significant and UMP can exclude a large number of rare IEMs using a single test with a rapid turn around time. This has potential to reduce costs and improve patient outcomes (see *Potential cost savings associated with urinary metabolic profiling*).

Laboratory accreditation and training

In Australia, UMP is considered to be reliable only when tests are performed by expert laboratories with appropriate National Association of Testing Authorities (NATA) accreditation (expert opinion, advisory panel). NATA accreditation requires demonstration of appropriate instrumentation, methodology and interpretative skills by laboratories.

Skilled medical and scientific staff are required to enable UMP to be conducted accurately. The Human Genetics Society of Australasia (HGSA) maintains certification processes that recognise these skills (HGSA 2008). The HGSA provides a training program and professional certification in biochemical genetics (HGSA 2008). With appropriate training and experience, clinicians can interpret tests, and all metabolic physicians and metabolic fellows-in-training can access laboratories to train in test interpretation (expert opinion, advisory panel).

Potential impact of delayed or missed diagnoses if urinary metabolic profiling is not available

Assessments of tests for the diagnosis of IEMs do not lend themselves to the application of an evidence-based approach. These conditions are rare, and there are challenges in conducting well-designed diagnostic test studies. As a consequence, evidence presented in this report is not considered to adequately represent the potential benefit of UMP on clinical outcomes.

Many IEMs can be fatal or cause severe mental and/or physical impairment if undiagnosed. Early diagnosis and appropriate treatment (where available) have the potential to extend life and avoid severe health consequences. Where the health consequences of missed or late diagnoses are severe, the resulting chronic condition is likely to be associated with significant costs for ongoing patient management.

This assessment considers the nine most common IEMs. If UMP was unavailable, and an IEM was suspected, it is possible that a definitive diagnosis would require many more tests, including but not limited to those listed in Appendix I. This has the potential to delay diagnoses and increase the associated costs in both the short and long term.

The diagnosis of an IEM can have significant implications for family planning decisions. When an IEM is diagnosed, genetic counselling for the family is one of the consequences of making the diagnosis. It is usual to offer prenatal diagnosis for future pregnancies during the diagnostic process, providing parents with the option of considering termination should IEM be detected prenatally.

Because of the potentially serious consequences of a missed diagnosis, the sensitivity of a test used for detection of IEMs is more important than the test's specificity. In most cases, the risks associated with treating a patient for an IEM based on a false positive result are minimal (expert opinion, advisory panel). On the other hand, the risks associated with a missed diagnosis are potentially much more severe. If a diagnostic test returns a false negative result, and treatment is withheld on this basis, there is a significant risk that the patient will develop serious mental and/or physical disabilities. The management of patients with an IEM who remain untreated can incur substantial social and economic costs.

Applicability of technology

The studies evaluated for this assessment applied a variety of UMP technologies. There is some variation among technologies used in included studies and those currently used in Australian clinical practice (see Table 2), but they are considered to be minor and unlikely to affect the applicability of the included studies (expert opinion, advisory panel).

The patient journey

Urinary metabolic profiling (UMP) is a non-invasive test performed on patients' urine samples. The UMP procedure is therefore not considered to impose safety issues for patients. Alternative tests often require extended analysis and interpretation time compared to UMP testing and are unlikely to cover such a broad range of rare disorders. Therefore, potential delayed diagnosis associated with using other tests may lead to further long term health and cost consequences. Inborn errors of metabolism (IEM) can be diagnosed or excluded by undertaking UMP. When diagnoses are confirmed, patients' first degree relatives may also be tested for the condition to determine carrier status. The results have important implications for the future health of family members and for family planning decisions and resources are re-directed from searching for a diagnosis to focus on disease management. Management may include nutritional changes, medication, surgical intervention, organ transplantation and other interventions that are focussed on the specific needs of each patient.

Research recommendations

After reviewing the body of evidence addressing each research question the evaluators developed specific research recommendations using a modified EPICOT (evidence, population, intervention, comparison, outcome, time stamp) format (Brown et al 2006). The research recommendations also address the prior test element. The research recommendations were formulated to address the identified gaps in the body of evidence for the use of urinary metabolic profiling (UMP) to detect inborn errors of metabolism (IEMs) following newborn screening (Table 32).

A systematic review of the evidence identified two studies allowing a comparison of UMP with plasma carnitine analysis, plasma acylcarnitine analysis and mutation analysis (Carpenter et al 2001, Waddell et al 2006). These studies were not designed as comparative diagnostic test studies.

Table 32 Research recommendations for assessment of urinary metabolic profiling to detect genetic metabolic disorders following newborn screening

Element	Description
Evidence	Two studies reported the use of UMP for newborns with screening results indicative of MCADD. MCADD diagnosis was made on the basis of UMP, plasma carnitine analysis and mutation analysis
	Patients with elevated acylcarnitine were positive for at least one copy of the A985G mutation
	Newborns with high levels of octanoylcarnitine detected by MS/MS were suspected of having MCADD. This was confirmed among all patients with increased urinary hexanoyglycine and plasma octanoylcarnitine
Population	Asymptomatic newborns with a positive screening test
Prior tests	Routine newborn screening tests[a]
Intervention/test	UMP[b]: by HPLC, GC/MS, TLC, HVE, MS/MS
Comparator[a]	UMP not available: See Appendix I for alternative tests
Outcome	Change in clinical outcomes[c]
	Change in clinical management[d]
	Diagnostic accuracy [e]
	Safety outcomes[f]
Time stamp	October 2007

Abbreviations: GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MS/MS, tandem mass spectrometry; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] Tandem mass spectrometry result indicative of possible (genetic) metabolic disorder; manual chemical fluorescence test for galactose metabolites (to detect galactosaemias): this test is not performed in Victoria

[b] High performance liquid chromatography (quantitative for amino acids); gas liquid chromatography (semi-quantitative for organic acids); high voltage electrophoresis (qualitative for amino acids); tandem mass spectrometry (quantitative for amino acids); thin layer chromatography (qualitative for amino acids

[c] Survival (overall survival); morbidity (disease progression)

[d] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD)—avoidance of catabolism, emergency regimen; glutaric aciduria I (GA1)—emergency regimen, low protein diet, carnitine medication; ornithine transcarbamylase(OTC) deficiency—low protein diet, complex medications, liver transplantation for severe cases; non-ketotic hyperglycinaemia (NKH)—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; methylmalonic aciduria (MMA)—vitamin B_{12} in some cases, low protein diet; maple syrup urine disease (MSUD)—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDH, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GAI, OTC deficiency, MMA, and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis

[e] Sensitivity and specificity estimates, positive and negative likelihood ratios, summary diagnostic measures (eg diagnostic odds ratio, summary receiver operating characteristics)

[f] Safety outcomes were considered not applicable because UMP is non-invasive and requires only patient urine samples

The research recommendations outlined in Table 33 were formulated to address the assessment of urinary metabolic profiling of individuals with clinical presentation suggestive of genetic metabolic disorder.

A systematic review of the evidence identified one study (Korman et al 2007) that was considered to provide comparative evidence for UMP for this research question. This study was not designed as a diagnostic test study.

The research recommendations outlined in Table 34 were formulated to address the assessment of urinary metabolic profiling of at-risk family members of patients with specific metabolic diseases.

A systematic review of the evidence did not identify any evidence that enabled the performance of UMP to be compared with other tests.

The systematic review of the evidence also identified several studies where the performance of UMP was examined in known cases and controls subjects (Landaas and Jakobs 1977, Tsai et al 1980, Giugliani et al 1987, Bonham Carter et al 1991, Pitt 1993, Bonafé et al 2000, Costa et al 2000, Kuhara et al 2001, Schor et al 2002, Paik et al 2005). These studies were not designed as diagnostic case-control studies and offer inadequate patient characteristic data. These studies were considered to provide evidence for the assessment of urinary metabolic profiling in both individuals with clinical presentation suggestive of genetic metabolic disorder (Table 33), and at-risk family members of patients with specific metabolic diseases (Table 34).

Table 33 Research recommendations for assessment of urinary metabolic profiling for individuals with clinical presentation suggestive of genetic metabolic disorders

Element	Description
Evidence	One study showed that there was an increased urinary excretion of GA and 3-HGA in patients who were symptomatic for GA1. Plasma free carnitine deficiency was marked in all patients who had elevated 3-HGA and elevated GA excretion. All patients had a mutation in the GCDH gene
	Three studies showed that patients with MCADD had elevated levels of urinary acylglycines compared with controls
	One study showed that 2-HV was the most abundant metabolite in the urine of the MSUD patient, followed by leucine and valine and methylmalonic acid was elevated in the urine of MMA patients, both in comparison with controls
	Patients with GA1 had elevated excretion of urinary 3-HGA in comparison with controls
	Patients with homocystinuria had elevated urinary levels of homocystine and methionine in comparison with controls
	Patients with lactic acidosis had elevated excretion of urinary 2-HV, lactate and 3-HB in comparison with controls
	MS/MS was able to confirm patients diagnosed with MCADD from known controls
	TLC was able to confirm the diagnosis for patients known to have cystinuria from known controls
	HPLC was able to confirm diagnosis for patients known to have NKH
	No studies were found on any of the technologies assessing OTC deficiency in known cases and controls
Population	Individuals with clinical presentation[a] and/or test results suggestive of genetic metabolic disorders
Prior tests	Tests[b] to investigate potential reasons for non-specific symptoms
Intervention/test	UMP[c] by HPLC, GC/MS, TLC, HVE, MS/MS
Comparator	UMP not available: See Appendix I for alternative tests
Outcome	Change in clinical outcomes[d]
	Change in clinical management[e]
	Diagnostic accuracy[f]
	Safety outcomes[g]
Time stamp	October 2007

Abbreviations: 2-HV, 2-hydroxyisolvalerate; 3-HGA, 3-hydroxyglutaric acid; 3-HB, 3-hydroxybutyrate; GA, glutaric acid; GA1, glutaric aciduria type I; GCDH, glutaryl CoA dehydrogenase; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MS/MS, tandem mass spectrometry; MSUD, maple syrup urine disease; NKH, non-ketotic hyperglycinaemia; OTC, ornithine transcarbamylase; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] Possible symptoms include encephalopathy, seizures, hypotonia, dystonia and spasticity, vomiting, failure to thrive, jaundice and other signs of liver failure, cardiomyopathy, rhabdomyolysis and renal calculi, skeletal abnormalities, dislocation of the lenses, abnormal hair. This list is not exhaustive because of the wide range of possible symptoms that may be caused by genetic metabolic disorders. Examples of specific symptoms highly suggestive of a metabolic disorder in an acute presentation include clinically unexplained encephalopathy; coma and increased respirations, with metabolic acidosis

[b] Tests used will vary depending on the particular symptoms, but routine investigations include: blood tests for electrolytes, glucose, ammonia, lactate, acid base, urea, creatinine, calcium, magnesium, phosphorus, creatine kinase, lead; liver function tests; full blood count; thyroid function tests, imaging abdominal ultrasound; magnetic resonance imaging of the head , skeletal survey; electroencepholgram for patients with seizures. This is not an exhaustive list because of the wide range of possible symptoms that may be encountered in clinical presentations suggestive of genetic metabolic disorder

[c] HPLC, quantitative for amino acids; GC/MS, semi-quantitative for organic acids; HVE, qualitative for amino acids; MS/MS, quantitative for amino acids; TLC, qualitative for amino acids

[d] Survival (overall survival); morbidity (disease progression)

[e] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; MCADD—avoidance of catabolism, emergency regimen; GA1—emergency regimen, low protein diet, carnitine medication; OTC deficiency—low protein diet, complex medications, liver transplantation for severe cases; NKH—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; MMA—vitamin B_{12} in some cases, low protein diet; MSUD—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDH deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GA1, OTC, MMA and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis

[f] Sensitivity and specificity estimates; positive and negative likelihood ratios; summary diagnostic measures (eg diagnostic odds ratio, summary receiver operating characteristics)

[g] Safety outcomes: Considered not applicable because UMP is non-invasive and requires only patients' urine samples

Table 34 Research recommendations for assessment of urinary metabolic profiling for at-risk family members of patients with specific genetic metabolic diseases

Element	Description
Evidence	One study provided evidence that UMP by GC/MS can be used to exclude diagnosis of MCADD in close family members of patients known to have the condition
	One study provided evidence that UMP by GC/MS can be used to confirm diagnosis by mutational analysis in close family members of patients known to have GA1)
	Three studies showed that patients with MCADD had elevated urinary levels acylglycines compared with controls
	One study showed that 2-HVA was the most abundant metabolite in the urine of the MSUD patient, followed by leucine and valine and methylmalonic acid was elevated in the urine of MMA patients, both in comparison with controls
	Urinary 3-HGA excretion was elevated in GA1 patients in comparison with control
	There were elevated urinary levels of homocystine and methionine in patients with homocystinuria in comparison with controls
	Elevated urinary excretion of 2-HV, lactate and 3-HB were detected in patients with lactic acidosis in comparison with controls
	MS/MS was able to confirm patients diagnosed with MCADD from known controls
	TLC was able to confirm the diagnosis for patients known to have cystinuria from known controls
	HPLC was able to confirm diagnosis for patients known to have NKH
	No studies were found on any of the technologies assessing OTC deficiency in known cases and controls
Population	At-risk family members of a patient with a specific genetic metabolic disease
Prior tests	No prior tests
Intervention/test	UMP [a] by HPLC, GC/MS, TLC, HVE, MS/MS
Comparator[a]	UMP not available: See Appendix I for alternative tests
Outcome	Change in clinical outcomes[b]
	Change in clinical management[c]
	Diagnostic accuracy[d]
	Safety outcomes[e]
Time stamp	October 2007

Abbreviations: 2-HV, 2-hydroxyisolvalerate; 3-HGA, 3-hydroxyglutaric acid; 3-HB, 3-hydroxybutyrate; GA1, glutaric aciduria type I; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; HVE, high voltage electrophoresis; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MS/MS, tandem mass spectrometry; MSUD, maple syrup urine disease; NKH, non-ketotic hyperglycinaemia; OTC, ornithine transcarbamylase; TLC, thin layer chromatography; UMP, urinary metabolic profiling

[a] HPLC, quantitative for amino acids; GC/MS, semi-quantitative for organic acids; HVE, qualitative for amino acids; MS/MS, quantitative for amino acids; TLC, qualitative for amino acids

[b] Survival (overall survival); morbidity (disease progression)

[c] Treatments vary depending on the specific genetic metabolic disorder: cystinuria—hydration, medication; MCADD—avoidance of catabolism, emergency regimen; GAI—emergency regimen, low protein diet, carnitine medication; OTC deficiency—low protein diet, complex medications, liver transplantation for severe cases; NKH—no effective treatment in most instances—treatment for late onset includes low protein diet, benzoate, dextromethorphan; homocystinuria (CβS deficiency)—pyridoxine, betaine, low protein, other medications; MMA—vitamin B_{12} in some cases, low protein diet; MSUD—low protein, branched chain-free amino acid supplement; lactic acidosis—ketogenic diet for PDH deficiency, vitamin cocktails possibly useful. Genetic counselling for pre-natal diagnosis is a component of the management of GAI, OTC deficiency, MMA; and MSUD. Genetic counselling is also a component of the management of cystinuria, homocystinuria, and lactic acidosis

[d] Sensitivity and specificity estimates; positive and negative likelihood ratios; summary diagnostic measures (eg diagnostic odds ratio, summary receiver operating characteristics)

[e] Safety outcomes: Considered not applicable because UMP is non-invasive and requires only patients' urine samples

Conclusions

Safety

Urinary metabolic profiling (UMP) is a non-invasive test conducted on patients' urine samples. The UMP procedure is not considered to present safety issues for patients.

Effectiveness

A linked evidence approach was undertaken to evaluate the diagnostic effectiveness of UMP and its impact on patient management and treatment.

There were 13 studies identified for inclusion in the analysis (Bonafé et al 2000, Bonham Carter et al 1991, Carpenter et al 2001, Costa et al 2000, Giugliani et al 1987, Kuhara et al 2001, Korman et al 2007, Landaas and Jakobs 1977, Paik et al 2005, Pitt 1993, Schor et al 2002, Tsai et al 1980, Waddell et al 2006).

Of the included studies, results reorted by Korman et al (2007) enabled comparison of UMP with a pre-specified comparator test. Findings from Carpenter et al (2001) and Waddell et al (2006) informed consideration of the role of UMP in the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency (MCADD) in screened newborns. Korman et al (2007) investigated UMP to establish its applicability in the detection of glutaric aciduria type I (GA1). These studies were considered to provide significant evidence to support UMP use for diagnosing inherited metabolic disorders in newborns whose screening results are positive (Carpenter et al 2001, Waddell et al 2006) and among symptomatic patients (Korman et al 2007). Results however provided limited evidence because the studies were not designed as diagnostic test investigations or direct comparisons with other tests. The study by Korman et al (2007) was subject to selection bias for the purpose of assessing the diagnostic performance of UMP because patient inclusion was based on results of the reference standard.

The remaining studies (Bonafé et al 2000, Bonham Carter et al 1991, Costa et al 2000, Giugliani et al 1987, Kuhara et al 2001, Landaas and Jakobs 1977, Paik et al 2005, Pitt 1993, Schor et al 2002, Tsai et al 1980) enabled the results of UMP conducted on urine samples from patients with previously diagnosed inborn errors of metabolism (IEMs) to be compared with those from healthy controls. These results indicated that UMP is a reliable means of diagnosing the IEMs considered in this assessment, but the reliability of this evidence was limited by studies' designs. Inherent spectrum bias, introduced by including patients with known IEMs, and inadequate blinding of samples to researchers were common problems among these studies. Overall, the quality of the available evidence was poor.

A quality assurance/educational program study was also identified that involved sending blinded reference samples of urine from patients with known IEMs to each of the six laboratories undertaking UMP testing in Australia (ASIEM quality assurance/educational program 1992–2007, JR Harrison, personal communication, May 2008). Of the six laboratories that participated in the study five correctly diagnosed all disorders, including reporting negative results for two normal urine samples containing compounds which

may give false positive results and two samples from patients in whom no evidence for an IEM had been found. Only one laboratory reported a false negative result for ornithine transcarbamylase deficiency. These findings appear to support UMP use to detect IEMs. The ASIEM study was limited by the small number of samples tested for each IEM by each laboratory. Details about the patients who supplied the samples and the technologies used by the laboratories were not reported, and criteria for test positivity were unavailable. The case-control study design precluded determination of the success of UMP to diagnose IEMs where there is clinical suspicion, but no confirmed diagnoses. Based on these results, it was not possible to make categorical conclusions about UMP test specificity and sensitivity for diagnosing the IEMs considered in this assessment.

Cost-effectiveness

The systematic literature review revealed a lack of appropriate comparative clinical evidence associated with UMP technologies currently used in Australia. It was therefore not feasible to conduct either cost-effectiveness or cost-utility analyses. A budget impact analysis was conducted to estimate the total financial implications to the Medicare Benefits Schedule associated with introducing UMP technologies for detection of genetic metabolic disorders for three patient populations: asymptomatic newborns with positive screening results, people with clinical presentations suggestive of genetic metabolic disorders, and at-risk family members of patients with specific genetic metabolic disorders. The average cost of a UMP test was estimated to be $131.50. This estimate was based on data provided by four of the six laboratories that are associated with teaching hospitals from those Australian states that conduct UMP testing.

According to the most recently available private laboratory data, UMP tests were performed for approximately 11,150 patients in Australia annually. Based on the unit costs and total number of UMP tests performed, the potential financial implications associated with UMP tests were estimated to be $1,466,225 ($1,070,400–$2,140,800) annually and not expected to increase significantly over the next five years.

Despite the absence of appropriate comparative evidence, it is important to consider the potential cost savings associated with UMP testing. In the absence of UMP testing, a number of other tests would be required to investigate suspected IEMs, resulting in increased test costs. Furthermore, alternative tests often require extended analysis and interpretation time compared with UMP testing. Depending on the particular IEM, a delay in applying alternative tests and implementing therapy could result in severe health consequences including mental and/or physical disability, with associated long-term management costs.

Advice

MSAC has considered the strength of the evidence in relation to the safety, effectiveness and cost-effectiveness of urinary metabolic profiling compared with alternative investigations for the detection of metabolic disorders for the three following indications:

1. Asymptomatic newborns with a positive screening result suggestive of metabolic disorder.

2. Individuals with a clinical presentation suggestive of a metabolic disorder.

3. At-risk family members of patients with specific genetic metabolic disorder.

The evidence was limited by three factors:

- the rarity of the various metabolic disorders such that conventional high quality comparative diagnostic test studies are not possible
- the large number of metabolic disorders that can be diagnosed by urinary metabolic profiling
- that urinary metabolic profiling has been standard practice in Australia for many years.

MSAC finds that urinary metabolic profiling is either as safe or safer than alternative investigations to diagnose metabolic disorders.

MSAC finds that urinary metabolic profiling is effective in diagnosing metabolic disorders and is likely to be more effective than alternative investigations in allowing a timely diagnosis, especially in patients with undifferentiated presentations where a metabolic disorder is suspected or needs to be excluded.

Given the lack of high quality comparative evidence of effectiveness, a cost-effectiveness analysis could not be conducted and a budget impact analysis was performed. Whilst it is likely that downstream costs incurred by alternative investigations or delayed diagnosis are avoided by the use of urinary metabolic profiling, these could not be formally costed. MSAC considers it is likely that urinary metabolic profiling in carefully selected patients is cost-effective.

The current funding arrangements adequately capture the target population. MSAC advises that current public funding arrangements within the health care system should continue to be supported for this procedure.

—The Minister for Health and Ageing noted this advice on 8 December 2008—

Appendix A MSAC terms of reference and membership

MSAC's terms of reference are to:

- advise the Minister for Health and Ageing on the strength of evidence pertaining to new and emerging medical technologies and procedures in relation to their safety, effectiveness and cost-effectiveness and under what circumstances public funding should be supported
- advise the Minister for Health and Ageing on which new medical technologies and procedures should be funded on an interim basis to allow data to be assembled to determine their safety, effectiveness and cost-effectiveness
- advise the Minister for Health and Ageing on references related either to new and/or existing medical technologies and procedures, and
- undertake health technology assessment work referred by the Australian Health Ministers' Advisory Council (AHMAC) and report its findings to AHMAC.

The membership of MSAC comprises a mix of clinical expertise covering pathology, nuclear medicine, surgery, specialist medicine and general practice, plus clinical epidemiology and clinical trials, health economics, consumers, and health administration and planning:

Member	Expertise or affiliation
Dr Stephen Blamey (Chair)	general surgery
Associate Professor John Atherton	cardiology
Associate Professor Michael Cleary	emergency medicine
Associate Professor Paul Craft	clinical epidemiology and oncology
Dr Kwun Fong	thoracic medicine
Professor Richard Fox	medical oncology
Dr William Glasson	ophthalmology
Professor Jane Hall	health economics
Professor John Horvath	Chief Medical Officer, Department of Health and Ageing
Associate Professor Terri Jackson	health economics
Professor Brendon Kearney	health administration and planning
Associate Professor Frederick Khafagi	nuclear medicine
Associate Professor Ray Kirk	health research
Dr Ewa Piejko	general practice
Dr Ian Prosser	haematology
Ms Sheila Rimmer	consumer health issues
Dr Judy Soper	radiology
Professor Ken Thomson	radiology
Dr David Wood	orthopaedics

Appendix B Advisory panel

Advisory panel for MSAC application 1114
Urinary metabolic profiling for the detection of metabolic disorders

Associate Professor John Atherton (Chair) Cardiology	Member of MSAC
Associate Professor Donald Perry-Keene (Second chair) Endocrinology	Former Member of MSAC
Mr John Rodney Harrison Genetic medicine	Australian Institute of Medical Scientists
Dr Walid Jammal General practice	Royal Australian College of General Practitioners
Mr Brian Stafford Consumer health	Consumers' Health Forum Nominee
Clinical Professor Bridget Wilcken Genetic medicine	Human Genetics Society of Australasia
Dr John C Coakley Pathology	Royal College of Pathologists of Australasia

Appendix C Quality criteria

Study design	Quality checklist
Systematic review	Was the research question specified? Was the search strategy documented and adequate? Were the inclusion and exclusion criteria specified, appropriate and applied in an unbiased way? Was a quality assessment of included studies undertaken? Were the methods of the study appraisal reproducible? Were the characteristics and results of the individual studies summarised? Were the methods for pooling the data appropriate? Were sources of heterogeneity explored? Was a summary of the main results and precision estimates reported?
Studies evaluating effectiveness of an intervention on health outcomes	
Randomised controlled trial	Were the inclusion and exclusion criteria specified? Was the assignment to the treatment groups really random? Was the treatment allocation concealed from those responsible for recruiting subjects? Was there sufficient description about the distribution of prognostic factors for the treatment and control groups? Were the groups comparable at baseline for these factors? Were outcome assessors blinded to the treatment allocation? Were the care providers blinded? Were the subjects blinded? Were all randomised participants included in the analysis? Was a point estimates and measure of variability reported for the primary outcome?
Cohort study	Were subjects selected prospectively or retrospectively? Was the intervention reliably ascertained? Was there sufficient description about how the subjects were selected for the new intervention and comparison groups? Was there sufficient description about the distribution of prognostic factors for the new intervention and comparison groups? Were the groups comparable for these factors? Did the study adequately control for potential confounding factors in the design or analysis? Was the measurement of outcomes unbiased (ie blinded to treatment group and comparable across groups)? Was follow up long enough for outcomes to occur? What proportion of the cohort was followed-up and were there exclusions from the analysis? Were drop-out rates and reasons for drop-out similar across intervention and unexposed groups?

Study design	Quality checklist
Case-control study	Was there sufficient description about how subjects were defined and selected for the case and control groups?
	Was the disease state of the cases reliably assessed and validated?
	Were the controls randomly selected from the source of population of the cases?
	Was there sufficient description about the distribution of prognostic factors for the case and control groups? Were the groups comparable for these factors?
	Did the study adequately control for potential confounding factors in the design or analysis?
	Was the new intervention and other exposures assessed in the same way for cases and controls and kept blinded to case/control status?
	How was the response rate defined?
	Were the non-response rates and reasons for non-response the same in both groups?
	Was an appropriate statistical analysis used?
	If matching was used, is it possible that cases and controls were matched on factors related to the intervention that would compromise the analysis due to over-matching?
Case series	Was the study based on a representative sample selected from a relevant population?
	Were the criteria for inclusion and exclusion explicit?
	Did all subjects enter the survey at a similar point in their disease progression?
	Was follow up long enough for important events to occur?
	Were the techniques used adequately described?
	Were outcomes assessed using objective criteria or was blinding used?
	If comparisons of sub-series were made, was there sufficient description of the series and the distribution of prognostic factors?
Study of diagnostic accuracy	Was the spectrum of patients representative of the patients who will receive the test in practice?
	Were selection criteria clearly described?
	Is the reference standard likely to correctly classify the target condition?
	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?
	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?
	Did patients receive the same reference standard regardless of the index test result?
	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?
	Was the execution of the index test described in sufficient detail to permit replication of the test?
	Was the execution of the reference standard described in sufficient detail to permit its replication?
	Were the index test results interpreted without knowledge of the results of the reference standard?
	Were the reference standard results interpreted without knowledge of the results of the index test?
	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?
	Were uninterpretable/ intermediate test results reported?
	Were withdrawals from the study explained?

Appendix D Studies included in the review

Table 35 Characteristics and results of studies assessing urinary metabolic profiling

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Bonafé et al (2000) Argentina, Italy, Switzerland Case-control Blinding, NR[a]	**Cases**: Urine samples were collected from patients with known MCADD (n = 5 patients, 15 samples; age range, NR; gender, NR) Details of tests used to diagnose MCADD, NR **Controls**: Details, NR (n = 54 samples, number of patients, NR; age range = < 1 week–15 years; gender, NR). Details of control selection, NR	**Urine sample**: Sampling time, NR; samples were derivatised to acylglycine methylesters, acylglycine butylesters or acetylated acylglycine butylesters **Instrumentation**: ESI-MS/MS was carried out on a Perkin-Elmer SCIEX API 365 LC/MS/MS system **Internal standard**: 50 µL of internal standard contained 150 ng d3-acetylglycine, 20 ng d3-propionylglycine, 30 ng d7-butyrylglycine, 30 ng d9-valerylglycine and 20 ng d3-heanoylglycine in methanol; all internal standards were 2H-labelled in the actyl group **Creatinine measurement**: Creatinine concentrations were measured by the Jafe-modified kinetic method by a Beckman Synchron CX5 analyser **Reference standard**: NR	Urinary acylglycine excretion compared between cases and controls **Cases**: C_6-G: 31.4 (11.8–103.2) C_7-G: 3.8 (1.3–17.8) 7-HOG or 8-HOG: 1.1 (0–16.7) PPG: 2.6 (0.8–3.6.6) SG: 1.7 (0–11.7) (n = 15 samples from MCADD patients) (Results shown as median [range] in mmol/mol creatinine, per number of samples) **Controls**: C_6-G: NR C_7-G: ND 7-HOG or 8-HOG: ND PPG: ND SG: ND (n = 54 samples) (Results shown as median [range] in mmol/mol creatinine, per number of samples)	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported **Applicability**: Applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Bonham Carter et al (1991) UK Case-control Blinding, NR[a]	**Cases**: Urine samples were collected from patients with known MCADD (n = 5 patients, 15 samples; age range, NR; gender, NR) Details of tests used to diagnose MCADD, NR **Controls**: Details, NR (n = 54 samples, number of patients, NR; age range = < 1 week–15 years; gender, NR). Details of control selection, NR	**Urine sample**: Sampling time, NR; samples were derivatised to acylglycine methylesters, acylglycine butylesters or acetylated acylglycine butylesters **Instrumentation**: ESI-MS/MS was carried out on a Perkin-Elmer SCIEX API 365 LC/MS/MS system **Internal standard**: 50 µL of internal standard contained 150 ng d3-acetylglycine, 20 ng d3-propionylglycine, 30 ng d7-butyrylglycine, 30 ng d9-valerylglycine and 20 ng d3-heanoylglycine in methanol; all internal standards were 2H-labelled in the actyl group **Creatinine measurement**: Creatinine concentrations were measured by the Jafe-modified kinetic method by a Beckman Synchron CX5 analyser **Reference standard**: NR	Urinary acylglycine excretion compared between cases and controls **Patients with MCADD:** HG: 14.09–56.56 OG: 0.28–1.24 3-PPG: 6.07–87.75 SG: 8.23–130.79 (µg/mg creatinine, range)(n = 3: results for 3 members of a family who had MCADD) **Patients without MCADD:** HG: 0.38–1.13 OG: ND–0.02 3-PPG: 0.03–0.28 SG: 0.13–0.37 (µg/mg creatinine, range)(n=6: results for 6 members of a family who did not have MCADD) **Controls:** HG: 0.03–1.15 OG: ND–0.17 3-PPG: 0.01–0.09 SG: 0.02–1.53 (µg/mg creatinine, range) (n = 29)	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls not reported **Applicability**: Applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Carpenter et al (2000) Australia Prospective cohort Consecutive recruitment Blinding NR April 1998 to March 2001	Newborns with initial octanoylcarnitine levels MS/MS who had further analysis of urinary organic acids for investigation of suspected MCADD Median age at screening was 3 days; >99% of newborns sampled before day 6 (n = 17[b] of 275,653 consecutive neonates undergoing newborn screening)	**Urine sample:** Sampling time, centrifugation and/or filtration and pH adjustment, NR; organic acids extracted with ethyl acetate and converted to trimethylesters **Instrumentation:** Hewlett Packard 5890 GC and 5971 MSD; reference compounds, NR **Internal standard:** NR **Creatinine measurement:** NR **Additional tests:** Mutation analysis, NR Plasma carnitine analysis; test details, NR	Urinary acylglycine and plasma acylcarnitines measured; mutational analysis **Urinary acylglycines:** Hexanoyl: ND–grossly elevated Suberyl: ND–grossly elevated **Plasma carnitines:** (cut-off values) C6: 0.2–1.3 (<0.1) C8: 1.1–5.6 (<0.3) C10:1: 0.2–2.5 (<0.3) (µmol/L) **A985G mutational analysis**: 4/17 patients homozygous for A985G; 8/17 patients heterozygous for A985G; 12/17 patients had no A985G mutation	NA

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Costa et al (2000) Portugal and Netherlands Case-control Blinding, NR[a]	**Cases:** Patients with proven MCADD (n = 10) Details of tests used to diagnose MCADD, NR **Controls:** Children unaffected by IEM (age range 2 days–12 years, n = 19) Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling time NR; pH adjusted to 1–2 with 2N HCl and saturation with NaCl; details of sample filtration or centrifugation, NR **Instrumentation:** GC/MS—610 ATI-Unicam GC coupled to an Automass series 1 ATI-Unicam mass spectrometer (Cambridge) Reference compounds, details NR **Internal standard:** [2H7]BG, [2H7]IBG,[13C2]2MBG, [2H3]IVG, [13C2]HG, [2-13C,15N]SG, and [2-13C,15N]PPG **Creatinine measurement:** NR **Reference standard:** Tests used to diagnose MCADD, NR	Urinary acylglycine excretion compared between cases and controls **Cases:** BG = 0.40 (0.14–0.91); IBG = 1.3 (0.078–3.6); 2 MBG = 0.81 (0.20–2.0); IVG = 2.4 (0.28–6.7); HG = 41 (5.5–122); PPG = 8.9 (0.10–28); SG = 87 (5.2–359) (n = 10) (acylglycine concentrations in mmol/mol creatinine, mean and range) **Controls:** BG = 0.041 (0.007–0.12); IBG = 0.36 (0.003–1.5); 2MBG = 0.20 (0.002–0.58); IVG = 0.37 (0.027–0.92); HG = 0.3 (0.014–0.83); PPG = 0.033 (<0.002–0.15); SG = 0.14 (0.024–0.52) (n = 19) (acylglycine concentrations in mmol/mol creatinine, mean and range) **Reproducibility** [b]: Intra-assay variability: ranged from V = 0.62% for 2 MBG to V = 5.62% for BG **Inter-assay variability:** Ranged from V = 4.67% for 2 MBG to V = 13.8% for BG	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Spectrum bias Inadequate data reporting: details of tests used to diagnose MCADD and confirm absence of IEM in controls NR **Applicability**: Applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Giugliani et al (1987) Brazil Case-control Blinding, NR[a]	**Cases:** Cystinuria heterozygotes (age range, NR; n = 32) Tested positive on prior HPLC **Controls:** Normal phenotype, (age range NR, n = 23) Unclear whether controls were type I or non-cystinuric patients Prior testing by HPLC, unclear	**Urine sample:** Sampling time random, details NR; 6 mol/L HCL acidification; details of sample filtration or centrifugation, NR **Instrumentation:** TLC, NR; reference compounds NR **Internal standard:** NR **Creatinine measurement:** Details, NR **Reference standard:** Cases—prior HPLC, with automatic amino acid analyser; test details, NR	**Cases:** 31 subjects tested positive [a] (n = 32) **Controls:** 20 subjects tested negative (n = 23) **Sensitivity:** 96.9% **Specificity:** 87.0%	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Potential spectrum bias Inadequate reference standard **Applicability**: Applicable
Korman et al (2007) Israel Direction unclear Consecutive recruitment, unclear Blinding, NR 5 year period ending mid-2004	Patients diagnosed with GA1 Investigated in response to acute encephalopathy (n = 3); neurological symptoms (n = 6); macrocephaly and history of mother's pregnancy termination due to anencephaly and congenital abnormalities (n = 1); macrocephaly and suspicion of GA1 (n = 1); and close family relative of known GA1 patient (n = 1) (age range 7 days–30 years) (n = 12)	**Urine sample:** Ethylacetate/diethyl extraction and trimethylsilyl derivatisation; sample timing, NR **Instrumentation:** GC/MS; details of system and running methods NR **Internal standard:** NR **Creatinine measurement:** NR **Reference standard:** Mutational analysis of GCDH gene on genomic DNA from peripheral blood; 11 exons and flanking intronic regions of GCDH gene analysed by PCR; further details, NR **Comparator test**: Blood spot analysed for acylcarnitine by MS/MS, further details NR	Urinary excretion of GA and 3-HGA compared with plasma carnitine levels and GCDH mutational analysis **Urinary levels:** GA: 6–13344 mmol/mol creatinine; 4/12 patients had elevated levels that were not quantified 3-HGA: 15–197 mmol/mol creatinine; 4/12 patients had elevated levels that were not quantified PPV = 100% **Plasma carnitines**: Free carnitines: 2.3–18.1 µmol/L (normal range 25–35 µmol/L) Total carnitines: ND–32.7 µmol/L (normal range 35–45 µmol/L) PPV = 100% **Mutation analysis**: 8/12 patients homozygous for mutation; 4/12 patients heterozygous for mutation	NHMRC III-2 C1, Q3, P1 **Quality***:* Poor Selection bias No blinding Review bias **Applicability**: Applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Kuhara et al (2000) Japan Case-control Blinding, NR[a]	**Cases**: Symptomatic homocystinuria patients (age range, 4–20 years; n = 4) Prior tests for case selection: 2 unclear, 1 diagnosed enzymatically (details, NR), 1 diagnosed enzymatically and using UMP (details, NR) **Controls**: Normal based on neonatal screening tests (age range, NR; n = NR) Testing used during neonatal screening, unclear	**Urine sample**: Sampling time, NR; samples were treated with urease at 37°C for 10 minutes to remove excess urea, deproteinised with ethanol, centrifuged to remove precipitate and trimethylsilylated by adding a BSFTA and TMCS mixture then heating at 80°C for 30 minutes **Instrumentation**: GC/MS using bench-top HP GC-MSD (HP6890/MSD 5973) Separation was carried out on a fused-silica DB-5 (30 m x 0.25 mm ID) with a 0.25 µm film thickness of 5% phenylmethylsilicone **Internal standard**: Known amounts of stable, isotope-labelled creatinine, methionine, cystine, homocystine, uracil, orotate and methylmalonate **Creatinine measurement**: Using d3-creatinine as an internal standard, the value of endogenous creatinine and creatine were determined and the evaluation of metabolite levels relative to creatinine was reported **Reference standard**: NR	**Cases**: Homocystine: 4.35–26.36 Methionine: 11.8–30.73 Cystine: 0.97–14.78 Methylmalonate: 0.48–2.04 Uracil: 3.38–13.00 Orotate: 0.43–9.60 Cystathionine: 0.00–15.60 Creatinine: 7.13–21.66 (Results shown in mmol/mol creatinine, range) **Controls**: Homocystine: ND Methionine: 3.00 Cystine: 7.70 Methylmalonate: 1.83 Uracil: 11.80 Orotate: 6.36 Cystathionine: 0–3.4 (Results shown in mmol/mol creatinine, mean or range)	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Potential spectrum bias Inadequate reference standard **Applicability**: Applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Landaas and Jakobs (1977) Norway and Germany Case-control Blinding, NR[a]	**Cases:** Patients acutely ill with lactic acidosis or combined lactic and ketoacidosis (age range 1–27 years; n = 5) Details of tests used to diagnose patients, NR **Controls:** Healthy members of study laboratory staff (age range 21–30 years; n = 9) Details of tests to confirm absence of IEM, NR	**Urine sample:** 24 hour collections not made due to rapid change in clinical state of patients; adjusted to pH 1 by 6 M HCl; details of sample filtration or centrifugation, NR **Instrumentation:** GC/MS—Varian 1440 gas chromatograph with Varian 112 mass spectrometer; Varian 2740 gas chromatograph with double focusing mass spectrometer (Varian MAT, type 311 A) Reference compounds, details NR **Internal standard:** Malonic acid **Creatinine measurement:** NR **Reference standard:** Details of tests used to diagnose cases and confirm absence of IEM in controls, NR	**Cases**[b]: 2HV = 44.2 (2–94); lactate = 2032 (1040–4760); 3HB = 13623 (23–43000) (mean and range, mg/g creatinine) (n = 5) **Controls**: 2HV = not detectable; lactate = 10–85; 3HB = 3–20 (range[c], mg/g creatinine) (n = 9)	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Potential spectrum bias Inadequate reporting: Details of tests used to select cases and controls NR **Applicability**: Applicable
Paik et al (2005) South Korea Case-control Blinding, NR[a]	**Cases:** Patients with MSUD (age unclear; n = 1), MMA (age unclear; n = 1) Details of tests used to diagnose patients, NR **Controls:** Normal individuals (age range, 0.25–1.25 years, n = 3) Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling time, NR; samples (equivalent to 0.25 mg creatinine) adjusted to pH ≥12 after internal addition of standard; samples subjected to sequential EOC/MO/TBDMS reactions; details of sample filtration or centrifugation, NR **Instrumentation:** GC/MS Agilent 6890 gas chromatograph interfaced to Agilent 5973 mass selective detector reference compound[c] **Internal standard:** NR **Creatinine measurement:** NR **Reference standard:** Details of tests used to diagnose cases and confirm absence of IEM in controls, NR	**Cases:** MSUD patient–in retention index spectra 2-hydroxyvaleric acid was most abundant, followed by leucine and valine; MMA patient–MMA extremely elevated compared with normal average retention index spectra. The second most prominent metabolite was glycine (n = 1 MSUD; n = 1 MMA) **Controls:** Urinary excretion patterns normal (n = 3)	NHMRC III-3 CX, Q3, P1 **Quality**: Poor Details of tests used to diagnose cases and confirm absence of IEM in control patients not reported One case for each IEM **Applicability**: Applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Pitt (1993) Australia Case-control Blinding, NR[a]	**Cases:** MCADD subjects (age range, NR; n = 8) Diagnoses confirmed by DNA mutation analysis and/or a positive phenylpropionate loading test; further details of both tests, NR **Controls:** Subjects typically with mild developmental delay (similar age to MCADD subjects, age range NR, n = 15) Metabolic screens normal; test details, NR	**Urine sample:** Sampling time random, details NR. Details centrifugation &/or filtration, NR, Urine acidified with 6 mol/L HCl; organic acids converted to TMS derivatives **Instrumentation:** GC/MS, instrument details NR. Reference compounds: IG, HG, 4HMG, 7HOG, 8HOG, SG **Internal standard:** NR **Creatinine measurement:** NR **Reference standard:** Cases—diagnoses confirmed by DNA mutation analysis and/or a positive phenylpropionate loading test; further details of both tests, NR	**Cases[b]:** Acute phase (n = 4): SG: range 11–50 approx[c] HG: range 10– 0 approx[c] IG: 3–6 approx[c] 4MHG: 2–4 approx[c] (µmol/mol creatinine) Asymptomatic (n = 6): SG: ND–10 approx[c] HG: 1–9 approx[c] IG: 0.4–3 approx[c] 4MHG: 0.1–1.5 approx[c] (µmol/mol creatinine) **Controls:** IG, 4-MHG, 8HOG, 7HOG, SG, and HG undetectable (n = 15)	NHMRC III-3 CX, Q2, P1 **Quality**: Medium Spectrum bias **Applicability**: Applicable
Schor et al (2002) Netherlands Case-control Blinding, NR[a]	**Case:** Patient with GA1 (age NR) Glutaryl CoA-dehydrogenase deficiency confirmed enzymatically; test details, NR **Controls:** Children unaffected by IEM (age range, NR; n = 12) Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling time, NR; adjusted to pH <1 by 6 M HCl; saturated with NaCl and centrifuged—supernatant applied to OASIS SPE column, 3-HGA eluate converted to triflouroacetyl-di-PFB-3-HGA **Instrumentation:** GC/MS—Hewlett Packard 5890 series GC, Hewlett Packard 5989B mass spectrometer **Reference compounds:** NR **Internal standard:** $^{2}H_{4}$-3-HGA **Creatinine measurement:** NR **Reference standard:** Cases—Glutaryl CoA-dehydrogenase deficiency confirmed enzymatically; test details, NR; controls—details of tests to confirm absence of IEM, NR	**Case:** 3-HGA = 262 (mmol/mol creatinine) **Controls:** 3-HGA range = 0.88–4.5 (mmol/mol creatinine) (n = 12) **Reproducibility:** 3-HGA concentration: Intra assay[b] = 2.6 ±0.09 (n = 5) Inter assay[c] = 2.6±0.16 (n = 8) (µmol/l)	NHMRC III-3 CX, Q3, P2 **Quality:** Poor Single case included No prespecified criteria for test positives No blinding **Applicability:** applicable

Author (year) Country Study design	Population characteristics	Test characteristics	Study outcomes	Study quality
Tsai et al (1980) USA Case-control Blinding, NR[a]	**Cases:** Recessive cystinuria (aged between 4 months and 3 years; exact age, NR; n = 1); NKH patients (aged between 4 months and 3 years; exact age, NR; n = 2) Details of tests used to diagnose patients, NR **Controls:** Healthy and hospitalised newborns (newborn–3 weeks, n = 38) and infants (4 months–3 years, n = 68); excluded individuals with diseases known to cause changes in urine amino acid excretion Details of tests to confirm absence of IEM, NR	**Urine sample:** Sampling untimed, details NR; adjusted to pH 2.3 with 5 mol/L HCl; sample aliquots centrifuged and filtered (0.45 µm Millipore filter) **Instrumentation:** HPLC—121 M fully automated analyser (Beckman Instruments,) with a single 2.8x205 mm column of Beckman AA-10 ion-exchange resin **Reference compounds**: Standard calibration mixtures of 40 amino acids, glutamine and homocystine added **Internal standard:** NR **Creatinine measurement:** Determined by Jaffé reaction with an IL 919 analyser (Instrumentation Laboratory); further details, NR **Reference standard:** Details of tests used to diagnose patients and confirm absence of IEM in controls, NR	**Cases:** Cystinuria [a] (recessive): arginine 41–90; ½-cystine 572–1200; lysine 1230–5400; ornithine 25–182 (n = 1) NKH[b] glycine 68450–72450 (n = 2) (amino acid levels in µmol/g creatinine) **Controls:**[c] Cystinuria patient comparison**:** arginine 0–75; ½-cystine 39–347 (181±78); lysine 12–677 (195±135); ornithine 0–125 (µmol/g creatinine) (n = 68) NKH patients comparison**:** glycine 1013–26814 (7172± 5480) (µmol/g creatinine) (n = 38)	NHMRC III-3 CX, Q3, P1 **Quality**: Poor No prespecified criteria for test positives No blinding **Applicability**: Applicable
Waddell et al (2006) Australia Prospective, unclear Consecutive recruitment, unclear Blinding, NR April 1998 to October 2004	Newborns with initial octanoylcarnitine levels ≥1 µmol/L on newborn screening by MS/MS who had further analysis of urinary organic acids for investigation of suspected MCADD Screening performed at 48–72 hours of age (n = 36 of 592,785 neonates undergoing newborn screening)	**Urine sample:** Sampling time and preparation NR **Instrumentation:** GC/MS; further details NR **Internal standard:** NR **Creatinine measurement:** NR **Additional tests:** Mutation analysis by PCR and sequencing; further details, NR Plasma acylcarnitine analysis by MS/MS; further details, NR	Urinary hexanoylglycine and plasma carnitines measured; mutational analysis **Urinary hexanoylglycine**: ND–grossly increased **Plasma acylcarnitines**: 0.3–9.3 (µmol/L) Normal value or cut-off for MCADD not reported. Note that Carpenter et al (2001) reported cut-off values for plasma carnitines of <0.1 µmol/L for C6 and <0.3 µmol/L for C8 and C10:1 **Mutational analysis**: 35/36 patients homozygous for mutation; one patient had several genetic polymorphisms, but no variations considered pathogenic were reported	NA

Abbreviations: BG, butyrylglycine; EOC/MO/TBDMS, ethoxycarbonyl/methoxime/*tert*-butyldimethylsiyl; GA1, glutaric aciduria type I; GC/MS, gas chromatography and mass spectrometry; HG, hexanoylglycine; HB, hydroxybutyrate; 3-HGA, 3-hydroxyglutaric acid; NKH, non-ketotic hyperglycinaemia; HMG, methylhexanoylglycine; HOG, hydroxyoctanoylglycine; HPLC, high performance liquid chromatography; HV hydroxyisovalerate; IEM, inborn error of metabolism; IBG, isobutyrylglycine; IG, isocaproylglycine; IVG, isovalerylglycine; MBG, methylbutyrylglycine; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; NA, not applicable; ND, not detected; MHG, methylhexanoylglycine; MMA, methylmalonic aciduria; MS, mass spectrometer; MSUD, maple syrup urine disease; NR, not reported; PFB, pentafluourobenzyl; PPG phenylpropionylglycine; PPV, positive predictive value; SG, suberylglycine; SPE, solid phase extraction; Sn, sensitivity; Sp, specificity; TLC, thin layer chromatography; TMS, trimethysilyl; UMP, urinary metabolic profiling

[a] Either details of blinding between tests used to select cases, or controls or operators blinded to whether samples were from cases or controls

[b] Includes unpublished data for 5 newborns (B Wilcken, personal communication, June 2008)

[c] Reference values determined from ethoxycarbonyl/methoxime/*tert*-butyldimethsilyl derivatives of 186 amino, carboxylic and ketoacid standards

Appendix E Excluded studies

Excluded: Case study

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Appendix F Literature search

Search strategies were developed and applied to identify relevant studies of urinary metabolic profiling for the detection of inborn errors of metabolism. The Medline and EMBASE databases were search using the EMBASE.com interface. The PreMedline database was searched using the PubMed interface. The CDSR, DARE, CENTRAL, CMR, HTA, NHSEED databases were searched using the Cochrane Library interface. The search results for EMBASE.com are presented in Table 36. The results from the Cochrane Library are presented in Table 37. PreMedline search results are presented in Table 38.

Table 36 EMBASE.com search results for urinary metabolic profiling for detection of inborn errors of metabolism (25 October 2007)

	Keywords/search history	Results
1	'thin layer chromatography'/exp	49,300
2	'high performance thin layer chromatography'/exp	1,216
3	'high voltage electrophoresis':de	57
4	'electrophoresis'/exp	210,985
5	'high voltage':ab,ti	4,819
6	#4 AND #5	442
7	'high voltage electrophoresis':ab,ti	291
8	'thin layer chromatography':ab,ti OR 'thinlayer chromatography':ab,ti	14,955
9	'skin layer chromatography':ab,ti OR 'thin film chromatography':ab,ti	4
10	tlc:ab,ti OR hptlc:ab,ti OR hve:ab,ti	10,369
11	#1 OR #2 OR #3 OR #6 OR #7 OR #8 OR #9 OR #10	60,844
12	'amino acid analysis'/exp	8,209
13	'amino acid'/exp/dd_an	33,315
14	aa:ab,ti OR aas:ab,ti OR 'aminoacid analysis':ab,ti OR 'amino acid analysis':ab,ti	35,742
15	'amino acid assay':ab,ti OR 'aminoacid assay':ab,ti	27
16	'amino acid determination':ab,ti OR 'aminoacid determination':ab,ti	95
17	'aminoacid separation':ab,ti OR 'figlu test':ab,ti	8
18	'formimino glutamic acid excretion test':ab,ti OR 'formiminoglutamic acid excretion test':ab,ti	2
19	'forminoglutaminic acid excretion test':ab,ti OR 'formiminoglutaminic acid test':ab,ti	0
20	#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19	75,434
21	'tandem mass spectrometry'/exp	9,074
22	'tandem *1 mass spectrometry':ab,ti	9,610
23	'mass spectrometry mass spectrometry':ab,ti	253
24	MS/MS:ab,ti OR 'ms ms':ab,ti	9,454
25	#21 OR #22 OR #23 OR #24	17,402
26	'gas liquid chromatography'/exp	5,911
27	'gas chromatography'/de	66,912
28	'gas liquid chromatography':ab,ti OR 'gas chromatography':ab,ti	40,689
29	'gas liquid chromatogram':ab,ti OR 'gas liquid partition chromatography':ab,ti	20
30	'gas chromatograph mass spectrometer':ab,ti	284
31	GC–MS:ab,ti OR 'gc ms':ab,ti OR glc:ab,ti	19,853
32	#26 OR #27 OR #28 OR #29 OR #30 OR #31	93,076
33	'high performance liquid chromatography'/exp	132,043
34	'high performance liquid chromatography':ab,ti	144,498

	Keywords/search history	Results
35	'hplc':ab,ti	3
36	'high performance gel chromatography':ab,ti	0
37	'high speed liquid chromatography':ab,ti	0
38	'high pressure liquid chromatography':ab,ti	2
39	#33 OR #34 OR #35 OR #36 OR #37 OR #38	144,499
40	'ion exchange chromatography'/exp	28,353
41	'ion exchange chromatography':ab,ti	28,354
42	'cation exchange chromatography':ab,ti	37
43	'ion exchange column chromatography':ab,ti	0
44	'ionexchange chromatography':ab,ti	0
45	'ligand exchange chromatography':ab,ti	1
46	#40 OR #41 OR #42 OR #43 OR #44 OR #45	28,390
47	#39 OR #46	170,665
48	#11 OR #20 OR #25 OR #32 OR #47	378,843
49	'cystinuria'/exp	1,350
50	cystinuria:ab,ti	947
51	#49 OR #50	1,479
52	'medium-chain acyl coenzyme a dehydrogenase'/exp	474
53	'acyl coenzyme a dehydrogenase'/exp	1,068
54	'medium-chain acyl coenzyme a dehydrogenase':de	505
55	mcad:ab,ti OR 'medium-chain acyl-CoA dehydrogenase':ab,ti	704
56	mcadd:ab,ti OR 'medium-chain acyl dehydrogenase':ab,ti	30
57	'medium-chain acyl coenzyme a dehydrogenase':ab,ti	89
58	'mcaca dehydrogenase':ab,ti OR 'enzyme deficiency':ab,ti	1,526
59	#52 OR #53 OR #54 OR #55 OR #56 OR #57 OR #58	3,183
60	'aciduria'/exp	1,067
61	'acid urine':ab,ti OR aciduria:ab,ti OR 'urinary acid excretion':ab,ti OR 'urine acidity':ab,ti	2,290
62	#60 OR #61	2,643
63	'glutaric acid'/exp	733
64	'glutaric acid':ab,ti OR glutarate:ab,ti OR 'pentanedioic acid':ab,ti	737
65	#63 OR #64	1,237
67	#62 AND #65	283
68	'glutaryl coenzyme a dehydrogenase'/exp	227
69	'glutaric aciduria':ab,ti OR ga1:ab,ti OR gcdh:ab,ti	995
70	'glutaryl coenzyme a dehydrogenase':ab,ti OR 'glutaryl coa dehydrogenase':ab,ti	187
71	#67 OR #68 OR #69 OR #70	1,152
72	'disorders of amino acid and protein metabolism'/de	2,298
73	'ornithine carbamoyltransferase'/de	2,445
74	'ornithine *1 transferase':ab,ti OR ornithinecarbamoyltransferase:ab,ti	338
75	'citrulline phosphorylase':ab,ti OR 'e.c. 2.1.3.3':ab,ti OR 'ec 2.1.3.3':ab,ti OR 'enzyme 2.1.3.3':ab,ti	95
76	'ornithine transcarbamoylase':ab,ti OR 'ornithine carbamoyltransferase':ab,ti	559
77	'ornithine transcarbamylase':ab,ti OR 'ornithyl carbamyl transferase':ab,ti	944
78	'ornithine carbamyltransferase':ab,ti OR 'ornithinecarbamylic transferase':ab,ti	47
79	'ornithin carbamyl transferase':ab,ti OR 'ornithine carbamyl transferase':ab,ti	163
80	#72 OR #73 OR #74 OR #75 OR #76 OR #77 OR #78 OR #79	4,927
81	'hyperglycinemia'/de	390

	Keywords/search history	Results
82	hyperglycinaemia:ab,ti OR hyperglycinemia:ab,ti	521
83	#81 OR #82	628
84	'homocystinuria'/exp	1,978
85	'cystathionine beta synthase'/exp	880
86	'cystathionine beta synthase deficiency':ab,ti	87
87	homocystinemia:ab,ti OR homocystinaemia:ab,ti	70
88	homocystinuria:ab,ti OR 'mckusick 23620':ab,ti	1,313
89	'cystathionine beta synthetase':ab,ti OR 'cystathionine beta-synthase':ab,ti	657
90	'e.c. 4.2.1.22':ab,ti OR 'ec 4.2.1.21':ab,ti OR 'ec 4.2.1.22':ab,ti	19
91	'serine sulfhydrase':ab,ti OR 'serine sulphhydrase':ab,ti	17
92	#84 OR #85 OR #86 OR #87 OR #88 OR #89 OR #90 OR #91	3,100
93	'methylmalonic aciduria'/exp	309
94	'methylmalonic acid'/exp	1,148
95	'methylmalonyl coenzyme a mutase'/exp	408
96	'methylmalonate excretion':ab,ti OR 'methylmalonate urine level':ab,ti	9
97	'methylmalonic acid':ab,ti OR 'methylmalonic aciduria':ab,ti	943
98	mma:ab,ti OR 'e.c. 5.4.99.2':ab,ti OR 'ec 5.4.99.2':ab,ti	2,145
99	'methyl malonate':ab,ti OR 'methyl malonic acid':ab,ti OR methylmalonate:ab,ti	221
100	'methylmalonyl coenzyme a mutase':ab,ti OR 'methylmalonic coa mutase':ab,ti	31
101	'methylmalonyl coa mutase':ab,ti OR 'methylmalonyl coa coa carbonylmutase':ab,ti	285
102	'methylmalonyl coa isomerase':ab,ti OR 'methylmalonyl coenzyme a carbonylmutase':ab,ti	2
103	#93 OR #94 OR #95 OR #96 OR #97 OR #98 OR #99 OR #100 OR #101 OR #102	3,841
104	'maple syrup urine disease'/exp	1,041
105	'branched chain ketoaciduria':ab,ti OR 'branched chain ketonuria':ab,ti	37
106	leucinosis:ab,ti OR 'maple syrup disease':ab,ti OR 'mckusick 24860':ab,ti	83
107	'maple syrup urine disease':ab,ti OR msud:ab,ti	754
108	#104 OR #105 OR #106 OR #107	1,191
109	'lactic acidosis'/exp	5,474
110	'lactic acidosis':ab,ti OR lactacidosis:ab,ti OR 'lactate acidosis':ab,ti	4,280
111	'lactic acid acidosis':ab,ti OR 'lactic acidoses':ab,ti	49
112	#109 OR #110 OR #111	7,211
113	#51 OR #59 OR #71 OR #80 OR #83 OR #92 OR #103 OR #108 OR #112	25,504
114	'cystine'/exp	4,964
115	'diamino acid'/exp	35,637
116	'dibasic acid':de	4
117	'dibasic amino acid':de	3
118	'lysine'/exp	25,797
119	'arginine'/exp	39,324
120	'ornithine'/exp	3,866
121	'amino acid'/exp	713,532
122	dibasic:ab,ti	1,106
123	#121 AND #122	307
124	cystin?:ab,ti OR cystine?:ab,ti OR dicysteine:ab,ti OR mesocystine:ab,ti OR cystinate:ab,ti	6,104
125	'diamino acid':ab,ti OR diaminoacid:ab,ti OR 'diamino amino acid':ab,ti	150
126	'diaminocaproic acid':ab,ti OR 'diaminohexanoic acid':ab,ti OR 'lysyl residue':ab,ti	206
127	arginin:ab,ti OR arginine:ab,ti OR 'arginyl residue':ab,ti OR argivene:ab,ti	54,250
128	ornithin:ab,ti OR ornithine:ab,ti OR bioarginina:ab,ti OR 'l a 60 45':ab,ti	12,286

	Keywords/search history	Results
129	'dibasic amino acid':ab,ti OR 'dibasic amino acids':ab,ti	290
130	#114 OR #115 OR #116 OR #117 OR #118 OR #119 OR #120 OR #123 OR #124 OR #125 OR #126 OR #127 OR #128 OR #129	116,770
131	'acylcarnitine'/exp	938
132	'glycine derivative'/exp	1,524
133	acylcarnitine?:ab,ti OR acylglycine?:ab,ti	591
134	#131 OR #132 OR #133	2,683
135	'glutaric acid'/exp	733
136	'3 hydroxyglutarate':de	3
137	'3 hydroxyglutaric acid':de	55
138	glutaric AND 'acid'/exp AND derivative	34
139	'glutaric acid':ab,ti OR glutarat:ab,ti OR 'pentanedioic acid':ab,ti OR glutarate?:ab,ti	373
140	'3 hydroxyglutarate':ab,ti OR '3 hydroxyglutaric acid':ab,ti	71
141	#135 OR #136 OR #137 OR #138 OR #139 OR #140	1,024
142	'orotic acid'/exp	2,734
143	'amino acid'/exp	713,532
144	'orotic acid':ab,ti OR 'orotic acids':ab,ti OR orotate:ab,ti OR oroturic:ab,ti	2,095
145	lactin:ab,ti OR lactinium:ab,ti OR orodin:ab,ti OR orotyl:ab,ti OR 'vitamin b13':ab,ti	51
146	aminoacid:ab,ti OR 'amino acid':ab,ti OR 'amino acids':ab,ti OR aminoacyl:ab,ti OR aminoacids:ab,ti	280,911
147	#142 OR #143 OR #144 OR #145 OR #146	886,017
148	'glycine'/exp	29,014
149	'amino acetic acid':ab,ti OR aminoacetate:ab,ti OR 'aminoacetic acid':ab,ti	80
150	glycine:ab,ti OR glycocal:ab,ti OR glycocol:ab,ti OR glycocoll:ab,ti OR glycolixir:ab,ti	32,258
151	'aminoethanoic acid':ab,ti OR 'sodium glycinate':ab,ti OR 'sucre de gelatine':ab,ti	24
152	#148 or #149 or #150 or #151	48,225
153	'homocysteine'/exp	12,055
154	'homocystine'/exp	428
155	'methionine'/exp	22,556
156	betahomocystein:ab,ti OR homocysteine:ab,ti OR homocystine:ab,ti	11,064
157	liquimeth:ab,ti OR pedameth:ab,ti OR oradash:ab,ti OR racemethionine:ab,ti	2
158	acimetion:ab,ti OR methionine:ab,ti OR levomethionine:ab,ti OR lobamine:ab,ti	29,158
159	menin?:ab,ti OR meonine:ab,ti OR methiolate:ab,ti OR methionin?:ab,ti	29,269
160	methurine:ab,ti OR metione:ab,ti OR methidin:ab,ti	0
161	#153 OR #154 OR #155 OR #156 OR #157 OR #158 OR #159 OR #160	52,893
162	'methylmalonic aciduria'/exp	309
163	'methylmalonic acid'/exp	1,148
164	'methylmalonyl coenzyme a mutase'/exp	408
165	'methylmalonate excretion':ab,ti OR 'methylmalonate urine level':ab,ti	9
166	'methylmalonic acid':ab,ti OR 'methylmalonic aciduria':ab,ti	943
167	mma:ab,ti OR 'e.c. 5.4.99.2':ab,ti OR 'ec 5.4.99.2':ab,ti	2,145
168	'methyl malonate':ab,ti OR 'methyl malonic acid':ab,ti OR methylmalonate:ab,ti	221
169	'methylmalonyl coenzyme a mutase':ab,ti OR 'methylmalonic coa mutase':ab,ti	31
170	'methylmalonyl coa mutase':ab,ti OR 'methylmalonyl coa coa carbonylmutase':ab,ti	285
171	'methylmalonyl coa isomerase':ab,ti OR 'methylmalonyl coenzyme a carbonylmutase':ab,ti	2
172	#162 OR #163 OR #164 OR #165 OR #166 OR #167 OR #168 OR #169 OR #170 OR #171	3,841
173	'branched chain amino acid'/exp	37,603

	Keywords/search history	Results
174	'oxoacid'/exp	43,886
175	alloisoleucine:ab,ti OR isoleucin?:ab,ti OR leucin?:ab,ti OR valine:ab,ti OR isovaline:ab,ti	38,840
176	oxoacid:ab,ti OR 'keto acid':ab,ti OR 'keto acids':ab,ti OR ketoacid?:ab,ti	2,194
177	#173 OR #174 OR #175 OR #176	105,980
178	'lactic acid'/exp	31,903
179	'alanine'/exp	23,392
180	'hydroxypropionic acid':ab,ti OR 'lactic acid':ab,ti OR epilyt:ab,ti OR lactate:ab,ti	65,338
181	'aminopropionic acid':ab,ti OR alanin?:ab,ti OR levoalanine:ab,ti	38,870
182	#178 OR #179 OR #180 OR #181	124,950
183	#130 OR #134 OR #141 OR #147 OR #152 OR #161 OR #172 OR #177 OR #182	1,059,055
184	#113 AND #183	15, 206
185	#48 AND #184	1, 442

Table 37 Cochrane Library search results for urinary metabolic profiling for detection of inborn errors of metabolism (1 November 2007)

	Keywords/search history	Results
1	MeSH descriptor Cystine, this term only	31
2	MeSH descriptor Amino Acids, Diamino, this term only	3
3	MeSH descriptor Lysine explode all trees	230
4	MeSH descriptor Arginine explode all trees	770
5	MeSH descriptor Ornithine explode all trees	77
6	MeSH descriptor Amino Acids, this term only	1187
7	(dibasic)	19
8	(#6 AND #7)	0
9	(cystin? or cystine? or dicysteine or mesocystine or cystinate)	92
10	"diamino acid" or diaminoacid or "diamino amino acid"	0
11	"diaminocaproic acid" or "diaminohexanoic acid" or "lysyl residue"	0
12	(arginin or arginine or "arginyl residue" or argivene)	2021
13	(ornithin or ornithine or bioarginina or "l a 60 45")	182
14	"dibasic amino acid" or "dibasic amino acids"	2
15	(#1 OR #2 OR #3 OR #4 OR #5 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13OR #14)	2492
16	(acylcarnitine)	24
17	(acylcarnitine? or acylglycine?)	7
18	(#16 OR #17)	28
19	MeSH descriptor Glutarates explode all trees	21
20	"glutaric acid" or glutarat or "pentanedioic acid" or glutarate?	6
21	"3 hydroxyglutarate" or "3 hydroxyglutaric acid"	0
22	(#19 OR #20 OR #21)	21
23	MeSH descriptor Orotic Acid, this term only	19
24	MeSH descriptor Amino Acids explode all trees	11326
25	"orotic acid" or "orotic acids" or orotate or oroturic	38
26	(lactin or lactinium or orodin or orotyl or "vitamin b13")	5
27	(aminoacid or "amino acid" or "amino acids" or aminoacyl or aminoacids)	3845
28	(#23 OR #24 OR #25 OR #26 OR #27)	13318
29	MeSH descriptor Glycine explode all trees	324
30	"amino acetic acid" or aminoacetate or "aminoacetic acid"	4
31	(glycine or glycocal or glycocol or glycocoll or glycolixir)	649
32	"aminoethanoic acid" or "sodium glycinate" or "sucre de gelatine"	8
33	(#29 OR #30 OR #31 OR #32)	735
34	MeSH descriptor Homocysteine explode all trees	577
35	MeSH descriptor Homocystine, this term only	13
36	MeSH descriptor Methionine explode all trees	283
37	(betahomocystein or homocysteine or homocystine)	947
38	(liquimeth or pedameth or oradash or racemethionine)	1
39	(acimetion or methionine or levomethionine or lobamine)	478
40	(menin? or meonine or methiolate or methionin?)	487
41	(methurine or metione or methidin)	0
42	(#34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41)	1418
43	MeSH descriptor Methylmalonic Acid, this term only	25
44	MeSH descriptor Methylmalonyl-CoA Mutase, this term only	0
45	"methylmalonate excretion" or "methylmalonate urine level"	0
46	"methylmalonic acid" or "methylmalonic aciduria"	35
47	(mma or "e.c. 5.4.99.2" or "ec 5.4.99.2")	66
48	"methyl malonate" or "methyl malonic acid" or methylmalonate	6
49	"methylmalonyl coenzyme A mutase" or "methylmalonic coa mutase"	0
50	"methylmalonyl CoA mutase" or "methylmalonyl coa coa carbonylmutase"	0
51	"methylmalonyl coa isomerase" or "methylmalonyl coenzyme a carbonylmutase"	0
52	(#43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51)	84
53	MeSH descriptor Amino Acids, Branched-Chain explode all trees	804

	Keywords/search history	Results
54	MeSH descriptor Keto Acids explode all trees	446
55	(alloisoleucine or isoleucin? or leucin? or valine or isovaline)	1112
56	(oxoacid or "keto acid" or "keto acids" or ketoacid?)	122
57	(#53 OR #54 OR #55 OR #56)	1665
58	MeSH descriptor Lactic Acid, this term only	1187
59	MeSH descriptor Alanine explode all trees	203
60	"hydroxypropionic acid" or "lactic acid" or epilyt or lactate	4861
61	"aminopropionic acid" or alanin? or levoalanine	2377
62	(#58 OR #59 OR #60 OR #61)	7071
63	(#15 OR #18 OR #22 OR #28 OR #33 OR #42 OR #52 OR #57 OR #62)	21954
64	MeSH descriptor Cystinuria, this term only	4
65	(cystinuria)	11
66	(#64 OR #65)	11
67	MeSH descriptor Acyl-CoA Dehydrogenase, this term only	7
68	(MCADD or "medium-chain acyl-CoA dehydrogenase")	22
69	(mcadd or "medium-chain acyl dehydrogenase")	9
70	"medium-chain acyl coenzyme a dehydrogenase"	3
71	"mcaca dehydrogenase" or "enzyme deficiency"	17
72	(#67 OR #68 OR #69 OR #70 OR #71)	42
73	MeSH descriptor Glutarates explode all trees with qualifier: urine	0
74	"acid urine" or aciduria or "urinary acid excretion" or "urine acidity"	251
75	(#73 OR #74)	251
76	"glutaric acid" or glutarate or "pentanedioic acid"	4
77	(#75 AND #76)	0
78	MeSH descriptor Glutaryl-CoA Dehydrogenase, this term only	0
79	"glutaric aciduria" or ga1 or gcdh	6
80	"glutaryl coenzyme A dehydrogenase" or "glutaryl CoA dehydrogenase"	0
81	(#77 OR #78 OR #79 OR #80)	6
82	MeSH descriptor Ornithine Carbamoyltransferase, this term only	3
83	MeSH descriptor Ornithine Carbamoyltransferase Deficiency Disease, this termonly	0
84	"ornithine near/1 transferase" or ornithinecarbamoyltransferase	0
85	"citrulline phosphorylase" or "e.c. 2.1.3.3" or "ec 2.1.3.3" or "enzyme 2.1.3.3"	0
86	"ornithine transcarbamoylase" or "ornithine carbamoyltransferase"	4
87	"ornithine transcarbamylase" or "ornithyl carbamyl transferase"	1
88	"ornithine carbamyltransferase" or "ornithinecarbamylic transferase"	0
89	"ornithin carbamyl transferase" or "ornithine carbamyl transferase"	0
90	(#82 OR #83 OR #84 OR #85 OR #86 OR #87 OR #88 OR #89)	4
91	MeSH descriptor Hyperglycinemia, Nonketotic, this term only	0
92	(hyperglycinaemia or hyperglycinemia)	0
93	(#91 OR #92)	0
94	MeSH descriptor Homocystinuria, this term only	2
95	MeSH descriptor Cystathionine beta-Synthase, this term only	5
96	"cystathionine beta synthase deficiency"	1
97	(homocystinemia or homocystinaemia)	1
98	(homocystinuria or "mckusick 23620")	10
99	"cystathionine beta synthetase" or "cystathionine beta-synthase"	5
100	"e.c. 4.2.1.22" or "ec 4.2.1.21" or "ec 4.2.1.22"	0
101	"serine sulfhydrase" or "serine sulphhydrase"	0
102	(#94 OR #95 OR #96 OR #97 OR #98 OR #99 OR #100 OR #101)	15
103	MeSH descriptor Methylmalonic Acid, this term only	25
104	MeSH descriptor Methylmalonyl-CoA Mutase, this term only	0
105	"methylmalonate excretion" or "methylmalonate urine level"	0
106	"methylmalonic acid" or "methylmalonic aciduria"	35
107	(mma or "e.c. 5.4.99.2" or "ec 5.4.99.2")	66
108	"methyl malonate" or "methyl malonic acid" or methylmalonate	6

	Keywords/search history	Results
109	"methylmalonyl coenzyme A mutase" or "methylmalonic coa mutase"	0
110	"methylmalonyl CoA mutase" or "methylmalonyl coa coa carbonylmutase"	0
111	"methylmalonyl coa isomerase" or "methylmalonyl coenzyme a carbonylmutase"	0
112	(#103 OR #104 OR #105 OR #106 OR #107 OR #108 OR #109 OR #110 OR#111)	84
113	MeSH descriptor Maple Syrup Urine Disease, this term only	1
114	"branched chain ketoaciduria" or "branched chain ketonuria"	0
115	(leucinosis or "maple syrup disease" or "mckusick 24860")	0
116	"maple syrup urine disease" or msud	7
117	(#113 OR #114 OR #115 OR #116)	7
118	MeSH descriptor Acidosis, Lactic, this term only	44
119	MeSH descriptor Acidosis, this term only	168
120	MeSH descriptor Lactates, this term only	1028
121	(#119 AND #120)	24
122	"lactic acidosis" or lactacidosis or "lactate acidosis"	123
123	"lactic acid acidosis" or "lactic acidoses"	2
124	(#118 OR #121 OR #122 OR #123)	159
125	(#66 OR #72 OR #81 OR #90 OR #93 OR #102 OR #112 OR #117 OR #124)	314
126	(#63 AND #125)	223
127	MeSH descriptor Chromatography, Thin Layer, this term only	49
128	MeSH descriptor Electrophoresis explode all trees	482
129	"high voltage"	72
130	(#128 AND #129)	0
131	"high voltage electrophoresis"	0
132	"thin layer chromatography" or "thinlayer chromatography"	76
133	"skin layer chromatography" or "thin film chromatography"	0
134	(tlc or hptlc or hve)	237
135	(#127 OR #130 OR #131 OR #132 OR #133 OR #134)	339
136	MeSH descriptor Amino Acids explode all trees with qualifier: analysis	0
137	(aa or aas or "aminoacid analysis" or "amino acid analysis")	5775
138	"amino acid assay" or "aminoacid assay"	0
139	"amino acid determination" or "aminoacid determination"	3
140	"aminoacid separation" or "figlu test"	1
141	"formimino glutamic acid excretion test" or "formiminoglutamic acid excretion test"	0
142	"forminoglutaminic acid excretion test" or "formiminoglutaminic acid test"	0
143	(#136 OR #137 OR #138 OR #139 OR #140 OR #141 OR #142)	5779
144	MeSH descriptor Tandem Mass Spectrometry, this term only	15
145	MeSH descriptor Mass Spectrometry explode all trees	493
146	(tandem)	466
147	(#145 AND #146)	92
148	(tandem near/1 "mass spectrometry")	185
149	"mass spectrometry mass spectrometry"	4
150	(MS/MS or "ms ms")	51
151	(#144 OR #147 OR #148 OR #149 OR #150)	229
152	MeSH descriptor Chromatography, Gas explode all trees	471
153	"gas liquid chromatography" or "gas chromatography"	1030
154	"gas liquid chromatogram" or "gas liquid partition chromatography"	0
155	"gas chromatograph mass spectrometer"	0
156	(GC–MS or "gc ms" or glc)	167
157	(#152 OR #153 OR #154 OR #155 OR #156)	1242
158	MeSH descriptor Chromatography, High Pressure Liquid, this term only	1824
159	"high performance liquid chromatography"	1962
160	“hplc”	2031
161	"high performance gel chromatography"	0
162	"high speed liquid chromatography"	1
163	"high pressure liquid chromatography"	376

	Keywords/search history	Results
164	(#158 OR #159 OR #160 OR #161 OR #162 OR #163)	4452
165	MeSH descriptor Chromatography, Ion exchange explode all trees	20
166	"ion exchange chromatography"	14
167	"cation exchange chromatography"	0
168	"ion exchange column chromatography"	0
169	"ionexchange chromatography"	0
170	"ligand exchange chromatography"	31
171	(#165 OR #166 or #167 or #168 or #169 or #170)	4480
172	(#164 OR #171)	4191
173	(#135 OR #143 OR #151 OR #157OR #172)	11,694
174	(#126 AND #173)	17

Table 38 PreMedline search results for urinary metabolic profiling for detection of inborn errors of metabolism (18 December 2007)

	Keywords/search history	Results
1	Search cystin*[tiab] OR cystine*[tiab] OR cystinate[tiab]	7956
2	Search dicysteine[tiab] OR mesocystine[tiab]	46
3	Search "diamino acid"[tiab] OR diaminoacid[tiab]	161
4	Search "diamino amino acid"[tiab] OR "diaminocaproic acid"[tiab]	0
5	Search "diaminohexanoic acid"[tiab] OR "lysyl residue"[tiab]	240
6	Search arginin[tiab] OR arginine[tiab]	61711
7	Search "arginyl residue"[tiab] OR argivene[tiab]	154
8	Search ornithin[tiab] OR ornithine[tiab]	12648
9	Search bioarginina[tiab] OR "l a 60 45"[tiab]	1
10	Search "dibasic amino acid"[tiab] OR "dibasic amino acids"[tiab]	302
11	Search #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10	79912
12	Search acylcarnitine*[tiab] OR acylglycine*[tiab]	1253
13	Search "glutaric acid"[tiab] OR glutarat[tiab]	266
14	Search "pentanedioic acid"[tiab] OR glutarate*[tiab]	495
15	Search "3 hydroxyglutarate"[tiab] OR "3 hydroxyglutaric acid"[tiab]	59
16	Search #13 OR #14 OR #15	769
17	Search "orotic acid"[tiab] OR "orotic acids"[tiab]	1196
18	Search orotate[tiab] OR oroturic[tiab]	928
19	Search lactin[tiab] OR lactinium[tiab] OR orodin[tiab]	28
20	Search orotyl[tiab] OR "vitamin b13"[tiab]	16
21	Search aminoacid[tiab] OR "amino acid"[tiab] OR "amino acids"[tiab]	287717
22	Search aminoacyl[tiab] OR aminoacids[tiab]	5361
23	Search #17 OR #18 OR #19 OR #20 OR #21 OR #22	293359
24	Search "amino acetic acid"[tiab] OR "aminoacetic acid"[tiab]	28
25	Search aminoacetate[tiab]	34
26	Search glycine[tiab] OR glycocal[tiab] OR glycocol[tiab]	35889
27	Search glycocoll[tiab] OR glycolixir[tiab]	76
28	Search "aminoethanoic acid"[tiab]	0
29	Search sodium glycinate[tiab] OR "sucre de gelatine"[tiab]	13
30	Search #24 OR #25 OR #26 OR #27 OR #28 OR #29	36026
31	Search betahomocystein[tiab] OR homocysteine[tiab]	10507
32	Search homocystine[tiab]	306
33	Search liquimeth[tiab] OR pedameth[tiab]	2
34	Search oradash[tiab] OR racemethionine[tiab]	0
35	Search acimetion[tiab] OR methionine[tiab]	33856
36	Search levomethionine[tiab] OR lobamine[tiab]	1
37	Search menin*[tiab] OR meonine[tiab]	66945
38	Search methiolate[tiab] OR methionin*[tiab]	34222
39	Search methurine[tiab] OR metione[tiab] OR methidin[tiab]	0
40	Search #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39	109350
41	Search "methylmalonate excretion"[tiab] OR "methylmalonate urine level"[tiab]	13
42	Search "methylmalonic acid"[tiab] OR "methylmalonic aciduria"[tiab]	954
43	Search mma[tiab] OR "e.c. 5.4.99.2"[tiab] OR "ec 5.4.99.2"[tiab]	2075
44	Search "methyl malonate"[tiab] OR methylmalonate[tiab]	208
45	Search methyl malonic acid[tiab]	16

	Keywords/search history	Results
46	Search "methylmalonyl coenzyme A mutase"[tiab]	37
47	Search methylmalonic coa mutase[tiab]	0
48	Search "methylmalonyl CoA mutase"[tiab]	309
49	Search methylmalonyl coa coa carbonylmutase[tiab]	0
50	Search "methylmalonyl coa isomerase"[tiab]	3
51	Search methylmalonyl coenzyme a carbonylmutase[tiab]	0
52	Search #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51	3146
53	Search alloisoleucine[tiab] OR isoleucin*[tiab] OR leucin*[tiab]	37925
54	Search valine[tiab] OR isovaline[tiab] OR oxoacid[tiab]	11037
55	Search "keto acid"[tiab] OR "keto acids"[tiab] OR ketoacid*[tiab]	5526
56	Search #53 OR #54 OR #55	49494
57	Search "lactic acid"[tiab] OR lactate[tiab]	66206
58	Search hydroxypropionic acid[tiab] OR epilyt[tiab]	5
59	Search "aminopropionic acid"[tiab] OR alanin*[tiab] OR levoalanine[tiab]	43674
60	Search #57 OR #58 OR #59	106556
61	Search #11 OR #12 OR #16 OR #23 OR #30 OR #40 OR #52 OR #56 OR #60	597809
62	Search #11 OR #12 OR #16 OR #23 OR #30 OR #40 OR #52 OR #56 OR #60 Limits: MEDLINE	573084
63	Search #61 NOT #62	24725
64	Search cystinuria[tiab]	925
65	Search mcad[tiab] OR "medium-chain acyl-CoA dehydrogenase"[tiab]	682
66	Search mcadd[tiab] OR "medium-chain acyl dehydrogenase"[tiab]	29
67	Search "medium-chain acyl coenzyme a dehydrogenase"[tiab]	87
68	Search "mcaca dehydrogenase"[tiab] OR "enzyme deficiency"[tiab]	1336
69	Search #65 OR #66 OR #67 OR #68	2054
70	Search "acid urine"[tiab] OR aciduria[tiab]	2138
71	Search urinary acid excretion[tiab] OR "urine acidity"[tiab]	66
72	Search #70 OR #71	2204
73	Search "glutaric acid"[tiab] OR glutarate[tiab] OR "pentanedioic acid"[tiab]	738
74	Search #72 AND #73	80
75	Search "glutaric aciduria"[tiab] OR ga1[tiab] OR gcdh[tiab]	888
76	Search "glutaryl coenzyme A dehydrogenase"[tiab]	17
77	Search glutaryl CoA dehydrogenase[tiab]	183
78	Search #74 OR #75 OR #76 OR #77	977
79	Search ornithine[tiab] AND transferase[tiab]	515
80	Search ornithinecarbamoyltransferase[tiab]	1
81	Search "citrulline phosphorylase"[tiab] OR "e.c. 2.1.3.3"[tiab]	6
82	Search ec 2.1.3.3[tiab] OR "enzyme 2.1.3.3"[tiab]	114
83	Search "ornithine transcarbamoylase"[tiab]	145
84	Search ornithine carbamoyltransferase[tiab]	479
85	Search "ornithine transcarbamylase"[tiab]	945
86	Search ornithyl carbamyl transferase[tiab]	1
87	Search "ornithine carbamyltransferase"[tiab]	53
88	Search ornithinecarbamylic transferase[tiab]	0
89	Search "ornithin carbamyl transferase"[tiab]	3
90	Search ornithine carbamyl transferase[tiab]	162
91	Search #79 OR #80 OR #81 OR #82 OR #83 OR #84 OR #85 OR #86 OR #87 OR #88 OR #89 OR #90	2095

	Keywords/search history	Results
92	Search hyperglycinaemia[tiab] OR hyperglycinemia[tiab]	441
93	Search "cystathionine beta synthase deficiency"[tiab]	122
94	Search homocystinemia[tiab] OR homocystinaemia[tiab]	62
95	Search homocystinuria[tiab] OR "mckusick 23620"[tiab]	1167
96	Search "cystathionine beta synthetase"[tiab]	17
97	Search cystathionine beta-synthase[tiab]	732
98	Search "e.c. 4.2.1.22"[tiab] OR "ec 4.2.1.21"[tiab] OR "ec 4.2.1.22"[tiab]	0
99	Search "serine sulfhydrase"[tiab] OR "serine sulphhydrase"[tiab]	16
100	Search #93 OR #94 OR #95 OR #96 OR #97 OR #98 OR #99	1770
101	Search "branched chain ketoaciduria"[tiab] OR "branched chain ketonuria"[tiab]	38
102	Search leucinosis[tiab] OR "mckusick 24860"[tiab]	40
103	Search maple syrup disease[tiab]	35
104	Search "maple syrup urine disease"[tiab] OR msud[tiab]	712
105	Search #101 OR #102 OR #103 OR #104	787
106	Search "lactic acidosis"[tiab] OR lactacidosis[tiab] OR "lactate acidosis"[tiab]	3867
107	Search "lactic acid acidosis"[tiab] OR "lactic acidoses"[tiab]	40
108	Search #106 OR #107	3895
109	Search #52 OR #64 OR #69 OR #78 OR #91 OR #92 OR #100 OR #105 OR #108	15723
110	Search #52 OR #64 OR #69 OR #78 OR #91 OR #92 OR #100 OR #105 OR #108 Limits: MEDLINE	14973
111	Search #109 NOT #110	750
112	Search #63 AND #111	0

Table 39	HTA websites searched in this review
Australia	Australian Safety and Efficacy Register of New Interventional Procedures—Surgical (ASERNIP-S) http://www.surgeons.org/Content/NavigationMenu/Research/ASERNIPS/default.htm
	Centre for Clinical Effectiveness, Monash University http://www.med.monash.edu.au/healthservices/cce/evidence/
	Health Economics Unit, Monash University http://chpe.buseco.monash.edu.au
Austria	Institute of Technology Assessment / HTA unit http://www.oeaw.ac.at/ita/e1-3.htm
Canada	Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS) http://www.aetmis.gouv.qc.ca/site/index.php?home
	Institute of Health Economics (IHE) http://www.ihe.ca/index.html
	Canadian Coordinating Office for Health Technology Assessment (CCHOTA) http://www.ccohta.ca/entry_e.html
	Canadian Health Economics Research Association (CHERA/ACRES) – Cabot database http://www.mycabot.ca
	Centre for Health Economics and Policy Analysis (CHEPA), McMaster University http://www.chepa.org
	Centre for Health Services and Policy Research (CHSPR), University of British Columbia http://www.chspr.ubc.ca
	Health Utilities Index (HUI) http://www.fhs.mcmaster.ca/hug/index.htm
	Institute for Clinical and Evaluative Studies (ICES) http://www.ices.on.ca
Denmark	Danish Institute for Health Technology Assessment (DIHTA) http://www.dihta.dk/publikationer/index_uk.asp
	Danish Institute for Health Services Research (DSI) http://www.dsi.dk/engelsk.html
Finland	FINOHTA http://finohta.stakes.fi/EN/index.htm
France	L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES) http://www.anaes.fr/
Germany	German Institute for Medical Documentation and Information (DIMDI) / HTA http://www.dimdi.de/dynamic/en/index.html
The Netherlands	Health Council of the Netherlands Gezondheidsraad http://www.gr.nl/adviezen.php
New Zealand	New Zealand Health Technology Assessment (NZHTA) http://nzhta.chmeds.ac.nz/
Norway	Norwegian Knowledge Centre for the Health Services http://www.kunnskapssenteret.no/index.php?show=38&expand=14,38
Spain	Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III"I/Health Technology Assessment Agency (AETS) http://www.isciii.es/htdocs/en/index.jsp
	Catalan Agency for Health Technology Assessment (CAHTA) http://www.aatrm.net/html/en/Du8/index.html
Sweden	Swedish Council on Technology Assessment in Health Care (SBU) http://www.sbu.se/www/index.asp
	Center for Medical Health Technology Assessment (CMT) http://www.cmt.liu.se/english?l=en
Switzerland	Swiss Network on Health Technology Assessment (SNHTA) http://www.snhta.ch/home/portal.php
United Kingdom	National Health Service Quality Improvement: Scotland (NHS QIS) http://www.nhshealthquality.org/nhsqis/43.0.140.html
	National Health Service Health Technology Assessment (UK) / National Coordinating Centre for Health Technology Assessment (NCCHTA) http://www.hta.nhsweb.nhs.uk/
	University of York NHS Centre for Reviews and Dissemination (NHS CRD) http://www.york.ac.uk/inst/crd/
	National Institute for Clinical Excellence (NICE) http://www.nice.org.uk/
United States	Agency for Healthcare Research and Quality (AHRQ) http://www.ahrq.gov/clinic/techix.htm
	Harvard School of Public Health—Cost-Utility Analysis Registry http://www.tufts-nemc.org/cearegistry/
	US Blue Cross/ Blue Shield Association Technology Evaluation Center http://www.bcbs.com/consumertec/index.html

Appendix G ASIEM quality assurance/educational program

Since the early 1990s, the Australasian Society for Inborn Errors of Metabolism (ASIEM), a special interest group of the Human Genetics Society of Australasia (HGSA), has conducted a quality assurance/educational program to assess the accuracy of UMP for detection of IEMs, including six of the nine considered in this assessment.

This program is operated in approximately annual cycles (a series) with up to 12 samples in each series. Current practice is to include 12 samples for analysis at monthly intervals.

Reference samples of urine from people previously diagnosed with IEMS are sent to the six laboratories in Australia that undertake UMP testing. Normal samples are also included to detect false positives. Laboratories are advised of the age, gender and symptoms for each patient as would be expected in the real-life situation. The samples are tested and the results evaluated by the convenors as either correct (1) or incorrect (0) diagnoses.

Objectives for inclusion of samples in a series:

- be of an adequate volume to allow distribution among the participating laboratories
- allow a definitive diagnosis to be made
- be challenging for laboratories; for example, no cystinuria samples were included because cystinuria is easily diagnosed.

Table 40 presents results from each of the six laboratories of IEMs tested.

Some results have been omitted from the table because they were from patients with disorders for which UMP is not a diagnostic test (MPS testing has been included in some series) or inadequate data were available.

Table 40 Results of the Australian Society for Inborn Errors of Metabolism quality assurance education program 1992–2007

		Laboratory					
Ref	**Disorder**	**1**	**2**	**3**	**4**	**5**	**6**
1992-01	Normal (on Valproate—mimics NKH)	1	1	1	1	1	1
1992-03	Normal (contained interfering compounds)	1	1	1	1	1	1
1992-04	Glycerol kinase deficiency	1	1	1	1	1	1
1992-05	Argininosuccinate lyase deficiency	1	1	1	1	0	1
1992-06	Methylcrotonyl-CoA carboxylase deficiency	1	1	1	1	1	1
1992-07	Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)	1	1	1	1	1	1
1994-01	b-Ketothiolase deficiency	0	1	1	1	1	1
1994-02	Citrullinaemia	1	1	1	1	1	1
1995-01	Glutaricaciduria type 1	1	1	1	1	1	1
1995-02	Hypophosphatasia	1	1	1	1	1	1
1995-03	b-Ketothiolase deficiency	0	1	1	1	1	1
1995-04	Succinate semialdehyde dehydrogenase deficiency	1	1	1	1	1	1
1995-06	Non-ketotic hyperglycinaemia	1	1	1	1	1	1
1995-07	Hyperlysinaemia	1	1	1	1	1	1
1997-01	Succinate semialdehyde dehydrogenase deficiency	1	1	1	1		1
1997-02	Hyperoxaluria type 1	1	1	1	1	1	1
1997-03	Ornithine transcarbamylase deficiency (heterozygote)	1	1	1	1	1	1
1997-05	Tyrosinaemia type 2	1	1	1	1	1	1
1997-07	Argininosuccinate lyase deficiency	1	1	1	1	1	1
1997-08	b-Ketothiolase deficiency	1	1	1	1	1	1
1998-01	Propionyl-CoA carboxylase deficiency	1	1	1	1	1	1
1998-02	Tyrosinaemia type 2	1	1	1	1	1	1
1998-03	Sulfite oxidase/molybdenum co-factor deficiency	1	1	1	1	0	0
1998-04	Isovaleryl-CoA dehydrogenase deficiency	1	1	1	1	1	1
1998-07	Homocystinuria	1	1	1	1	1	1
1998-08	2-Hydroxyglutaric aciduria	1	1	1	1	1	1
1999-01	Argininosuccinate lyase deficiency	1	1	0	1	1	1
1999-02	Long-chain hydroxyacyl-CoA dehydrogenase def.	1	1	1	1	1	1
1999-03	Maple syrup urine disease	1	1	1	1	1	1
1999-04	Glutaric aciduria type I	1	1	1	1	1	1
1999-05	Barth syndrome	1	1	1	1	1	1
1999-06	Hyperoxaluria type 2	1	1	1	1	1	1
1999-07	Glutathione synthetase deficiency	1	1	1	1	1	1
1999-08	Homogentisic acid oxidase deficiency	1	1	1	1	1	1
2000-01	Biotinidase deficiency/holocarboxylase synthetase deficiency	1	1	1	1	1	1
2000-02	GGT deficiency	0	0	0	1	0	0
2000-03	Non-ketotic hyperglycinaemia	1	1	1	1	1	1
2000-05	Glycerol kinase deficiency	1	1	1	1	1	1
2000-06	Cystathionine b-synthase deficiency (homocystinuria)	1	1	1	1	1	1
2000-07	Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	1	1	1	1	1	1
2000-08	2-Aminoadipic aciduria	1	0	1	1	1	1

Ref	Disorder	Laboratory					
		1	2	3	4	5	6
2000-10	Ornithine transcarbamylase deficiency (heterozygous)	1	1	1	1	0[a]	1
2000-11	Hawkinsonuria	1	1	1	1	1	1
2002-01	Fumarase deficiency	1	1	1	1	1	1
2002-02	Multiple acyl-CoA dehydrogenase deficiency	1	0	1	1	0	1
2002-03	Normal	1	1	1	1	1	1
2002-05	Glycerol kinase deficiency	1	1	1	1	1	1
2002-07	Argininosuccinate lyase deficiency	1	1	1	1	1	1
2002-10	Aromatic L-amino acid decarboxylase deficiency	1	1	1	0	1	1
2002-11	Succinic semialdehyde dehydrogenase deficiency	1	1	1	1	1	1
2002-12	Homocystinuria	1	1	1	1	1	1
2003-01	Tyrosinaemia type 1	1	1	1	1	1	1
2003-02	Malonyl-CoA decarboxylase deficiency	1	1	1	1	1	0
2003-03	Fumarase deficiency	1	1	1	1	0	1
2003-05	Glutaricaciduria type 1	1	1	1	1	1	1
2003-06	Short bowel syndrome	1	1	1	1	1	1
2003-07	Isovaleryl-CoA dehydrogenase deficiency	1	1	1	1	1	1
2003-08	Methylmalonic aciduria	1	1	1	1	1	1
2003-10	Citrullinaemia type 1	1	1	1	1	1	1
2003-11	Phenylketonuria	1	1	1	1	1	1
2004-01	Glutaric aciduria type 1	1	1	1	1	1	1
2004-03	Hereditary oroticaciduria (not OTC deficiency)	b	1	1	1	0	1
2004-04	Hypophosphatasia	1	1	1	1	1	1
2004-05	Methylmalonic aciduria	1	1	1	1	1	1
2004-08	Tyrosinaemia type 1 *	1	1	1	1	1	1
2004-11	3-Methylcrotonyl-CoA carboxylase deficiency	1	1	1	1	1	1
2005-01	Sulfite oxidase/molybdenum co-factor deficiency	1	1	1	1	1	1
2005-02	Normal	1	1	1	1	1	1
2005-03	Homocystinuria *	1	1	1	1	1	1
2005-05	HMG-CoA lyase deficiency	1	1	1	1	1	1
2005-06	Tyrosinaemia type 1 *	1	1	1	1	1	1
2005-07	Argininosuccinate lyase deficiency	1	1	1	1	1	1
2005-08	L-2-hydroxyglutaricaciduria	1	1	1	1	1	1
2005-09	Hypophosphatasia	1	1	1	1	1	1
2005-11	Homogentisic acid oxidase deficiency	1	1	1	1	1	1
2005-12	b-Ketothiolase deficiency	1	1	1	1	1	1

Key: 1 = correct diagnosis; 0 = incorrect diagnosis
[a] There is no explanation for this result
[b] No result due to technical problems

Appendix H Inherited metabolic disorders

Table 41 Incidence and natural history for some disorders detectable by urinary metabolic screen

Disorder and approximate incidence [a]	Detected at newborn screening	Presentation	Diagnostic metabolites	Detected by	Treatment	Inheritance
Alkaptonuria 1:4,000,000	No	Dark coloration of urine on exposure to air, arthritis later in life	Homogentisic acid	Organic acids	Symptomatic	Autosomal recessive
Argininosuccinate lyase deficiency (ASA) 1:400,000[b]	Possibly[c]	Episodes of hyperammonaemia, protein intolerance	Argininosuccinate and anhydrides	Amino acids	Protein restriction. Prevention of catabolism	Autosomal recessive
Argininosuccinate synthase deficiency (citrullinaemia) 1:400,000	Yes[c]	Neonatal or later onset hyperammonaemia, protein intolerance	Citrulline and glutamine	Amino acids	Protein restriction. Prevention of catabolism. Benzoate or phenylbutyrate therapy, severe presentations may require liver transplant	Autosomal recessive
Argininaemia (arginase deficiency) Unknown, very rare	Possibly[c]	Spasticity of lower limbs, psychomotor retardation and seizures. Very rare	Arginine	Amino acids	Protein restriction	Autosomal recessive
Aromatic L-amino acid decarboxylase deficiency Unknown, very rare	No	Extrapyramidial movement disorder, often preceded by oculogyric crises accompanied by convergence spasm	Vanillactate	Organic acids	Treatment with dopamine agonists and MAO inhibitors has some effect. Early diagnosis and treatment improves outcome	Autosomal recessive
Barth syndrome Unknown, very rare	No	Dilated cardiomyopathy, skeletal mysopathy, growth failure	3-methylglutaconate	Organic acids	None proven effective	X-linked
Canavan disease Unknown, very rare	No	Developmental delay, seizures, macrocephaly	N-acetylaspartate	Organic acids	None available, prenatal diagnosis possible	Autosomal recessive
Carbamoylphosphate synthetase deficiency 1:500,000 [b]	No	Neonatal onset severe hyperammonaemia	Glutamine, normal orotate	Amino acids and organic acids	Severe neonatal form unlikely to respond to treatment	Autosomal recessive
Cobalamin defects (inherited or acquired through deficiency), multiple defects described 1:500,000	Some[c]	Megaloblastic anaemia, neurological disease	Homocystine methylmalonate	Amino acids and organic acids	Depending on exact defect most are responsive to vitamin B_{12}	Inherited defects all autosomal recessive
Cystinuria 1:10,000	No	Nephrolithiasis	Lysine, ornithine, cystine	Amino acids	Increased fluid intake, alkalisation of urine	Autosomal recessive

Disorder and approximate incidence [a]	Detected at newborn screening	Presentation	Diagnostic metabolites	Detected by	Treatment	Inheritance
Dihydropyrimidine dehydrogenase deficiency Unknown, very rare	No	Mostly benign but treatment with 5-flurouracil can be fatal	Thymine, uracil	Organic acids	Avoid 5-flurouracil treatment	Autosomal recessive
Glutaric acidaemia type 1 1:700,000 [b]	Yes[c]	Macrocephaly, progressive dystonia	Glutarate, 3-hydroxyglutarate	Organic acids	Pre-symptomatic treatment with carnitine and lysine restriction prevents neurological crises with good outcome	Autosomal recessive
Glutathione synthetase deficiency Unknown, very rare	No	Severe metabolic acidosis, haemolytic anaemia	Pyroglutamate	Organic acids	Antioxidants and free radical scavengers. Outcome generally poor	Autosomal recessive
Glycerol kinase deficiency Unknown, very rare	No	May be benign as an isolated defect but more often presents as part of a contiguous gene deletion involving Duchenne muscular dystrophy and congenital adrenal hypoplasia	Glycerol	Organic acids	Treatment of adrenal insufficiency as required	X-linked
Hartnup disease 1:33,000	No	Usually benign. Occasional skin lesions	Neutral amino acids	Amino acids	Nicotinamide may be given for skin lesions if present	Autosomal recessive
Histidinaemia 1:10,000	No	Benign	Histidine	Amino acids	Non required	Autosomal recessive
HMG CoA lyase deficiency 1:1,500,000	Yes[c]	Vomiting, lethargy, hypoglycaemia and metabolic acidosis	3-hydroxy-3-methylglutarate, 3-methylglutaconate, and 3-hydroxyisovalerate	Organic acids	High carbohydrate diet, with IV glucose during episodes of catabolism	Autosomal recessive
Homocystinuria, (Cystathionine beta synthase deficiency) 1:180,000 [b]	Possibly[c]	Mental retardation, eye, skeletal and vascular problems. Thromboembolism is most common cause of death in untreated individuals	Homocystine, mixed disulphides	Amino acids	Treatment with vitamin B_6, protein restriction and/or betaine. Good clinical and biochemical response to treatment in most cases	Autosomal recessive
Hyperlysinaemia Unknown, very rare	No	Benign	Lysine	Amino acids	Non required	Autosomal recessive
Hyperornithinaemia Unknown, very rare	No	Gyrate atrophy of the choroid and retina	Ornithine	Amino acids	Various treatments have been tried, none proven effective in the long term	Autosomal recessive

Disorder and approximate incidence [a]	Detected at newborn screening	Presentation	Diagnostic metabolites	Detected by	Treatment	Inheritance
Hypophosphatasia 1:100,000	No	Neonatal form is lethal with hypomineralisation of bone. Infantile form presents with hypomineralisation and rickets, childhood form is milder with early loss of deciduous teeth. Adult form is milder still with osteomalacia	Phosphoethanolamine	Amino acids	None proven	Severe forms autosomal recessive, later onset forms may be dominantly inherited
Isovalericacidaemia 1:700,000 [b]	Yes[c]	Neonatal onset of vomiting, lethargy, metabolic acidosis, hyperammonaemia. Intermittent milder forms are also found	3-hydroxyisovalerate, isovalerylglycine	Organic acids	Protein restriction, carnitine supplementation and prevention of catabolism. Severe neonatal forms may not respond to treatment	Autosomal recessive
Lactic acidosis (primary mitochondrial defects) 1:16,000[d]	No	Wide spectrum of presentation— "any organ, any age"	Lactate, alanine	Organic acids, amino acids	Some cases are responsive to vitamin cocktails but generally poor long term outcome	Some nuclear encoded autosomal recessive, others mitochondrial DNA mutations passed down maternal line
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency 1:400,000 [b]	Yes[c]	Fatty acid oxidation defect presenting with episodic hypoglycaemia. Retinitis pigmentosa	Long- chain 3-hydroxydicarboxylic aciduria disproportionate to the degree of ketosis	Organic acids	Dietary restriction of long chain fat, supplemented with medium-chain triglycerides	Autosomal recessive
Lysinuric protein intolerance Unknown, very rare	No	Failure to thrive, enlarged liver and spleen, hypotonia and osteoporosis	Isolated lysinuria	Amino acids	Protein restriction and supplementation with oral citrulline	Autosomal recessive
Malonyl CoA decarboxylase deficiency Unknown, very rare	No	Seizures, hypotonia, diarrhoea, vomiting, metabolic acidosis, hypoglycaemia, ketosis and lactic acidaemia, and hypertrophic cardiomyopathy	Malonate and methylmalonate	Organic acids	None effective	Autosomal recessive
Medium-chain acyl-CoA dehydrogenase deficiency 1:10,000 to 1:20,000 [b]	Yes[c]	Commonest fatty acid oxidation defect, presents with episodic hypoglycaemia	Dicarboxylic aciduria with hexanoylglycine and suberylglycine	Organic acids	Avoidance of fasting , calorific support during intercurrent illness	Autosomal recessive

Disorder and approximate incidence [a]	Detected at newborn screening	Presentation	Diagnostic metabolites	Detected by	Treatment	Inheritance
Methylmalonic aciduria 1:180,000 [b]	Yes[c]	Metabolic ketoacidosis acidosis, hyperammonaemia presenting in neonatal period. Milder intermittent forms also exist	Methylmalonate and methylcitrate	Organic acids	Protein restriction, some milder cases responsive to vitamin B_{12}	Autosomal recessive
Maple syrup urine disease 1:180,000	Yes[c]	Metabolic ketoacidosis acidosis, hyperammonaemia presenting in neonatal period. Milder intermittent forms also exist	Branch chain amino acids, alloisoleucine, branched chain ketoacids	Amino acids, organic acids	Protein restriction and supplementation with branched chain free formula. Some milder cases responsive to thiamine	Autosomal recessive
3-methylcrotonyl CoA carboxylase deficiency 1:130,000 [b]	Yes[c]	Probably benign but may present with symptoms secondary to carnitine depletion	3-methylcrotonyl glycine, 3-hydroxyisovalerate	Organic acids	Carnitine supplementation if required	Autosomal recessive
3-methylglutaconyl hydratase deficiency Unknown, very rare	No	Delayed psychomotor development, metabolic acidosis during illness	3-methylgutaconate, 3-methylglutarate	Organic acids	Leucine restriction and carnitine therapy have been tried	Autosomal recessive
Multiple acyl-CoA dehydrogenase deficiency Unknown, very rare	Yes[c]	Fatty acid oxidation defect presenting with hypoglycaemia and metabolic acidosis. Severe neonatal form associated with congenital abnormalities	Short, medium and long chain dicarboxylic aciduria, butyryl, isovaleryl, hexanoyl and suberylglycine	Organic acids	Avoidance of fasting, calorific support during intercurrent illness. Some patients are responsive to riboflavin	Autosomal recessive
Multiple carboxylase deficiency (biotinidase or holocarboxylase synthetase) 1:300,000 [b]	Yes[c]	Episodic ketoacidosis, hyperammonaemia, skin rash, alopecia, developmental delay	Latate, 3-hydroxypropionate, 3-hydroxyisovalerate, 3-methylcrotonylglycine, methylcitrate	Organic acids	Biotin	Autosomal recessive
Non-ketotic hyperglycinaemia 1:150,000 [b]	No	Neonatal onset of seizures, hypotonia, respiratory depression	Glycine	Amino acids	Patients severely brain damaged, no proven effective therapy	Autosomal recessive
Ornithine carbamoyl transferase deficiency 1:105000 [b]	Possibly	Neonatal presentation with vomiting, lethargy leading to coma, severe hyperammonaemia. Milder late onset variants also occur	Glutamine, orotate	Amino acids organic acids	Protein restriction. Prevention of catabolism. Benzoate or phenylbutyrate therapy, severe cases may require liver transplant	X-linked
Phenylketonuria (PKU) (phenylalanine hydroxylase deficiency) 1:14,000	Yes	Severe mental retardation if not detected on newborn screening and treated. Confirmation rarely involves urine metabolic screen	Phenylalanine, phenyllactate, phenylpyruvate	Amino acids organic acids	Protein restriction, supplementation with phenylalanine free formula	Autosomal recessive

Disorder and approximate incidence [a]	Detected at newborn screening	Presentation	Diagnostic metabolites	Detected by	Treatment	Inheritance
Propionyl CoA carboxylase deficiency 1:800,000 [b]	Yes[c]	Metabolic ketoacidosis, hyperammonaemia presenting in neonatal period	3-hydroxypropionate, propionylglycine, methylcitrate	Organic acids	Protein restriction. Prevention of catabolism. Benzoate or phenylbutyrate therapy during hyperammonaemia, severe presentations may require liver transplant	Autosomal recessive
Short-chain acyl-CoA dehydrogenase deficiency 1:70,000 [b]	Yes[c]	Probably mostly benign	Ethylmalonate, butyrylglycine	Organic acids	Not required	Autosomal recessive
Succinic semialdehyde dehydrogenase deficiency Unknown, very rare	No	Mental retardation, hypotonia	4-hydroxybutyrate	Organic acids	Vigabatrin but long term efficacy is unproven	Autosomal recessive
Sulphite oxidase deficiency Unknown, very rare	No	Severe convulsions presenting in neonatal period	S-sulphocysteine	Amino acids	No proven therapy	Autosomal recessive
Tyrosinaemia type I (fumarylacetoacetase deficiency) 1:400,000 [b]	Some[c]	Acute liver failure, cirrhosis, renal Fanconi syndrome and peripheral neuropathy	Succinylacetone	Organic acids	NTBC therapy very effective. Long term need for liver transplant still unclear	Autosomal recessive
Tyrosinaemia type II 1:350,000 [b]	Yes[c]	Palmoplantar keratosis and painful corneal erosions with photophobia	Tyrosine, 4-hydroxyphenyllacate and 4-hydroxyphenylpyruvate	Amino acid organic acids	Tyrosine restricted diet	Autosomal recessive

Abbreviations: ASA, argininosuccinate lyase deficiency; CoA, co-enzyme A; DNA, deoxyribonucleic acid; HMG, 3-hydroxy-3-methylglutaric; IV, intravenous; MAO, monamine oxidase; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl) 1,3-cyclohexanedione; PKU, phenylketonuria

[a] Incidence figures are best estimates for the Australian population taken from the following references or from Scriver CR, Beaudet AL, Sly WS, Valle D [eds] The Metabolic and Molecular Bases of Inherited Disease, 8th ed. New York, NY, McGraw-Hill, 2001reatments are summaries of those discussed in reference 3

[b] Wilcken et al 2003

[c] Conditions that can be detected using tandem mass spectrometry based newborn screening but require confirmation by urinary metabolic screen

[d] Skaldal et al 2003

Appendix I Alternative tests where UMP is unavailable

Table 42 Comparator tests used where UMP is unavailable

Genetic metabolic disease	Patient population(s)	Alternative tests where UMP is unavailable (costs)
Cystinuria	Symptomatic patients	RPHPLC[#a]
	At-risk family members	Quantitative amino acid analysis ($100[b])
MCADD	Post-newborn screening	Plasma acylcarnitines ($88[c], $130[d])
	Symptomatic patients	Mutation analysis ($230–750[d])
Glutaric aciduria type I	Post-newborn screening	Plasma acylcarnitines* $88[c], $130[d])
	Symptomatic patients	Mutation analysis [#a]
		Enzymology ($870[#d])
Ornithine transcarbamylase deficiency	Symptomatic patients	Plasma amino acids ($100[b])
	At-risk family members	Mutation analaysis ($750[d])
		Enzymology ($265[*c])
Non-ketotic hyperglycinaemia	Symptomatic patients	Plasma and CSF amino acid analysis ($200[*b])
		Liver biopsy enzymology ($1130[*#d])
Homocystinuria	Symptomatic patients	Plasma amino acid analysis ($100[b])
Methylmalonic aciduria	Post-newborn screening	Enzyme analysis [#a]
	Symptomatic patients	
Maple syrup urine disease	Post-newborn screening	Plasma amino acid analysis ($100[b])
	Symptomatic patients	
	At-risk family members	
Lactic acidosis	Symptomatic patients	Enzyme analysis of muscle ($860[*e])
		Enzyme analysis of cultured cells $1160[*e])
		Mutation analysis ($160[*e], $400[*e], $750[*d])

Abbreviations: CSF, cerebrospinal fluid; MCAD, medium-chain acyl-CoA dehydrogenase; RPHPLC, reverse-phase high performance liquid chromatography
* Does not include the cost of obtaining the relevant test sample (skin fibroblasts, liver, muscle, CSF)
# Testing not available in Australia
[a] No costing available
[b] Medicare Benefits Schedule: Item 66756
[c] Genetic Medicine, Adelaide Women's and Children's Hospital
[d] New South Wales Biochemical Genetics Service, New Children's Hospital at Westmead
[e] Biochemical Genetics, Victorian Clinical Genetics Service

Notes: Mutation testing costs vary and depend on the frequency of the mutation and/or complexity of testing required
Some of these tests are not readily available, are high-cost items and hence are not routinely requested. Costs therefore are based on limited experiences and occurrences but are nonetheless indicative. UMP obviates the need to perform this testing in most cases
Packaging and freight costs are not included in the estimates—the figures quoted below are representative and will vary depending on departure point and destination, carrier used, urgency of delivery and nature of contents
Interstate: Approximately $50 to $100
Overseas: Approximately $1000 for overseas testing depending on destination and nature of required packaging. Costs for shipping with dry ice (for some testing this is required to preserve sample integrity) doubles shipment costs to more than $2000 (Genetic Medicine, Adelaide Women's and Children's Hospital)

Appendix J Further tests

Table 43 Potential further tests following an equivocal diagnosis of genetic metabolic disease

Genetic metabolic disease	Further test(s)
Cystinuria	Repeat UMP plus urine amino acid analysis
MCADD	Plasma/blood acylcarnitine profile; repeat UMP and single analyte analysis: hexanoylglycine; mutation analysis
Glutaric aciduria I	Repeat UMP and plasma/blood acycarnitine profile; repeat UMP and single analyte analysis: enzyme analysis (including *in vitro* co-factor/transport studies) mutation analysis (neither available in Australia)
Ornithine transcarbamylase deficiency	Repeat UMP and single analyte analysis: orotate; loading tests mutation analysis (effective for 80%)
Non-ketotic hyperglycinaemia	Plasma/CSF amino acids; single analyte analysis: enzyme analysis and/or mutation analysis (neither available in Australia)
Homocystinuria	Plasma total homocysteine: plasma methylmalonic acid: vitamin B_{12}: plasma amino acids; single analyte analysis
Methylmalonic aciduria	Plasma/blood acylcarnitine profile; single analyte analysis: vitamin B_{12}; plasma methylmalonic acid; enzyme analysis (including *in vitro* co-factor/transport studies). (not available in Australia).
Maple syrup urine disease	Plasma amino acids, repeat UMP, load tests (variant forms): enzyme assay in some
Lactic acidoses	Mutation analysis; enzyme analysis (including *in vitro* co-factor/transport studies)

Source: Advisory panel
Abbreviations: CSF, cerebrospinal fluid; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; UMP, urinary metabolic profiling

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