

## **Newborn screening for inborn errors of metabolism: a systematic review**

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**Health Technology Assessment  
NHS R&D HTA Programme**



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# Newborn screening for inborn errors of metabolism: a systematic review

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This report is one of a series covering acute care, diagnostics and imaging, methodology, pharmaceuticals, population screening, and primary and community care. It was identified as a priority by the Population Screening Panel (see inside back cover).

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## List of abbreviations

apo	apolipoprotein
CAH	congenital adrenal hyperplasia
CBA	cost-benefit analyses
CEA	cost-effectiveness analysis
CI	confidence interval
CID	collision-induced decomposition*
CNL	constant neutral loss
CPT	carnitine palmitoyl transferase
CUA	cost-utility analysis
CV	coefficient of variation
ELISA	enzyme-linked immunosorbent assay
FAB	fast atom bombardment
FH	familial hypercholesterolaemia
GAI	glutaric aciduria type 1
GALT	galactose-1-phosphate uridyl transferase
GOS	Great Ormond Street Hospital
HMGA	3-hydroxy-3-methylglutaric aciduria
HPA	non-PKU hyperphenylalaninaemia
IVA	isovaleric acidaemia
LCAD	long-chain acyl CoA dehydrogenase deficiency*
LDL	low-density lipoprotein
MACDD	multiple acyl CoA dehydrogenase deficiency
MCAD	medium-chain acyl CoA dehydrogenase
MDPH	Massachusetts Department of Public Health*
MMA	methylmalonic aciduria
MS	mass spectrometry
MSUD	maple syrup urine disease
NTBC	2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione
PA	propionic acidaemia
PABA	4-aminobenzoate
PCR	polymerase chain reaction
PKU	phenylketonuria
QALY	quality adjusted life-year
RIA	radioimmunoassay
RID	radial immunodiffusion
SCAD	short-chain acyl CoA dehydrogenase*
SD	standard deviation
TCA	tricarboxylic acid
TLC	thin-layer chromatography
UDP	uridine diphosphate

\* Used only in tables and appendices



## Executive summary

### Objectives

- To establish a database of literature and other evidence on neonatal screening programmes and technologies for inborn errors of metabolism.
- To undertake a systematic review of the data as a basis for evaluation of newborn screening for inborn errors of metabolism.
- To prepare an objective summary of the evidence on the appropriateness and need for various existing and possible neonatal screening programmes for inborn errors of metabolism in relation to the natural history of these diseases.
- To identify gaps in existing knowledge and make recommendations for required primary research.
- To make recommendations for the future development and organisation of neonatal screening for inborn errors of metabolism in the UK.

### How the research was conducted

There were three parts to the research.

- A systematic review of the literature on inborn errors of metabolism, neonatal screening programmes, new technologies for screening and economic factors. Inclusion and exclusion criteria were applied, and a working database of relevant papers was established. All selected papers were read by two or three experts and were critically appraised using a standard format. Seven criteria for a screening programme, based on the principles formulated by Wilson and Jungner (WHO, 1968), were used to summarise the evidence. These were as follows.
  - Clinically and biochemically well-defined disorder
  - Known incidence in populations relevant to the UK
  - Disorder associated with significant morbidity or mortality
  - Effective treatment available
  - Period before onset during which intervention improves outcome
  - Ethical, safe, simple and robust screening test
  - Cost-effectiveness of screening
- A questionnaire which was sent to all newborn screening laboratories in the UK.

- Site visits to assess new methodologies for newborn screening.

The classical definition of an inborn error of metabolism was used (i.e. a monogenic disease resulting in deficient activity in a single enzyme in a pathway of intermediary metabolism).

### Research findings

#### Inborn errors of metabolism

- Phenylketonuria (PKU) (incidence 1:12,000) fulfilled all the screening criteria and could be used as the 'gold standard' against which to review other disorders despite significant variation in methodologies, sample collection and timing of screening and inadequacies in the infrastructure for notification and continued care of identified patients.
- Of the many disorders of organic acid and fatty acid metabolism, a case can only be made for the introduction of newborn screening for glutaric aciduria type 1 (GA1; estimated incidence 1:40,000) and medium-chain acyl CoA dehydrogenase (MCAD) deficiency (estimated incidence 1:8000– 1:15,000). Therapeutic advances for GA1 offer prevention of neurological damage but further investigation is required into the costs and benefits of screening for this disorder. MCAD deficiency is simply and cheaply treatable, preventing possible early death and neurological handicap. Neonatal screening for these diseases is dependent upon the introduction of tandem mass spectrometry (tandem MS). This screening could however also simultaneously detect some other commonly-encountered disorders of organic acid metabolism with a collective incidence of 1:15,000.
- Neonatal screening for congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (incidence 1:17,000) has been shown to be beneficial in other countries and similar benefits should accrue in the UK. A national programme of neonatal screening for CAH would be justified, with reassessment after an agreed period.
- Biotinidase deficiency is of low incidence in the UK (estimated 1:100,000), but this may be outweighed by the simplicity of the screening methodology and the benefits in prevention

of serious neurological disease in patients with profound biotinidase deficiency. This question requires further investigation and a national neonatal screening programme would be justified, with reassessment after an agreed period.

- Neonatal screening for galactosaemia (incidence 1:44,000) has been based upon prevention of neonatal mortality. However, evidence suggests that, despite early treatment, long-term outcome is poor with neurological dysfunction and a high incidence of ovarian failure in females. The accepted criteria are not currently met by galactosaemia and newborn screening is not justified.
- The accepted criteria for a neonatal screening programme are not currently met by non-PKU amino acidopathies (including tyrosinaemia type 1, homocystinuria and maple syrup urine disease), familial hypercholesterolaemia, peroxisomal disorders, urea cycle defects, trace metal disorders, purine or pyrimidine disorders, or lysosomal disorders.

### Screening technologies

- Automation of all or parts of the screening process is technically possible but some current methodologies are not amenable to automation. Fully automated neonatal screening utilising time-resolved fluorescence is currently being developed.
- Current molecular (DNA) techniques do not permit the simultaneous screening of large numbers of mutations and can be very expensive. At present there is no indication for newborn screening for inborn errors of metabolism using these techniques.
- Tandem MS can be considered as the most important of the new technologies for newborn screening for inborn errors of metabolism. It has the potential for simultaneous multi-disease screening for selected disorders of amino acid and organic acid metabolism using a single analytical technique and is complementary to immunoassay-based methods for congenital hypothyroidism (CH) and CAH screening. The technology has been demonstrated to be robust (accurate, sensitive, lack of false-positives) and suitable for the reliable detection of PKU and some other inborn errors of metabolism. However, introduction of new technologies for neonatal screening must be determined by the perception and evidence for the need for screening for each disorder or group of related disorders and by the need for the new technology in existing programmes. Of those disorders detectable by tandem MS in addition to PKU, evidence has identified only GA1 and MCAD

deficiency as disorders for which a case for newborn screening can be made. Further, evidence for the utility of tandem MS in prospective neonatal screening for inborn errors of metabolism has come from only one source, based on relatively small numbers screened. Thus this technology requires further evaluation through primary research in the UK with prospective screening of more than 1,000,000 neonatal infants for PKU, GA1, MCAD deficiency (and possibly other selected disorders) in order to validate fully the utility of tandem MS for newborn screening for inborn errors of metabolism.

### Economic evidence

- PKU screening provides a positive net monetary benefit to society and justifies the collection of blood samples from neonatal infants. There is insufficient economic evidence to support a change from current methodology to tandem MS-based screening solely for PKU. More information is needed on the cost-effectiveness of extending screening to other disorders. There is insufficient evidence to assess the economic value of screening for any other inborn errors of metabolism.

### Conclusions and recommendations

- Universal neonatal screening for PKU is worthwhile and should be continued. Cost-benefit analyses show that screening for PKU by itself justifies the collection and testing of neonatal blood spots.
- If the neonatal screening programme is to be expanded a clinical and supportive infrastructure for paediatric metabolism urgently needs to be established to provide adequate treatment and care for identified patients and their families.
- National programmes for neonatal screening for profound biotinidase deficiency and CAH would be justified on the evidence. If they were introduced, there would need to be structured, coordinated, on-going evaluation to ensure that they are cost-effective, with review after 5 years.
- Screening for MCAD deficiency should be seriously considered for inclusion in newborn screening programmes. Similarly, a case can be made for the introduction of newborn screening for GA1. The clinical effectiveness and cost-effectiveness of such screening would need to be carefully monitored, with review after 10 years. Such screening is dependent upon the introduction of tandem MS technology into newborn screening programmes. Tandem MS could simultaneously detect other selected disorders.

- There is however insufficient evidence at present for the widespread introduction of tandem MS technology into newborn screening programmes in the UK. Tandem MS for newborn screening for PKU, MCAD deficiency and GA1 should be further evaluated by primary research conducted over 5 years with a defined timetable and external and independent statistical, health economic and scientific monitoring and evaluation of the technology and programmes. This research should be conducted at four selected centres that have been identified as having the required infrastructure and appropriate expertise. During this primary research, and until reports are presented and decisions made, there should be an embargo on the introduction of tandem MS technology into newborn screening laboratories in the UK.
- There is no evidence to support a newborn screening programme for galactosaemia and any current newborn screening for galactosaemia should be discontinued.
- Screening for other inborn errors of metabolism is not warranted at this time.
- Technologies for fully automated immunoassay-based screening are not yet sufficiently developed. The benefits from a fully automated neonatal screening system remain to be demonstrated. These benefits will probably only be achieved if the range of tests is expanded from CH (and PKU) alone and this will in turn depend upon decisions about other diseases to which newborn screening should be extended.
- At present there is no indication for newborn screening using molecular techniques.



# Chapter I

## Introduction

### Neonatal screening for inborn errors of metabolism in the UK

A neonatal screening programme for phenylketonuria (PKU) was introduced in the UK 30 years ago on the basis of purely medical considerations, namely the prevention of disability and handicap through a simple test and subsequent dietary treatment. At that time methodologies for the measurement and analysis of health gains and cost-effectiveness were less well developed, and did not influence the introduction of the nationwide screening programme. Since then there has been a substantial increase in the number of identified inborn errors of metabolism (inherited metabolic diseases) and knowledge about these diseases is ever increasing.

For the purposes of this systematic review the classical definition of an inborn error of metabolism has been taken: that is, a monogenic disease resulting in deficient activity in a single enzyme in a pathway of intermediary metabolism. Thus included are disorders in the catabolic and synthetic pathways of carbohydrates, amino acids, organic acids and fatty acids, purines and pyrimidines, porphyrins, steroids, lipids, and bile acids and of the processes involved in the uptake, synthesis and utilisation of the essential co-factors for enzymes in these pathways, including trace metals and vitamins. Also included are disorders of lysosomal enzymes but other inherited disorders of, for example, membrane transport, connective tissue, blood and blood-forming tissues, the defence and immune systems and muscle and skin are excluded.

Each individual inborn error of metabolism is rare but the total incidence is significant. If undiagnosed and untreated, many of the affected children will be hospitalised for extensive periods, require intensive care, and need long-term institutional care. There will be a major impact on the families concerned and on healthcare provision. PKU is the inborn error of metabolism for which universal newborn screening is most generally accepted and applied. Each year in UK there are some 793,000 births and so with an incidence of PKU of about 1 in 12,000 live births, 60–70 children will be born with PKU. The numbers of newborn infants affected with some other inborn error

of metabolism, for which neonatal screening is not currently used, are similar. Thus each year in the UK, there will be born some 40 children with congenital adrenal hyperplasia (CAH), 50–60 with medium-chain acyl CoA dehydrogenase (MCAD) deficiency and 40–50 with disorders of organic acid metabolism. In total about 250 babies are born each year in the UK with an inborn error of metabolism as defined here (including PKU), and these disorders are thus of considerable public health significance with an impact on health care comparable to that of juvenile-onset diabetes.<sup>1</sup> With early neonatal detection and adequate treatment, the clinical and neurological effects may be prevented in some 75% of these children,<sup>1</sup> or more than 180 children per year for the UK. Left to clinical ascertainment, diagnosis is often late and may be difficult to achieve and frequently severe physical, developmental or neurological damage has occurred or begun by the time of diagnosis.

There is thus a perceived need for a more widespread, uniform neonatal screening programme for a broader range of inborn errors of metabolism with the goal of prevention of serious long-term morbidity and mortality. However, there is also pressure to screen for serious diseases for which early detection offers little, questionable or no benefit to the child.<sup>2</sup> Although they are outside the scope of the present review and definition of an inborn error of metabolism (see above), the latter can be exemplified by cystic fibrosis, for which there is still no consensus that detection in the neonatal period improves long-term outcome, and by Duchenne muscular dystrophy for which there is no effective treatment.<sup>2</sup> Despite this, screening for cystic fibrosis is established in four centres in the UK and there is a pilot programme of screening for Duchenne muscular dystrophy (see *Table 1*). In addition, there are frequent calls to screen for new disorders, often before there is sufficient evidence that early treatment improves outcome (for example, some of the organic acidurias) or even that the majority of such children actually need treatment at all (for example, MCAD deficiency).<sup>2</sup> Similarly, the development of new technologies that may have application in neonatal screening for inborn

TABLE 1 Disorders tested for in neonatal blood specimens in the UK (Streetly et al<sup>3</sup>)

Disorder	Annual number screened	Percentage of UK births	Number of laboratories	Established or pilot scheme
<b>PKU</b>	793,000	99	25	Established
<b>Congenital hypothyroidism</b>	793,000	99	25	Established
<b>Amino acid disorders</b>				
Homocystinuria (specific testing)	95,000	12	2	Established
Tyrosinaemia (specific testing)	25,000	3	1	Established
On chromatography*	268,000	34	9	Established
<b>Galactosaemia</b>				
Specific testing	70,000	9	1	Established
Incidental finding	67,000	8	1	Established
<b>Haemoglobinopathies</b>	71,000	9	3	2 established, 1 pilot
<b>Cystic fibrosis</b>	130,000	16	4	Established
<b>Duchenne muscular dystrophy</b>	20,000	3	1	Pilot
<b>Familial hyperlipidaemia</b>	4000	1	1	Pilot

\* Incidental findings; includes tyrosinaemia, maple syrup urine disease and histidinaemia.

errors of metabolism, for example, tandem mass spectrometry (tandem MS) and automated immunoassay, has brought further pressure to expand newborn screening to a much wider range of disorders in multi-disease neonatal screening programmes.

However, there is a lack of evidence and information regarding the need for extension of newborn screening to inborn errors of metabolism other than PKU and about the application and effectiveness of new technologies in newborn screening. It is most important not to add more tests or alter screening technologies without significant supportive evidence as to the clinical and financial impact of such changes. The relevant costs and consequences of altering existing newborn screening programmes need to be quantified before any decisions regarding the introduction of changes can be made. The Department of Health commissioned this systematic review under the NHS Health Technology Assessment Programme in order to obtain, summarise and evaluate the evidence on these issues, to reach conclusions based upon this evidence and to make recommendations for the future development of neonatal screening programmes in the UK.

## Programmes for neonatal screening in the UK

The current procedure for collecting samples for neonatal screening is well established. An infant will, normally at 6–14 days of age, have a heel-prick blood specimen taken by the midwife or the health visitor for a metabolic screening test. Dried blood spots or capillary blood are tested for PKU at one of 26 regional screening laboratories using either a bacterial inhibition assay (the Guthrie test), thin-layer chromatography (TLC) or fluorometry. A survey of NHS neonatal screening laboratory directors (see page 6) showed that the number of babies tested annually in each laboratory ranged from 2250 to 118,500. There is no statutory national coordination between regional screening programmes and there is considerable variation in the organisation of sample collection, testing and the way results are reported. In addition to PKU, the blood samples are used to test all infants for congenital hypothyroidism. Despite the lack of coordination, the coverage rate in the UK is very high, with over 99% of live newborn infants being tested for PKU and hypothyroidism.<sup>3,4</sup>

The heel-prick blood spots are also used in several places to test locally for other diseases



(see *Table 1*) although the siting of these tests is not necessarily related to the incidence of disease. These expanded programmes have not always been formally assessed against criteria of suitability for newborn screening programmes. Decisions on additional screening programmes must be based upon evidence of their effectiveness and cost-effectiveness.<sup>3</sup>

## Aims of the review

The aims of this review are as follows.

- To establish a database of literature and other evidence on neonatal screening programmes and technologies for inborn errors of metabolism.
- To undertake a systematic review of these data as a basis for evaluation of universal newborn screening for inborn errors of metabolism.
- To prepare an objective summary of the evidence on the appropriateness of various existing and possible neonatal screening programmes for inborn errors of metabolism in relation to the natural history of these diseases.
- To identify gaps in existing knowledge and make recommendations for required primary research.
- To make recommendations for the future development and organisation of universal neonatal screening for inborn errors of metabolism in the UK.

## Plan of investigation

The St George's consortium addressed two fundamental questions.

1. Which inborn errors of metabolism should be screened for?
2. What technologies should be used for screening?

Although there is room for improvement, in the review we made the assumption that the infrastructure for sample collection should not be

changed and that nothing should be done to compromise the high rate of coverage of current screening programmes.

A systematic and comprehensive review of the literature was undertaken to assess the feasibility, effectiveness and costs of neonatal screening for the following inborn errors of metabolism:

- PKU
- amino acidopathies
- disorders of carbohydrate metabolism
- disorders of organic acid metabolism
- fatty acid oxidation defects
- disorders of adrenal steroidogenesis
- lipoprotein disorders
- peroxisomal disorders
- disorders of the urea cycle
- respiratory chain/tricarboxylic acid (TCA) cycle disorders
- trace metal disorders
- purine/pyrimidine disorders
- lysosomal disorders.

The appropriateness of continuing or introducing neonatal screening programmes for these diseases was evaluated by addressing the following points:

- incidence of the disease in the UK
- sensitivity and specificity of the screening test
- health outcome of screening (i.e. the marginal gain in length or quality of life for patients detected through screening compared with those who present clinically)
- costs of the screening programme
- marginal costs of care and treatment for patients diagnosed through screening compared with those who present with disease
- outcomes and costs of false-positive and false-negative results.

The review group did not include the intangible (psycho-social) costs and benefits of screening within its remit, although these are clearly important factors which should be included when making any decisions about screening programmes. From the findings of the review detailed in chapters 3, 4 and 5, recommendations for the future of neonatal screening programmes in the UK are presented.



# Chapter 2

## Structure of the review

### Study design

The review was divided into three sections.

1. A formal systematic review of the literature.
2. A questionnaire to all newborn screening laboratories in the UK.
3. Site visits to assess new methodologies for newborn screening.

### Literature review

#### Study identification

The methods used in the literature review were chosen to minimise the possibility of bias in the identification, selection and appraisal of papers. Medline was searched, without language restrictions, back to the beginning of the database (i.e. from 1966), thus covering literature published before the implementation of a universal neonatal screening service in the UK, until June 1996. Firstly, a broad search was conducted, combining the exploded MeSH (medical subject heading) terms 'mass screening' and 'inborn errors of metabolism'. Further searches were then carried out using search terms as listed in the box below.

#### Search terms used to identify papers for the literature review

Each named individual error of metabolism (e.g. amino acid metabolism, inborn errors) *plus* one of the following terms:

- mass screening
- outcome
- incidence
- false-positive reactions
- false-negative reactions
- costs and cost analysis
- sensitivity and specificity

All search terms were entered both as keywords and as text words. Searches were also carried out combining inborn errors of metabolism with different screening technologies (e.g. mass spectrometry) and with economics. Citations were imported into a Reference Manager (Research Information

Systems) database, known as the **primary database**, and coded for subject. Similar searches were carried out on other computerised bibliographical databases, each covering the literature back to the beginning of each database: BIDS and Embase (Elsevier Science BV) from 1980, Science Citation Index from 1981 and Index to Scientific and Technical Proceedings from 1982. Manual searches were conducted of textbooks, conference proceedings, Index Medicus and Current Contents. Relevant citations were entered into the primary database. Information on 'grey' and unpublished literature was requested as part of a questionnaire sent to the directors of all screening laboratories in the UK (see page 6). The reference lists of articles were searched to identify additional relevant citations.

#### Study selection

The citations and abstracts of 1866 references identified by the above strategies were reviewed by the review coordinator (MJT) and a subject expert. Those with contents that fulfilled the following **exclusion** criteria were discarded:

- pure laboratory-based studies pre-1980
- methodologies not suitable for mass population screening
- not neonatal screening (unless outcome studies or incidence data)
- not well baby screening (unless outcome studies or incidence data).

The reasons for rejecting each paper were entered into the database. If there was any doubt as to whether or not a paper should be rejected it was retained. The 832 references that remained after the application of the exclusion criteria were copied to another database, identified as the **secondary database**.

The citations and abstracts held in the secondary database were re-evaluated by the review coordinator (MJT) and another subject expert. Where there was insufficient information in the abstract, or where there was no abstract, a copy of the full paper was obtained and assessed for inclusion.

Papers which met the following **inclusion** criteria were selected.

**I** Papers that contained data on population incidence, effectiveness of screening, health outcomes or screening and/or treatment costs for the following defined list of metabolic disorders.

1. PKU
2. Amino acidopathies
3. Disorders of carbohydrate metabolism
4. Disorders of organic acid metabolism
5. Fatty acid oxidation defects
6. Disorders of adrenal steroidogenesis
7. Lipoprotein disorders
8. Peroxisomal disorders
9. Disorders of the urea cycle
10. Respiratory chain/TCA cycle disorders
11. Trace metal disorders
12. Purine/pyrimidine disorders
13. Lysosomal disorders

**II** Methodology papers that described defined suitable screening technologies for blood samples.

1. Guthrie bacterial inhibition assay
2. Chromatography
3. Fluorometry
4. Radioimmunoassay
5. Enzymology
6. Tandem MS
7. Immunoassay based on time-resolved fluorometry
8. Molecular (DNA) approaches

The references selected by these inclusion criteria were copied into a third database, identified as the **working database**. These references were all retrieved in full so that they could be critically appraised.

### Study appraisal

The 407 papers in the working database were sub-divided by individual metabolic disease or closely related groups of disorders, multi-disease screening technologies and economic literature. For each category, a working group of two or three subject experts from the consortium was convened to review the literature. All selected papers were read by each expert in the group and were critically appraised using a standard checklist (see Appendix 2).

Each working group then met to pool their appraisals and to develop their conclusions in relation to seven criteria based on the Wilson and Jungner (WHO, 1968) criteria for screening programmes<sup>5</sup> (see box below).

### Screening criteria used for critical appraisal

- Clinically and biochemically well-defined disorder
- Known incidence in populations relevant to UK
- Disorder associated with significant morbidity or mortality
- Effective treatment available
- Period before onset during which intervention improves outcome
- Ethical, safe, simple and robust screening test
- Cost-effectiveness of screening\*

\* *The published economic evidence on the cost-effectiveness of screening is reviewed in chapter 5.*

These conclusions are summarised in a table for each disease or group of related diseases. The symbols used in the tables are as follows:

- ✓ where the criterion is completely fulfilled
- [✓] where there is still need for some further data
- ± where data and evidence are equivocal or incomplete
- ✗ where data are lacking, insubstantial or of dubious quality.

### Screening questionnaire

A questionnaire was sent to the directors of all neonatal screening laboratories in the UK, in collaboration with a group from SCHARR (Sheffield Centre for Health and Related Research) who were also undertaking a Department of Health commissioned review entitled *Neonatal metabolic screening: cost, yield and effects on outcome*. A copy of the full questionnaire is contained in Appendix 3. The box below lists the information which was requested in three key areas: activity, resources and research and development.

There was a disappointing response: although reminders were sent out, only 15 out of the 28 directors contacted returned a completed survey. A further three wrote specifically to confirm that they would not be filling in the questionnaire.

### Laboratory visits

Site visits were made to the laboratories in the USA and the UK where tandem MS is being developed or applied for neonatal screening of metabolic disorders. The sites visited were:

- Duke University Medical School, North Carolina, USA, where the technique of tandem MS was

<p align="center"><b>Information requested in the screening laboratory questionnaire</b></p>
<p><b>Activity</b></p> <ul style="list-style-type: none"> <li>- the number of babies screened by the laboratory</li> <li>- the disorders for which the babies were screened</li> <li>- the methods used to screen for each disease</li> </ul>
<p><b>Resources</b></p> <ul style="list-style-type: none"> <li>- the number of staff, by grade, involved in the different stages of the screening process</li> <li>- the amount of laboratory space devoted to screening</li> <li>- the cost of replacement of the equipment used for screening</li> <li>- the proportional use of screening equipment for each disease</li> <li>- consumable costs</li> </ul>
<p><b>Research and development</b></p> <ul style="list-style-type: none"> <li>- information on any pilot studies, unpublished laboratory reports, theses, etc</li> <li>- putative plans for expansion of the neonatal screening programme</li> </ul>

initially introduced and where methodologies have been extensively developed

- Neogen Inc., Pennsylvania, USA, where the technique has been extended and where the only prospective screening applied to well baby screening has been undertaken
- Institute of Child Health, University of London
- Sir James Spence Institute of Child Health, University of Newcastle upon Tyne.

The two visits in the USA were made in conjunction with Dr Rodney Pollitt from the SCHARR review group.

A visit was made to Wallac in Finland to assess the future of automation of neonatal screening using AUTODELFIA® (dissociation-enhanced lanthanide fluorescence immunoassay) methodology.



## Chapter 3

# Reviews of inborn errors of metabolism

## Phenylketonuria

### Introduction

PKU is the clinical disease that results from an inborn error of metabolism affecting activity of the hepatic enzyme phenylalanine hydroxylase which converts the amino acid phenylalanine to tyrosine, the precursor of dopamine and noradrenaline. It is characterised by hyperphenylalaninaemia and manifests primarily as neurological damage impairing cognitive development. Like many inborn errors of metabolism, there is a spectrum of reduced enzyme activity, from absent to marginally reduced, that correlates with disease severity. PKU is thus arbitrarily defined as phenylalanine levels above 1000  $\mu\text{mol/l}$  at which residual enzyme activity is absent or nearly absent, and the risk of neurological damage without treatment approaches 100%.<sup>6</sup> Non-PKU hyperphenylalaninaemia (HPA) is defined as levels between 120  $\mu\text{mol/l}$  (upper limit normal) and 1000  $\mu\text{mol/l}$ , which are much less likely to cause clinical disease. However, reports from the Medical Research Council in the UK have taken PKU as blood phenylalanine levels in excess of 240  $\mu\text{mol/l}$ <sup>7</sup> or 480  $\mu\text{mol/l}$ .<sup>8</sup>

In patients with PKU who are not treated, delayed development usually first becomes noticeable between 6 and 12 months of age, by which time up to 50 IQ points will have been lost. Only rarely do symptoms lead to diagnosis before this stage. Effective therapy to lower raised blood phenylalanine levels by dietary restriction of phenylalanine prevents progressive, irreversible cognitive damage but does not reverse pre-existing damage, and so the earlier treatment is commenced the better the ultimate outcome. Some 1–2% of cases of hyperphenylalaninaemia are due to disorders in the metabolism of the enzyme co-factor, tetrahydrobiopterin, which is also required for the production of the neurotransmitters (dopamine, noradrenaline and 5-hydroxytryptamine).<sup>6</sup> These cases require treatment in addition to dietary phenylalanine restriction. In the UK, all cases of hyperphenylalaninaemia are investigated for these defects by a combination of total biopterin and dihydropteridine reductase assay in eluates from the neonatal screening blood spots.<sup>9</sup>

The current UK screening programme for PKU was established on the basis of recommendations from the Ministry of Health in 1969 in circular HM(69)72 for heel-prick blood samples obtained from all newborn infants aged between 6 and 14 days.<sup>10</sup> These recommendations also specifically indicated the Guthrie test as the method of choice, and highlighted the need for neonatal screening to be closely integrated with laboratory and clinical services for these patients.

### Incidence

The incidence in England and Wales of all forms of hyperphenylalaninaemia is 1:9200 live births; in Northern Ireland it is more than twice as high. Data from a review of the screening programme found that 78% of cases were due to PKU, defined as phenylalanine above 900  $\mu\text{mol/l}$ .<sup>7</sup> Therefore the estimated incidence of PKU in the UK is 1:12,000 live births.

### Outcome

Early diagnosis and treatment has reduced the risk of neurological handicap in PKU from about 80–90% to 6–8% of cases (general population risk, 2%).<sup>11</sup> In a recent cohort review, only 5% of 51 untreated patients followed for 20 years had IQ values greater than 68.<sup>12</sup> Studies of long-term outcome of patients with early diagnosis and treatment still show a mean IQ reduction of 0.5 standard deviation (SD) from the normal population. In addition there is an increased occurrence of subtle neuropsychiatric disturbances such as delayed speech, learning difficulties, and behavioural problems. It was believed that these abnormalities may be linked to the quality of phenylalanine control, especially in pre-school years, and this led to the recommendations emphasising the need for specialist services, and the importance of proper management of PKU before and during pregnancy.<sup>13</sup> Despite these problems, “most early treated children [with PKU] fall within the broad normal range of ability and attend ordinary schools”.<sup>13</sup> Reports of neurological deterioration in young adults off dietary supervision have led to the recommendation of treatment/supervision for life,<sup>13,14</sup> which will increase life-time treatment costs.

## Screening options

Today screening in the UK is, as far as possible, universal. The most recent estimates are that screening covers at least 99% of live-born infants surviving at 6–14 days.<sup>3,4</sup> Smith and colleagues,<sup>7</sup> although unable to determine the exact coverage, also concluded that coverage approached 100%, as they found no affected infants between 1984 and 1988 who had been missed because they had not been tested.

The dried blood spots are tested at local screening laboratories using the Guthrie bacterial inhibition test, thin-layer or paper chromatography or fluorometry. The Guthrie test is only semi-quantitative and difficult to automate, with different opinions being expressed about its precision and comparative cost.<sup>7,15,16</sup> These are evidenced by the various methodologies in current use, namely chromatography, fluorometry using ninhydrin, enzyme assays based on phenylalanine dehydrogenase and colorimetric/fluorometric detection, and most recently tandem MS. For fluorometry, sensitivities of 15–30  $\mu\text{mol/l}$ , with coefficient of variation (CV) of 10% are quoted, and false-positive rates of 0.02–0.64% on first screen are reported compared with 5% for the Guthrie assay, although second blood samples were required in 0.15% of cases for both the fluorometric and Guthrie methods.<sup>15,17</sup> Tandem MS has been demonstrated to have a sensitivity of 3  $\mu\text{mol/l}$  for phenylalanine with intra- and inter-assay CV of 6–10%. Discrimination is further enhanced by the simultaneous measurement of tyrosine, permitting calculation of a phenylalanine:tyrosine ratio which allowed the successful detection of all five false-positives generated by fluorometry.<sup>18</sup>

Some places (for example, Turkey<sup>19</sup>) still continue with the Guthrie test as the sole screening method, while others, for example, France (18 out of 22 laboratories),<sup>20</sup> the Soviet Union<sup>21</sup> and Hanover in Germany,<sup>17</sup> have abandoned it for fluorometry. In the UK, Smith and colleagues<sup>7</sup> found that of the 26 laboratories undertaking hyperphenylalaninaemia screening in 1988, nine used the Guthrie method, 11 used either paper chromatography or TLC and six used fluorometry. In the current review, of the 14 laboratories that replied to the questionnaire in 1996, seven used the Guthrie test, four used chromatography and three used fluorometry. Despite the lack of uniformity in approach to methodology in the UK, there is no objective evidence of any difference in outcome between the different methodologies.

A very few centres (probably two in the UK) take liquid blood samples into capillary tubes

for chromatography rather than carrying out direct blood spotting onto Guthrie cards. Lorey and Cunningham<sup>22</sup> found phenylalanine levels to be a mean of 9.3  $\mu\text{mol/l}$  lower for analysis of blood samples collected into a capillary tube in comparison with analysis of blood directly spotted onto filter paper. In considering the implications for quality control and standards set for cut-off values, the authors advised that “all other methods [apart from direct spotting] should be strongly discouraged if not eliminated entirely”. This potential variability from sample type is, however, minor compared with the variation in cut-off values used in the UK programme. Smith and colleagues<sup>7</sup> reported a range of threshold levels being used: 200  $\mu\text{mol/l}$  (two laboratories), 250  $\mu\text{mol/l}$  (two laboratories), 300  $\mu\text{mol/l}$  (one laboratory), 480  $\mu\text{mol/l}$  (one laboratory), and > 240  $\mu\text{mol/l}$  (remaining 20 laboratories).

There is variation between countries as to the exact timing of screening, but there is general agreement that treatment should preferably be instituted within about 3 weeks of birth. The review by Smith and colleagues<sup>7</sup> of the UK neonatal screening programme for PKU over the period 1984–1988 showed considerable regional variation with respect to the timing of the initial neonatal screening test and the time to start of treatment. The median age at initial screening varied between 6.5 and 11 days. The median age at start of treatment varied from 9.5 to 17 days, with a range of 6 to 55 days. Smith and colleagues<sup>7</sup> found that 8% of infants with severe PKU, and 42% with co-factor disorders, were still not on treatment by 20 days after birth. Since IQ falls linearly by 1 point for each week's delay in starting treatment, after other factors are allowed for (for example, severity of the disorder and quality of phenylalanine control),<sup>9</sup> Smith and colleagues<sup>7</sup> recommended that a reduction in the age of testing to 5–8 days seemed to have few disadvantages and ought to be considered.

Although the UK neonatal screening programme generally works in accordance with the Department of Health's recommendations there are significant regional variations in screening practice. While they did not formally assess these variations in terms of their impact on outcome measures, Smith and colleagues<sup>7</sup> recommended that variation should be reduced through an (effective) national screening policy as is already the case in France. Cappuccio and co-workers<sup>23</sup> confirmed the perception of a need for a better infrastructure for the notification and continued care and management of cases of PKU identified through the current neonatal screening programme.



## Conclusions

A review of the available literature confirms that there is universal agreement that neonatal screening for PKU is justified (*Table 2*). However, certain deficiencies have been identified which need to be addressed. Cost-benefit analyses (CBAs) in the literature have not included the cost of life-time therapy and rarely the cost of therapy during pregnancy. All reviewers seemed satisfied with their programmes. PKU is the classic example of an inborn error of metabolism which causes irreversible damage if not treated early, and one in which early treatment, maintained through childhood at least, results in almost all children developing normally. PKU continues to fulfil all the criteria for a screening programme. The national programme for PKU screening has prevented mental handicap and likely long-term residential care in some 1300 children over the past 20 years.

TABLE 2 PKU: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	✓
Period before onset during which intervention improves outcome	✓
Ethical, safe, simple and robust screening test	✓

## Amino acidopathies

### Introduction

Amino acids are a group of compounds which contain both an amino and acidic group. The term amino acidopathy is used to refer to a defect in amino acid metabolism that produces diagnostic changes in the concentration of that amino acid in body fluids. This defect can be either in the intracellular metabolic pathway which causes elevated levels of the amino acid in plasma and/or urine (depending on the degree of renal tubular reabsorption), or in cell membrane transport when there is amino aciduria but normal or low plasma levels. Amino acids are not only the building blocks of proteins, but also function as integral components of metabolic cycles and as neurotransmitters. Because of these many functions, and the quite different biochemistry of individual amino acids and their metabolites, clinical

features differ markedly between the various amino acidopathies. There is usually a spectrum of severity which is largely dependent on residual enzyme activity. Some amino acidopathies, for example, maple syrup urine disease (MSUD; branched-chain amino acidemia), can have a neonatal onset with life-threatening disease whereas the initial symptoms of others, for example, homocystinuria (cystathionine  $\beta$ -synthase deficiency), may develop only well into infancy and life-threatening events are delayed until young adulthood or later. Other amino acidopathies such as histidinaemia and hyperprolinaemia are completely or largely benign.

Newborn screening has been proposed for several amino acidopathies to prevent the development of life-threatening disease in early infancy and improve long-term outcome by early institution of effective maintenance therapy. Specific screening programmes have been proposed for MSUD, homocystinuria, and tyrosinaemia type 1 and have been implemented in some countries and in some states in North America. PKU is an amino acidopathy, but because of its special characteristics and because neonatal screening for the disorder is well established, we have considered it in a separate section (see page 9). Capillary blood-spot analysis will not detect cell membrane transport defects, such as cystinuria (causing renal stones) and iminoglycinuria (a benign condition), which have been the more common amino acidopathies (incidences of 1:10,000 to 1:20,000) detected in postnatal urine screening programmes.

### Incidence

Individually, amino acidopathies excluding PKU are rare but collectively they are probably of an equal incidence to PKU at about 1:5000 to 1:10,000 live births (*Table 3*).<sup>24,25</sup>

Well-baby neonatal screening programmes have been established for MSUD based on detection of raised blood levels of the branched-chain amino acids, usually leucine. Data from Scotland indicate that the disorder may have a lower incidence in the UK than elsewhere.<sup>27</sup> Newborn screening for homocystinuria has been on the basis of hypermethioninaemia but the UK data hide much regional variation with incidences of 1:126,000 live births in Manchester, 1:1,000,000 in Scotland, and 1:78,000 for Northern Ireland.<sup>34</sup> It is believed that there may be under-ascertainment of cases of homocystinuria by neonatal screening, especially for pyridoxine-responsive disease (approximately 50% of clinically detected cases).

**TABLE 3** Incidences of amino acidopathies: data from newborn screening programmes

Disorder	Worldwide incidence	UK incidence
MSUD	1:185,000 <sup>26</sup>	No cases detected in 467,448 newborn infants screened in Scotland <sup>27</sup>
Homocystinuria	1:291,000 <sup>28</sup>	1:234,000 <sup>29</sup>
Tyrosinaemia type I	No data available	1:105,000 <sup>30</sup>
Histidinaemia	1:11,500 <sup>31</sup>	1:11,000 <sup>32</sup>
Hyperprolinaemia	1:40,000 <sup>33</sup> to 1:100,000 <sup>25</sup>	No data available

Neonatal screening for tyrosinaemia type I is carried out in some high-risk populations such as the French-Canadian population in Quebec, Canada.<sup>35</sup> This disorder appears to be of higher incidence in certain parts of the UK, where it is largely associated with a specific genetic pool and the practice of consanguineous marriage.<sup>30</sup>

### Outcome

Presentation of amino acidopathies ranges from severe and fatal if untreated to mild in those disorders that are clinically significant, with the less severe disease being easier to treat. Some of the disorders respond to vitamin therapy and others are now recognised to be benign (e.g. histidinaemia<sup>32,36</sup>).

### MSUD

MSUD manifests a spectrum of presentation ranging from acute neonatal onset, most commonly at days 4–7 of life, through manifestation with developmental delay/convulsions in later infancy but extending up to young adulthood, to an intermittent encephalopathic presentation with intervening periods of good health. Long-term survival is now good but intellectual performance is correlated with the age at which metabolic control is achieved and the quality of control. Mean IQ for MSUD patients is well below normal, and much lower than for a comparable group of treated PKU patients.<sup>37</sup> Dietary therapy is for life, is difficult to maintain, and does not completely prevent recurrent metabolic crises which remain potentially fatal.<sup>38</sup>

### Homocystinuria

Homocystinuria, which is due to cystathionine  $\beta$ -synthase deficiency, causes significant morbidity

and mortality from a multi-system disease, with impaired vision, osteoporosis, neurological dysfunction, and thromboembolism. A retrospective case survey has indicated that disease manifestations increase with age, and are less severe in pyridoxine-responsive disease. Lens dislocation will have occurred in 50% of patients by the age of 8 years, and 50% of patients will have had a thromboembolic event by the age of 28 years. In a survey of late-treated patients, median IQ was 78 for pyridoxine-responsive cases and 56 for pyridoxine non-responsive cases.<sup>28</sup> A study showed that, over limited periods of follow-up, treatment in infancy with dietary restriction and pyridoxine prevented many complications, whereas disease manifestations failed to respond to similar therapy given at onset of symptomatic disease.<sup>28</sup> Greater difficulties are encountered in terms of compliance when such therapy is instituted outside the neonatal period.

### Tyrosinaemia type 1

Patients with tyrosinaemia type 1 (fumarylacetoacetase deficiency) present with liver failure, renal tubular dysfunction, and neuropathic crises. The disease may be of acute onset, occurring within weeks of birth, or may be more delayed with liver damage leading to cirrhosis and an increasing risk of hepatocellular carcinoma in survivors over the age of 2 years. Prognosis is uncertain as the disease may progress despite rigorous dietary therapy. The long-term efficacies of more recent therapies such as liver transplantation and 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione; NTBC, an inhibitor of 4-hydroxyphenylpyruvate oxygenase, are still under assessment.

### Screening options

#### MSUD

Neonatal screening of more than 6 million infants for MSUD by blood-spot analysis using a bacterial inhibition assay for leucine has been described.<sup>27</sup> The cut-off was set at 300  $\mu\text{mol/l}$  (normal 48–220  $\mu\text{mol/l}$ ), and a false-positive rate of 0.05%, and a very low false-negative rate, were reported. One case of intermittent disease was missed in this series. Intermittent disease is known to be associated with normal plasma levels when the patient is asymptomatic and later onset disease with less elevated leucine levels so that these phenotypes are likely to be more difficult to detect. Tandem MS (see page 39) offers a sensitivity of 2  $\mu\text{mol/l}$  for leucine + isoleucine, reduced false-positives and potentially fewer false-negatives. Using tandem MS in a prospective neonatal screening of 173,537 babies, Neogen Inc. identified two newborn infants with MSUD and

there were no false-positives and no known false-negative results (EW Naylor: personal communication, 1996/1997).

### **Homocystinuria**

Neonatal screening for homocystinuria currently has to rely on the detection of the associated secondary hypermethioninaemia. Although the bacterial inhibition assay for hypermethioninaemia is widely used, it is only semi-quantitative, with a sensitivity of 60  $\mu\text{mol/l}$  (normal  $20 \pm 8 \mu\text{mol/l}$ ). Whiteman and colleagues in a UK study concluded that using a bacterial inhibition assay to detect hypermethioninaemia on capillary blood spots was not justified because of the poor sensitivity of the assay and the absence of detectable hypermethioninaemia in the milder cases.<sup>29</sup> Single-dimensional paper chromatography or TLC requires liquid blood samples and has a sensitivity of about 200  $\mu\text{mol/l}$ .<sup>29</sup> Thus false-negatives are a recognised problem with both methods and hypermethioninaemia measured in this way is likely to be too unreliable for universal neonatal screening for homocystinuria. However, tandem MS (see page 39) offers sensitivity of 4  $\mu\text{mol/l}$  for methionine, a demonstrated ability to detect false-positives generated by the bacterial inhibition assay, and the potential to significantly reduce false-negatives.<sup>39</sup> Using tandem MS in a prospective neonatal screening of 173,537 babies, Neogen Inc. identified two infants with hypermethioninaemia; there were no known false-negative results (EW Naylor: personal communication, 1996/1997).

### **Tyrosinaemia type 1**

Tyrosinaemia type 1 has been detected as an incidental finding in screening programmes using chromatography as the preferred methodology, although there was a false-positive rate of 0.3%, and two of eight known cases were missed.<sup>30</sup> In areas of high incidence of tyrosinaemia type 1 specific determination of blood-spot tyrosine has been undertaken, for example, by fluorometry, with subsequent measurement of urinary succinyl-acetone for all newborn infants with high values indicated by fluorometry.<sup>35</sup> Using tandem MS in a prospective neonatal screening of 173,537 babies, Neogen Inc. identified one infant with hypertyrosinaemia (EW Naylor: personal communication, 1996/1997). The tandem MS technique can distinguish between hypertyrosinaemia and benign neonatal tyrosinaemia, which is often associated with hyperphenylalaninaemia (simultaneously determined). There is no clear indication in the UK for neonatal screening for tyrosinaemia type 1 despite the new and promising therapies of liver transplantation and NTBC therapy.<sup>40</sup>

We could identify only one paper in the literature that specifically addressed whether screening for amino acidopathies was cost-beneficial in a defined population relevant to the UK.<sup>25</sup> However, this study in New South Wales, Australia used screening of urine from 6-week-old infants by one-dimensional paper chromatography rather than neonatal blood-spot screening. The methods used had poor sensitivity for certain conditions. Benefit from early diagnosis was based on whether therapy was instituted and on a clinical but subjective assessment of improved outcome from therapy. Although non-PKU amino acidopathies had a total incidence of 1:10,000 (approximately the same as that of PKU), the authors concluded that the benefit from their early diagnosis compared poorly with that seen for PKU. Several issues were identified as detracting from benefits of screening for non-PKU amino acidopathies, including the low incidences of the conditions, the benign nature of some of the conditions detected, the non-availability of therapies that were effective or of proven benefit, and the inadequacies of, and difficulties in maintaining, unpalatable and unpleasant treatments over prolonged periods.

### **Conclusions**

Neonatal screening for non-PKU amino acidopathies does not meet all of the criteria required to justify a neonatal screening programme (*Table 4*). It is probable, however, that if tandem MS were introduced in the UK for screening for PKU and some other inborn errors of metabolism, some cases of amino acidopathies would also be identified during the course of such screening. There is no evidence that such a programme would be cost-beneficial at the present time and therefore there is no justification for including neonatal screening specifically for non-PKU amino acidopathies in the UK programme.

## **Galactosaemia**

### **Introduction**

Inherited deficiencies in three enzymes in the metabolic pathway of galactose have been described. Although all of these deficiencies are characterised by raised blood galactose levels ('galactosaemia'), each is associated with a distinct and different clinical condition. Galactosaemia is usually taken by convention to refer to the condition resulting from deficiency of galactose-1-phosphate uridyl transferase (GALT), an autosomal recessively inherited defect in the

TABLE 4 Amino acidopathies: summary of conclusions

Screening criteria	Criteria fulfilled		
	MSUD	Homocystinuria	Tyrosinaemia type I
Clinically and biochemically well-defined disorder	✓	✓	✓
Known incidence in populations relevant to UK	[✓]	[✓]	[✓]
Disorder associated with significant morbidity or mortality	✓	✓	✓
Effective treatment available	±	±	±
Period before onset during which intervention improves outcome	±	✓	±
Ethical, safe, simple and robust screening test	✓	✓	✓

conversion of galactose to glucose. GALT catalyses the transfer of galactose-1-phosphate to uridine diphosphate (UDP)-glucose to produce UDP-galactose with release of glucose-1-phosphate. Deficiency of GALT will therefore result in the accumulation of its substrate galactose-1-phosphate, and also galactose, the latter being the substrate for the preceding step which produces galactose-1-phosphate from galactose by phosphorylation from ATP catalysed by the enzyme galactokinase. The main dietary source of galactose is the milk sugar lactose (glucose and galactose), with much smaller amounts contributed by fruit and vegetables. There is now increasing evidence that galactose production from turnover of glycoproteins and glycolipids<sup>41</sup> is a significant source of the metabolic load of galactose, far exceeding that consumed from non-milk sources. A third enzyme, UDPgal 4-epimerase, is responsible for regenerating UDP-glucose from UDP-galactose to act as substrate for GALT.

In a North American study,<sup>42</sup> 87% of cases of galactosaemia were associated with the Q188R mutation (arginine substituted for glutamine at position 188), 46% being homozygous for the mutation and 40% heterozygous. A number of other mutant alleles exist which are associated with a reduction in GALT activity (partial deficiency). The commonest of these is the Duarte allele which is 5–10 times commoner than the galactosaemia allele and is associated with a 50% reduction in GALT activity in the homozygous state and a 75% reduction when heterozygous with the galactosaemia allele.

Clinically, galactosaemia is characterised by jaundice, failure to thrive, hepatosplenomegaly, deranged liver function, renal tubular defects

such as amino aciduria, susceptibility to infection, and albuminuria in the first few weeks of life. Cataracts have been observed within a few days of birth. If the disease is severe, undiagnosed, and untreated the liver disease progresses to cirrhosis with end-stage liver failure and death. Some patients have a more chronic course, with presentation some months after birth with delayed development, hepatomegaly, and cataracts. Sometimes the manifestation of the disease occurs in late childhood with mental retardation and cataracts. Introduction of a galactose-free diet will cause the resolution of all of the early manifestations of the disease, including cataracts, and prevent their recurrence. This initial resolution of organ dysfunction led to the expectation that dietary therapy could and would preclude the development of long-term sequelae related to the metabolic defect. Unfortunately this has not proved to be true, probably because of the significant endogenous turnover of galactose which is independent of manipulation of dietary intake.

Although many programmes have included neonatal screening for galactosaemia, it was not introduced in Norway after a 10-year retrospective case review,<sup>43</sup> and has not been introduced in The Netherlands or in 44 out of 50 states in the USA.<sup>44</sup>

### Incidence

There is only one study in the literature that assesses the incidence of galactosaemia in the UK.<sup>45,46</sup> This was a 3-year prospective case ascertainment study (1988–1990 inclusive), which used data from neonatal screening programmes for galactosaemia that were current for this period in Scotland and the Republic of Ireland and compared them with information on diagnosed cases in England, Wales and Northern Ireland where there were no such screening programmes. This study revealed

the incidence of galactosaemia in the UK to be 1:44,000 population which is within the range reported for Caucasian populations of 1:26,000 (Ireland) to 1:89,000 (Sweden); Japan has an incidence of 1:667,000. Schickling and colleagues<sup>47</sup> found that in North Germany the incidence of partial GALT deficiency (Duarte and other variants) was 1:9200 live births compared with 1:44,000 for galactosaemia, and Bowling and Brown<sup>48</sup> found an incidence of 1:3800 for the variants compared with 1:35,000 for galactosaemia in a study in Australia.

### Outcome

Studies from America<sup>49</sup> have reported death associated with *Escherichia coli* sepsis in up to 30% of neonates with galactosaemia where diagnosis and treatment were delayed until the second week of life. However, in the only published British study, which covered the years 1988–1991,<sup>45,46</sup> there were five cases of septicaemia out of 60 cases (13%) and one death (at 4 days of age).

Honeyman and colleagues<sup>45,46</sup> reviewed 60 patients with galactosaemia to assess the benefits of early treatment resulting from neonatal screening. They found that 93% of the 14 patients detected by screening were on dietary treatment by 15 days of age compared with 71% of the non-screened group. Among the screened group, one infant (2%) died aged 4 days, and four (28%) presented clinically before the screening result was available. The incidence of severe symptoms was similar in the screened and non-screened groups which indicated that screening had little impact on acute neonatal illness. The authors concluded that there was no current justification for neonatal screening for galactosaemia in the UK.

Prospective treatment in sibling pairs<sup>50</sup> has shown that treatment from birth in a subsequent sibling has produced the same long-term outcome as in the index sibling who commenced therapy much later. However, dietary therapy does reverse and prevent the development of liver and renal damage, cataract formation, and the immunodeficiency that makes neonates so susceptible to Gram-negative sepsis. On the other hand, it has no effect on long-term neurological damage, which may be progressive throughout childhood and into adulthood, and on the incidence of ovarian damage. In one study, 65% of patients aged more than 3 years had speech abnormalities,<sup>51</sup> and 83% of patients aged more than 12 years had an IQ less than 85. In another study 85% of the female patients had ovarian dysfunction.<sup>52</sup> In

addition to lowering of IQ, there are specific learning defects involving spacial and mathematical concepts, and more general problems such as short attention span, and behavioural problems.<sup>53</sup> More ominously, there are now case reports appearing of a progressive, neurodegenerative disorder in older children and adolescents with galactosaemia who present with cerebellar and extrapyramidal signs and symptoms.<sup>53</sup>

Neonatal screening will detect the partial deficiencies that are 5–10 times commoner than total GALT deficiency. These partial deficiencies are of doubtful clinical significance but are still treated in some institutions.<sup>52</sup> Galactose tolerance is reduced in these partial deficiencies, and in infants blood galactose and galactose-1-phosphate levels are often significantly elevated for several months as their diet is largely milk-based. Adults with these variant genotypes appear healthy. However, while Buist and Waggoner<sup>52</sup> accept the likely benign nature of these variants, they recommend treatment during infancy to prevent potential litigation. Gitzelman and Bosshard,<sup>54</sup> in reviewing their experience of 72 cases of partial GALT deficiency (presumed Duarte/galactosaemic heterozygotes), were unable to determine the validity of their pragmatic approach of a lactose-free diet for the first 4 months of life.

The recent, commercially sponsored, establishment of a galactosaemia register in the UK should provide data for prospective studies of patient outcome.

### Screening options

There is little doubt that the methodology for neonatal screening for galactosaemia is simple, robust, and practical. It uses a neonatal dried blood spot to determine either alone or in combination, galactose and galactose-1-phosphate levels, and activity of GALT. Schickling and colleagues<sup>47</sup> reported the use of a colorimetric enzymatic microassay for galactose and galactose-1-phosphate to screen 350,000 newborn infants. The false-positive rate was 0.028%, of which 40% were compound heterozygotes, mostly Duarte/galactosaemia. No false-negatives were reported, and the test was said to be 'inexpensive'. Bowling and Brown<sup>48</sup> evaluated a protocol based on a fluorescent enzymic assay for galactose and galactose-1-phosphate with secondary determination of GALT by the Beutler fluorescent assay and sugar TLC to exclude false-positives when the metabolite assay was above the predetermined cut-off. In screening of 207,000 newborn infants false-positives were stated to be 'acceptably low'

and no false-negatives were reported; costs were not given. Thibodeau and co-workers<sup>55</sup> reported on 299,420 newborn infants screened using the Beutler assay for GALT activity alone. The false-positive rate averaged 0.16% over 3 years (1989–1991) and there were no reported false-negatives. The Beutler assay is affected by humidity and temperature during transport of the blood spot and by the degree of drying of the blood spot; the authors were concerned that their false-negative rate might be higher during the cooler 4 months of the year although they had no objective evidence of this. Honeyman and colleagues<sup>45,46</sup> reported one false-negative (n = 14, 7%) in their series of neonatally screened infants in the UK.

### Conclusions

The incidence of galactosaemia in the UK is much lower than that of PKU or hypothyroidism. Galactosaemia does not produce the severity of mental handicap seen with PKU or hypothyroidism. There are different views in the literature of the value of neonatal screening for galactosaemia for a number of reasons: there is little evidence that the earlier institution of treatment allowed by neonatal screening improves long-term outcome;<sup>43,56,57</sup> the long-term outcome remains poor in spite of best available current treatment;<sup>58</sup> and there are as yet uncosted difficulties and uncertainties posed by the allelic variants producing partial GALT deficiency. The benefits of screening are currently related to possible prevention of acute neonatal illness in some cases, the facilitation of collection of epidemiological and outcome data, and improved patient support. On the basis of the evidence, the accepted criteria for a neonatal screening programme are not currently met by galactosaemia (*Table 5*) and therefore there is no justification at present for a neonatal screening programme in the UK.

**TABLE 5** Galactosaemia: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	✗
Period before onset during which intervention improves outcome	±
Ethical, safe, simple and robust screening test	±

## Disorders of organic acid metabolism: the organic acidurias and organic acidurias (including disorders of the mitochondrial respiratory chain)

### Introduction

Disorders of organic acid metabolism, more commonly referred to as organic acidurias or organic acidurias, comprise a diverse group of diseases the biochemistry of which encompasses several areas of intermediary metabolism. These include metabolic pathways associated with amino acid metabolism (particularly of L-leucine and other branched-chain amino acids, and of L-lysine and of aromatic amino acids), pathways associated with fatty acid metabolism and ketogenesis, and also pathways of pyruvate and carbohydrate metabolism including the TCA (or Krebs's) cycle. Such disorders may occur in or involve the cytosol and/or many different organelles including mitochondria, peroxisomes and microsomes. Some disorders, for example, biotinidase deficiency and disorders of mitochondrial fatty acid  $\beta$ -oxidation and of peroxisomes, are considered elsewhere in this review (see pages 19, 28 and 31).

The disorders share common features in that they are characterised by the accumulation in body tissues and fluids, particularly urine, of organic acids and their esters and conjugates. Those disorders that are associated with acyl CoA ester accumulation in mitochondria are also associated with acylcarnitine accumulation in body tissues and fluids, which offers important diagnostic opportunities for neonatal screening (see below and *Table 6*). The different disorders have similarities in chemistry and biochemistry that lead to common clinical characteristics, especially acute presentation in early life, with acidosis, ketosis, hypoglycaemia, hyperammonaemia, vomiting, convulsions and coma. The disorders are often lethal in the newborn infant and young child and survivors may be physically or mentally handicapped. Other patients may present later in childhood with established failure to thrive, neurological deterioration or with sudden acute and profound attacks associated with infections and trauma.<sup>59</sup>

### Incidence

Most of the disorders of organic acid metabolism are of autosomal recessive inheritance. Although the incidence of individual diseases may be low, taken collectively their overall incidence may be as high as 1:3000 live births. Included among these disorders are the most commonly encountered organic acidurias, including methylmalonic

TABLE 6 Acylcarnitines identified in some disorders of acyl CoA metabolism

Disorder/defect	Acyl CoA	Acylcarnitine*
Propionic acidaemia	Propionyl	Propionyl
Methylmalonic aciduria	Propionyl	Propionyl
Glutaric aciduria type I	Glutaryl	Glutaryl
3-Hydroxy-3-methylglutaric aciduria	3-Hydroxy-3-methyl-glutaryl	3-Methylglutaryl, 3-hydroxyisovaleryl
Isovaleric acidaemia	Isovaleryl	Isovaleryl, [3-hydroxyisovaleryl]
3-Methylcrotonylglycinuria	3-Methylcrotonyl	3-Methylcrotonyl, 3-hydroxyisovaleryl
Multicarboxylase deficiencies	3-Methylcrotonyl Propionyl	3-Hydroxyisovaleryl, [propionyl]
$\beta$ -Ketothiolase deficiency	Tiglyl	Tiglyl

\* Putative acylcarnitines expected to occur in these disorders are shown in brackets.

aciduria (MMA), propionic acidaemia (PA), isovaleric acidaemia (IVA), 3-hydroxy-3-methylglutaric aciduria (HMGA) and glutaric aciduria type 1 (glutaryl CoA dehydrogenase deficiency; GA1) with a collective incidence that may be estimated at around 1:15,000.

As its name implies, MMA is characterised by greatly increased excretion of methylmalonic acid into the urine. Substituted malonic acids are relatively unique in that they may be detected by simple colorimetric tests. Neonatal screening for MMA using urine samples collected at 3–4 weeks of age and chromatography with colorimetric development has been a feature of the screening programme in Massachusetts, USA, for many years.<sup>60</sup> Prospective screening of 293,535 neonatal infants in this way revealed four with MMA, giving an incidence of 1:73,000. The authors also drew attention to four other children with MMA who were identified on clinical grounds during the same period (from a total newborn population of 383,429, giving an overall incidence of 1:48,000) but who were not included in the prospective screening.

### Outcome

Patients with disorders of organic acid metabolism often have severe life-threatening episodes of acidosis, ketosis and hypoglycaemia. Long-term therapeutic approaches involve substrate restriction through dietary manipulation, use of co-factors at pharmacological levels, removal of substrate by conjugation or inhibition of endogenous synthesis and use of L-carnitine. Treatment modalities are continuously evolving and have resulted in much improvement in the prognosis of some conditions (for example, IVA, MMA, HMGA, and the co-factor-responsive disorders, including some forms of MMA and

multicarboxylase deficiency). This is particularly relevant to newborn screening for these disorders. However, treatment of other disorders of organic acid metabolism is fraught with difficulties, poor response and poor prognoses and for many disorders of organic acid metabolism, particularly disorders of the mitochondrial respiratory chain (the so-called 'mitochondrial myopathies'), there is no effective treatment available at present. In general, the disorders are still associated with high morbidity and mortality.

GA1 is perhaps unique among these disorders in that all patients described have had a period before onset during which it is believed that intervention could have significantly improved outcome. GA1 is associated with a particular and disabling generalised dystonic cerebral palsy and choreoathetosis in clinically-affected individuals. Other patients with the same biochemical disorder and abnormal organic aciduria, even siblings of clinically-evident index cases, may remain completely asymptomatic. Evidence is emerging that a combination of the biochemical defect with other, unknown, factors precipitates rapid and devastating neurological damage that is irreversible. Administration of L-carnitine to asymptomatic individuals appears to offer considerable benefit by preventing the occurrence of such damage and allowing normal development. There is some evidence that acute neurological damage will not occur beyond late childhood (H Morten, JBC de Klerk and others: personal communications at 2nd European workshop on glutaryl CoA dehydrogenase deficiency, Marburg, Germany, 1996).

### Screening options

As their collective name implies most disorders of organic acid metabolism are diagnosed by analysis

of body fluids for organic acids. Current methods are generally based upon gas chromatography and mass spectrometry and require extraction of the organic acids and conversion into thermally and chemically stable derivatives for chromatography.<sup>59</sup> They are quite unsuitable for large-scale newborn screening.

The acylcarnitines that accumulate in disorders of mitochondrial acyl CoA metabolism are characteristic of individual diseases. These are listed in *Table 6*.<sup>61</sup> They include all the most commonly encountered organic acidurias/acidaemias.

Analysis of blood samples and of dried blood spots for acylcarnitines using tandem MS has been shown to be an effective method for the identification of patients with these disorders of acyl CoA metabolism.<sup>62–65</sup> Prospective newborn screening for organic acidurias based on tandem MS analysis for acylcarnitines in dried ('Guthrie') blood spots has been carried out in a multi-racial population in Pittsburgh, USA (see page 42). The use of tandem MS for neonatal screening for acylcarnitines in blood spots appears to be a viable procedure.

## Conclusions

GA1 comes quite close to fulfilling the necessary criteria for newborn screening (*Table 7*), although there is some lack of information on absolute incidence and natural history. Prevention of the clinical manifestations of GA1 (and of MCAD deficiency – see page 19) alone could make newborn screening for these disorders cost-beneficial.

Screening criteria for the other common organic acidurias are less well fulfilled and

taken individually there is no justification for screening for them. Collectively, however, they would be observed during any screening by tandem MS for acylcarnitines and would be detected in such a screening programme for no additional screening cost. There would be clinical benefits for at least some of these patients and early identification would assist genetic counselling and prevention of further cases in the same families. Thus, if newborn screening for GA1 (and MCAD deficiency) using tandem MS was introduced, some additional benefits would accrue from detection of these other common organic acidurias.

For other disorders of organic acid metabolism knowledge is still emerging and they do not fulfil the screening criteria. They should not be considered for newborn screening at the present time.

Disorders of the mitochondrial respiratory chain fulfil none of the screening criteria and should not be considered for any newborn screening programme.

On consideration of the evidence a case could be made for the introduction of newborn screening for GA1, which would also detect some other common disorders of organic acid metabolism. Such screening would be dependent upon the introduction of tandem MS technology and would require careful monitoring, with review after 10 years because outcome data is required for the assessment of clinical effectiveness and cost-effectiveness.

**TABLE 7** Organic acidurias and organic acidaemias: summary of conclusions

Screening criteria	Criteria fulfilled					
	Disorders of acyl CoA metabolism					Mitochondrial respiratory chain disorders
	MMA	PA	IVA	GA1	HMGA	
Clinically and biochemically well-defined disorder	[✓]	[✓]	✓	[✓]	[✓]	✗
Known incidence in populations relevant to UK	±	±	±	±	±	✗
Disorder associated with significant morbidity or mortality	✓	✓	✓	✓	✓	✓
Effective treatment available	[✓]	±	✓	[✓]	✓	✗
Period before onset during which intervention improves outcome	±	±	±	✓	±	✗
Ethical, safe, simple and robust screening test	✓*	✓*	✓*	✓*	✓*	✗

\* By tandem MS only.



## Disorders of fatty acid metabolism (mitochondrial $\beta$ -oxidation)

### Introduction

The mitochondrial  $\beta$ -oxidation of fatty acids (< C<sub>24</sub>) comprises a complex series of steps including the activation of the long-chain fatty acids to their corresponding co-enzyme A esters, transport across the mitochondrial membranes and entry into the intra-mitochondrial  $\beta$ -oxidation spiral. Transport across the inner mitochondrial membrane is facilitated by L-carnitine and the action of carnitine palmitoyl transferases (CPT I and II) and a carnitine-acylcarnitine translocase. The  $\beta$ -oxidation process includes sequential FAD-dependent acyl CoA dehydrogenase, enoyl CoA hydratase, NAD<sup>+</sup>-dependent 3-hydroxyacyl CoA dehydrogenase and ketothiolase steps. Each step chain shortens the fatty acyl moieties by two carbons (release of acetyl CoA) with return of the residual molecule into the  $\beta$ -oxidation spiral. Specific enzymes exist for very-long-chain, (long-chain), medium-chain and short-chain fatty acyl moieties, at least for the acyl CoA dehydrogenases, and the pathway is further complicated by the occurrence of trifunctional protein combining the functions of some of these enzyme systems.

During the past 15 years inherited metabolic disorders caused by deficient activity of these enzyme systems have been identified at almost every stage of the  $\beta$ -oxidation process.<sup>59,66</sup> These disorders lead to accumulation of acyl CoA esters within the mitochondrion and of their corresponding L-carnitine esters inside and outside the mitochondrion. Precursor fatty acids also accumulate outside the mitochondrion and are oxidised alternatively on the microsomes to dicarboxylic and mono-hydroxy-monocarboxylic acids. Because of these accumulating species, disorders of fatty acid metabolism are frequently characterised by an abnormal dicarboxylic aciduria, sometimes associated with glycine conjugates, although during periods of remission in some disorders levels of these metabolites may be minimal or absent. Because of this, and because of the methods required for their analysis, the dicarboxylic acid and glycine-conjugated metabolites are unsuitable for neonatal screening.

However, in disorders of fatty acid metabolism, characteristic acylcarnitines accumulate in the blood with medium-chain and short-chain species being excreted into the urine (see *Table 8*). These compounds assume considerable diagnostic importance, particularly for disorders of long-chain fatty acid metabolism, when accumulation of long-chain

TABLE 8 Acylcarnitines identified in disorders of fatty acyl CoA metabolism

Disorder/defect	Acyl CoA	Acylcarnitine
MCAD deficiency	Octanoyl, hexanoyl	C <sub>6</sub> , C <sub>8:1</sub> , C <sub>8</sub> , C <sub>9</sub> , C <sub>10:1</sub>
LCAD, CPT II, [translocase*]	Palmitoyl, oleyl	C <sub>16:0</sub> , C <sub>18:1</sub>
SCAD deficiency	Butyryl, [hexanoyl*]	Butyryl
MACDD	Isovaleryl, isobutyryl 2-Methylbutyryl Glutaryl Fatty acyl	Isovaleryl Isobutyryl Methylbutyryl
* Putative, but expected to occur.		

acylcarnitines in the blood may be the primary initial diagnostic feature. They are thus also of potential importance for neonatal screening (see page 16).

All the known disorders of mitochondrial fatty acid  $\beta$ -oxidation are of autosomal recessive inheritance. Most of these disorders have been identified relatively recently and are apparently very rare. Their precise biochemical basis and associated abnormal metabolic processes, natural history and prognosis are essentially unknown. Success in treatment of these conditions is variable, depending upon the disorder concerned and the severity of presentation, and many are associated with high morbidity and mortality. However, others, for example, MCAD deficiency, once diagnosed may be relatively easy to treat by simple dietary manipulation and with L-carnitine, and these disorders have much better prognoses. MCAD deficiency is the most common of these disorders with a high estimated incidence varying between 1:8000 and 1:15,000 live-born infants. This, and other more common disorders of fatty acid metabolism, are considered in the following sections.

### MCAD deficiency

#### Introduction

Patients with MCAD deficiency may present acutely with acidosis, hyperammonaemia, profound life-threatening hypoketotic hypoglycaemia, profound hypo-carnitinaemia, encephalopathy and hepatomegaly and pathologically with severe microvesicular fatty infiltration of the viscera.<sup>59</sup> The age of initial presentation varies widely, with some patients presenting acutely in early infancy with all the features described above, while others present in later childhood primarily with episodic hypoglycaemia, or as adults with muscle weakness and fatigue. The clinical history often reveals milder episodes preceding the more acute presentation<sup>59</sup>

and significant symptoms may be present in the neonatal period.<sup>67</sup> These early symptoms are frequently overlooked, and later presentation with more severe symptoms often seems to be dependent upon dietary, environmental and other unknown factors. The disorder has been identified as underlying some cases of Reye's syndrome and some patients with MCAD deficiency may die suddenly and unexpectedly. Many patients appear to remain undiagnosed and others may well live on into adult life without any overt symptoms. Thus there is a considerable spectrum of age of presentation, from the newborn through early infancy, childhood and, very rarely, into adult life. Likewise the severity of presentation is highly variable and milder presentations with mild episodic hypoglycaemia or simple early fatigue on prolonged exercise may well be overlooked.

### Diagnosis and genetic basis

Diagnosis is relatively straightforward during an acute presentation, with a marked abnormal dicarboxylic aciduria, suberylglycerinuria and hexanoylglycerinuria with excretion of ( $\omega$ -1)-hydroxymonocarboxylic acids. Dec-4-enoic acid accumulates in the blood and the disorder is also characterised by accumulation of octanoylcarnitine and hexanoylcarnitine in the blood and excretion into the urine. However, during treatment and periods of remission in the asymptomatic subject with MCAD deficiency, most of these abnormal biochemical markers are absent and diagnosis is much more difficult. A residual suberylglycerinuria with dec-4-enoic acid in blood plasma may remain but in all cases examined, octanoylcarnitine remains at abnormal levels in blood plasma. It is this elevation of blood octanoylcarnitine that may form the basis of newborn screening using blood spots.

Direct measurement of MCAD activity usually shows complete absence of enzyme activity. Much work has been done on the molecular basis of MCAD deficiency and it has been shown that more than 88% of MCAD-deficient patients have a common point mutation at position 985 relative to the start codon of the gene, in exon 11. This results in an A to G conversion ( $A \rightarrow G_{985}$ ) causing a lysine to glutamate conversion in the peptide sequence at position 304 in the mature monomeric sub-unit. Genotypically, more than 80% of patients are homozygous for this mutation, with most others being compound heterozygotes for this and other, more rare, mutations. The high frequency of this point mutation suggests a founder effect and MCAD deficiency has a high frequency in and is exclusive to Caucasian populations, generally of North European origin.<sup>59,66</sup>

### Incidence

Preliminary screening of dried blood spots from newborn infants, using polymerase chain reaction (PCR)-based molecular methods, has revealed carrier frequencies of around 1:40 to 1:100 in Caucasian populations, giving a putative frequency of the homozygous individuals with this mutation at around 1:6500 to 1:20,000. Prospective screening of 80,371 newborn infants from a multi-racial population in Pittsburgh, USA for MCAD deficiency using tandem MS has been reported.<sup>68</sup> Nine newborn infants with MCAD deficiency were identified (incidence 1:9000), confirming the high incidence of this disorder in the general population. Subsequent molecular analysis showed that an unexpectedly high proportion (56%) were compound heterozygotes for the  $A \rightarrow G_{985}$  mutation and other, more rare mutations (compared with only 20% in clinically-ascertained cases). This may suggest that some compound heterozygotes represent some of the asymptomatic individuals and some of the individuals who die early and undiagnosed.

### Outcome

The  $A \rightarrow G_{985}$  allele frequency is high in comparison with the number of clinically and biochemically identified cases in many countries and it is evident that many cases remain undiagnosed. This may be because some clinically-presenting cases are missed, and also because there are relatively high numbers of asymptomatic individuals, as has been shown by the identification of asymptomatic older siblings following diagnosis of a younger index case. A current survey of reported cases of MCAD deficiency in the UK has shown that, allowing for the incidence of the carriers and the putative incidence for homozygotes obtained from preliminary newborn screening, relatively few of the expected cases are presenting clinically or being diagnosed (RJ Pollitt: personal communication, 1997). MCAD deficiency does not contribute to the aetiology of sudden infant death syndrome<sup>59</sup> and even allowing for misdiagnosed or undiagnosed cases, it seems that many individuals with MCAD deficiency remain asymptomatic. Very few of the reported mutations affect the active site of the enzyme, but rather act on the folding and assembly of the active homotetramer. It has been suggested that under appropriate conditions, considerable enzyme activity may occur in various organs *in vivo* despite almost complete absence of enzyme activity in cultured fibroblasts *in vitro*.<sup>66</sup>

Neonatal screening in Pittsburgh allowed the pre-symptomatic identification of patients, early treatment with a low-fat, high-carbohydrate diet, and genetic counselling of the parents involved.

Despite the dietary treatment, two of the nine MCAD infants died suddenly and unexpectedly, which illustrates the extent of the mortality associated with this condition. Additional therapy with L-carnitine appears to avoid such early infant deaths (D Finegold and H Morten: personal communications, 1996) and MCAD deficiency appears to be a preventable disorder.

### Screening options

Limited newborn screening for MCAD deficiency has been carried out using molecular techniques, generally PCR-based,<sup>69</sup> but this has been done with the objective of ascertaining the putative frequency of the A→G<sub>985</sub> allele. Despite the very high frequency of the A→G<sub>985</sub> allele, molecular techniques are generally unsuitable for universal newborn screening for MCAD deficiency (as for other inborn errors of metabolism, see page 39), because of the large number of other mutations that occur. Molecular assays for a large number of different mutations have not yet been developed<sup>66</sup> and molecular-based screening will not be sufficiently comprehensive.

Reports and suggestions have also been made for population screening for MCAD deficiency based upon detection of diagnostic organic acid and acylglycine conjugates in blood spots,<sup>70,71</sup> but these methods, which are based on gas chromatography-mass spectrometry, are cumbersome and quite unsuitable for large-scale studies.

Determination of octanoylcarnitine in blood has been shown to be a reliable method for the diagnosis of MCAD deficiency irrespective of symptomatology,<sup>72,73</sup> and tandem MS offers a rapid, reliable and relatively simple method for this analysis.<sup>73</sup> The success of prospective screening for MCAD deficiency using tandem MS has demonstrated the utility of this new approach to newborn screening.

### Conclusions: neonatal screening for MCAD deficiency

MCAD deficiency shows a high frequency in the general population, especially in Caucasians of North European origin. The disorder is associated with increased mortality and morbidity and is relatively easily and cheaply treated with simple dietary manipulation and L-carnitine. Treatment in infancy and childhood appears to be necessary to avoid acute encephalopathic episodes and current evidence suggests that for affected individuals who reach adulthood MCAD is associated with little or no risk. The occurrence of some individuals with MCAD deficiency who may (always) remain

asymptomatic and the possibility of (even minor) clinically-unwarranted treatment of some individuals have to be an important considerations when deciding policy for the diagnostic effort and resources required for a screening programme for MCAD deficiency.<sup>66</sup> There is a lack of knowledge on which to base these considerations at present, and the necessary information may come only from universal neonatal screening programmes. However, although some individuals remain asymptomatic, many others, if untreated, die suddenly and unexpectedly in infancy and early childhood, and survivors of acute episodes are often severely mentally handicapped. This situation is not unlike that for PKU and hyperphenylalaninaemia, and prevention of severe handicap and death in the larger proportion (estimated at > 70%) of affected individuals provides considerable justification for newborn screening for MCAD deficiency. Neonatal screening for MCAD deficiency is only practicable with use of tandem MS.

MCAD deficiency satisfies most of the criteria for newborn screening (*Table 9*) but there is still incomplete evidence about its natural history and outcome. Consideration of the arguments for and against newborn screening for MCAD deficiency lead to the conclusion that the balance is fairly strongly weighted in favour of screening. Thus screening for MCAD deficiency should be seriously considered for inclusion in neonatal screening programmes. Such screening would be dependent upon the introduction of tandem MS technology and would require careful monitoring, with review after 10 years as outcome data are required for assessment of clinical effectiveness and cost-effectiveness.

TABLE 9 MCAD deficiency: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	±
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	✓
Period before onset during which intervention improves outcome	✓
Ethical, safe, simple and robust screening test	✓*
* By tandem MS only.	

## Disorders of mitochondrial long-chain fatty acid oxidation

### Introduction and outcome

This section is primarily concerned with disorders of long-chain acyl CoA  $\beta$ -oxidation. Most of these disorders have been identified relatively recently and at present there are limited data on the precise biochemical basis, natural history, and prognosis of the disorders and on effective treatments. However, morbidity and mortality are usually high. Most of these disorders are associated defects in the  $\beta$ -oxidation of long-chain acyl moieties and may present with hypoketotic hypoglycaemia, encephalopathy, macro- and microvesicular fatty infiltration of the tissues and early death. Older patients may have hypotonia with lethargy, skeletal muscle weakness and cardiomyopathy, and neurological damage. In other patients a milder course may be encountered and treatments include high-carbohydrate, low-fat diets with medium-chain triglyceride supplements. The more frequently encountered disorders include (very)-long-chain-acyl CoA dehydrogenase deficiency and long-chain 3-hydroxyacyl CoA dehydrogenase deficiency. Others include the CPT I and II deficiencies and carnitine–acylcarnitine translocase deficiency.

Diagnosis of these conditions may be difficult, since all are associated with various forms of dicarboxylic aciduria or 3-hydroxydicarboxylic aciduria, with accumulation of the corresponding monocarboxylic acids in blood. Acylglycines are not usually observed. All of the disorders except for CPT I deficiency are associated with accumulation of long-chain acyl CoA esters and thus also with accumulation of the corresponding acylcarnitine esters. The long-chain acylcarnitines in particular are not excreted into the urine but accumulate in the tissues and blood. Thus identification, if not absolute differentiation and diagnosis, may be achieved by analysis of body fluids, particularly blood, for acylcarnitines. The optimal methods for this analysis are fast atom bombardment (FAB) ionisation or similar techniques and tandem MS, by which not only the molecular species can be identified but absolute confirmation of them as acylcarnitines can be obtained. Quantification may be achieved by use of appropriate deuterated internal standards.

### Incidence

All of these disorders are inherited as autosomal recessive conditions and, individually, they appear to be rare. The true incidence is unknown.

### Screening options

The disorders of mitochondrial long-chain fatty acid oxidation may collectively be identified in dried ('Guthrie') blood spots by acylcarnitine analysis using tandem MS. At least some affected individuals would be identified by this methodology if it were applied to screening for other disorders on the basis of blood acylcarnitines. However this method is unlikely to identify all affected individuals because long-chain acylcarnitines may not accumulate in the early neonatal period in all of these conditions and low-level accumulation may be difficult to detect (EW Naylor: Experience with tandem mass spectrometry, presentation to International Society for Neonatal Screening, Boston, October, 1996). Additionally, interpretation of some of the profiles obtained may be difficult and a clear-cut diagnosis may not be possible. Thus any screening programme for disorders of long-chain fatty acid  $\beta$ -oxidation is unlikely to be comprehensive. In any such programme a proactive decision would need to be taken on whether or not to include long-chain acylcarnitines in the analysis and profiling because of possible difficulties in comprehensive detection and in interpretation of the results.

### Conclusions: disorders of long-chain fatty acid oxidation

On the basis of the current knowledge of the incidence, natural history, treatment and outcome (*Table 10*) of disorders of mitochondrial fatty acid  $\beta$ -oxidation caused by single enzyme deficiencies (primarily long-chain fatty acid oxidation disorders), specific directed newborn screening for disorders of long-chain fatty acid oxidation cannot be advocated.

**TABLE 10** Disorders of mitochondrial long-chain fatty acid oxidation: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	X
Known incidence in populations relevant to UK	X
Disorder associated with significant morbidity or mortality	±
Effective treatment available	±
Period before onset during which intervention improves outcome	X
Ethical, safe, simple and robust screening test	✓*

\* By tandem MS only.

## Multiple acyl CoA dehydrogenase deficiency

### Introduction and outcome

Multiple acyl CoA dehydrogenase deficiency (MACDD) affects all of the mitochondrial acyl CoA dehydrogenases including glutaryl CoA dehydrogenase (hence an earlier designation of the disorder as 'glutaric aciduria type 2'). In addition to the common dicarboxylic aciduria seen in MCAD deficiency (except possibly suberylglycerinuria), MACDD is associated with accumulation and excretion of glutaric acid, 2-hydroxyglutaric acid and various acylglycine species including isovalerylglycine, methylbutyrylglycine and isobutyrylglycine. Clinical presentations are highly variable and include acute neonatal presentation associated with severe hypoketotic hypoglycaemia, metabolic acidosis, hepatomegaly, hypotonia, hyperammonaemia, convulsions and neurological symptoms and early death. Other cases, also neonatal, are associated with dysmorphic features, severe fatty infiltration of the tissues and grossly enlarged polycystic kidneys, with damage evident *in utero*. Still other cases present later in infancy and childhood or even in adult life, although acute episodes may occur and cardiomyopathy also occurs. Treatment is universally unsuccessful in the neonatal presentations but milder cases may respond to moderate reduction of protein and fat intake, and increased carbohydrate intake, riboflavin and L-carnitine.<sup>59</sup>

### Incidence

The incidence of MACDD is unknown. It is believed to be not uncommon, although very rare in comparison with MCAD deficiency.

### Screening options

The disorder is associated with the accumulation of a wide variety of short-chain acylcarnitine species and could be detectable from analysis of dried blood spots for acylcarnitines using tandem MS.

### Conclusions: MACDD

In view of the wide range of presentations, the early neonatal presentation of the more severely affected cases, the evidence for damage *in utero* in some such cases, and the unknown incidence (probably rare), natural history and relatively poor outcome of treatment (*Table 11*), specific newborn screening for MACDD is not advocated. However, it is probable that affected individuals would be detected during newborn screening using tandem MS.

TABLE 11 MACDD: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	X
Known incidence in populations relevant to UK	X
Disorder associated with significant morbidity or mortality	±
Effective treatment available	±
Period before onset during which intervention improves outcome	X
Ethical, safe, simple and robust screening test	✓*
* By tandem MS only.	

## Congenital adrenal hyperplasia

### Introduction

CAH is the collective term used to describe a group of autosomal recessively inherited enzyme defects of adrenal corticosteroid biosynthesis, so called because on necropsy they are characterised by adrenocortical hyperplasia. The two end-products of adrenal corticosteroid biosynthesis, cortisol and aldosterone, have several enzymic steps in common. Cortisol is an integral component of the stress response and is also necessary for normal cellular and physiological homeostasis including blood glucose control (glucocorticoid activity). Aldosterone regulates electrolyte and water homeostasis through renal sodium and potassium excretion (mineralocorticoid activity). Defects in corticosteroid biosynthesis lead to deficiency of the end-product which produces a compensatory stimulation of ACTH (adrenocorticotrophin) production which adds further to metabolite accumulation proximal to the block. The sex steroids also share initial common precursors with the corticosteroids. With a defect in corticosteroid biosynthesis, depending on the position of the block, there can be either an absence or an overflow of accumulated precursors into the sex steroid pathway which in the adrenal primarily produces androgens. The presentation of the different corticosteroid disorders, therefore, is determined by the accumulating precursors and the reduced activity of the deficient end-products.

The commonest form of CAH ( $\geq 90\%$  of cases) is caused by deficiency of the enzyme 21-hydroxylase which affects both the cortisol and aldosterone synthetic pathways and leads to accumulation

of its substrate 17  $\alpha$ -hydroxyprogesterone (17OHP) with overflow of metabolites into adrenal androgens. 17OHP can reliably be measured in dried blood collected onto filter paper as part of the 'Guthrie test' procedure. 21-Hydroxylase deficiency is the only inherited disorder of corticosteroid metabolism for which well-baby neonatal screening has been proposed.

The symptoms of 21-hydroxylase deficiency are those of glucocorticoid deficiency (hypoglycaemia, hypotension, shock and collapse), mineralocorticoid deficiency (dehydration, hyponatraemia, hyperkalaemia and acidosis) and virilisation. Symptomatic glucocorticoid and mineralocorticoid deficiency (so-called salt loss) usually presents in the neonatal period, with a peak at 3 weeks after birth within the range 3 days to 8 weeks. A very few cases present as early as 3–5 days. It is as yet unclear why not all patients develop salt-wasting disease and present later (i.e. late-onset disease) with the virilising effects of excessive androgens. Virilisation in the female classically presents with ambiguous genitalia in the neonatal period, but in late-onset disease there is a spectrum of effects ranging from sexual precocity with or without clitoral hypertrophy at any time during childhood to hirsutism in adulthood (1% of unselected hirsute women have CAH<sup>74</sup>). In the male there may be only subtle signs of genital virilisation (for example, increased scrotal pigmentation and rugosity) but late-onset disease may present as sexual precocity and accelerated growth that usually results in ultimate short stature despite therapy.

Applying the criteria for successful neonatal screening to CAH to establish unequivocal benefit is difficult and complex for a number of reasons. Female infants with CAH have genital virilisation that is usually detectable clinically so that there is no 'asymptomatic' window in these cases. In male infants, the equivalent virilisation is rarely obvious, and presentation with salt-losing crisis overlaps with the period before and during which the results of neonatal screening become available. In the case of late-onset disease, which causes significant morbidity in the affected individual, there are questions of how to manage mild elevations of 17OHP that might be indicative of late-onset disease, and of whether late-onset disease can be detected reliably by elevated 17OHP in the neonatal period. Current data on the incidence and natural history of late-onset disease in CAH come from clinically-detected (and thus selected) cases, and only neonatal CAH screening programmes can provide unbiased epidemiological and outcome data on which to

base a valid analysis of the benefits of neonatal screening in these groups of patients. These difficulties aside, neonatal screening for CAH is routine in at least 16 of the 52 state programmes in the USA<sup>44</sup> and in New Zealand, Israel, and Japan, with regional programmes in Canada, Spain and Germany. There are evaluative programmes in France, Italy, and Switzerland<sup>75</sup> and Sweden has recently added CAH to its established national neonatal screening programme after 10 years of assessment.<sup>76</sup> Three programmes have been discontinued, one in Rome and one in Portugal, both for financial reasons, and one pilot programme in Scotland that did not receive funding for continuation.

We identified only four studies that provided data relevant to an assessment of neonatal screening for CAH in the UK. Three of the studies, by Murtaza and colleagues,<sup>77</sup> by Viridi and Green together with Rudd,<sup>78,79</sup> and by Donaldson and co-workers,<sup>80</sup> were purely retrospective case surveys. Donaldson and co-workers<sup>80</sup> were firmly of the belief that a prospective study was needed to evaluate neonatal screening for CAH, whereas Viridi and Green<sup>78</sup> thought such a study was unnecessary, and Murtaza and colleagues<sup>79</sup> drew equivocal conclusions from the results of their survey. The fourth study was a prospective pilot study of neonatal screening for CAH combined with a retrospective case survey which concluded strongly in support of screening for CAH.<sup>81</sup>

## Incidence

Pang and Clark<sup>75</sup> have reviewed worldwide experience of neonatal screening for CAH up until 1991, including data from 13 countries, 29 programmes and almost 6.5 million newborn infants screened. The incidence of CAH in the general population varied from 1:7500 (Brazil) to 1 in 21,000 (New Zealand). European countries showed a broadly similar incidence, at between 1:10,000 and 1:14,000 live births.

In the four retrospective UK case studies, the incidence of CAH is quoted at 1:12,000<sup>77</sup> on the basis of 26 identified cases in Wales over the 11 years from 1966 to 1977 (but with probable under-ascertainment of male cases), at 1:6200 over 1 year (four cases out of 24,750 births) in the West Midlands,<sup>78</sup> and at 1:21,000 on the basis of 15 diagnosed cases in Scotland<sup>81,82</sup> over the 10 years 1974–1984. In the prospective study in Scotland the incidence of CAH was 1:17,100 in 119,690 infants tested, compared with the retrospective incidence of 1:20,900 (95% confidence interval (CI), 1:12,700 to 1:32,600).<sup>75,81,82</sup>

## Outcome

The retrospective analysis of 115 clinically diagnosed cases in the Birmingham (UK) area (1958–1985)<sup>78,79</sup> reported a male to female ratio approaching unity between 1970 and 1981, implying virtually complete clinical ascertainment. However, 66% of the total number of cases presented after 12 days of age by which time a neonatal screening result could be available to expedite and improve patient management. The authors rejected neonatal screening for CAH on the basis of purported adequate clinical diagnosis without consideration of the benefits of earlier diagnosis such as prevention of adrenal crisis, facilitation of correct sex assignment in virilised females, and earlier treatment of virilisation in males. Murtaza and co-workers<sup>77</sup> reported a smaller number of cases (26) in Wales over a shorter period (1966–1977) which overlaps with the periods covered by the Birmingham study<sup>78,79</sup> and the (UK) South Western Region study (1968–1988) of Donaldson and colleagues.<sup>80</sup> Murtaza and co-workers reported a female predominance of clinically ascertained cases which they believed was due to male salt-losers dying before a diagnosis was made.<sup>77</sup> However, a neonatal screening programme was dismissed as not cost-effective because “unlike cases of phenylketonuria... infants [with CAH] die and do not become long-term financial burdens to the community”. Such a conclusion clearly neglects the intrinsic value of human life which, in health economic terms, is now an essential component of decision making. Donaldson and colleagues,<sup>80</sup> who retrospectively reviewed 63 cases in the South Western Region, believed a prospective pilot CAH screening study should be carried out because they found delay in starting treatment, gender mis-assignment at birth (17% of girls), and a previously unreported finding of learning difficulties in cases of salt-wasting disease (30% of boys, 21% of girls). The sex ratio for cases was unity, as in the study by Viridi and Green,<sup>78</sup> implying near complete case ascertainment despite the different conclusions of these two studies.

The only prospective study in the UK was undertaken in Scotland.<sup>75,81,82</sup> Seven cases were identified (five boys, two girls), all with salt-loss. In four of the infants the CAH screening results were available before there was clinical suspicion of disease, and in two infants the early stages of adrenal (salt-losing) crisis were apparent on reaching hospital. The false-positive rate was 0.04% with no false-negatives as far as could be determined (119,690 babies screened).

Worldwide, neonatal screening has been shown capable of preventing salt-wasting adrenal crises in newborn infants and gender mis-assignment in virilised neonatal girls, and of detecting the more severe cases of late-onset disease.<sup>75</sup> This worldwide experience is agreement with the UK studies of Donaldson<sup>80</sup> and Wallace,<sup>81,82</sup> but not with that of Viridi and Green.<sup>79</sup>

## Screening options

Simple and robust assays based on 17OHP measurement are now commercially available with an achievable false-positive rate of less than 0.2% (mostly low birth weight and premature infants). False-negatives comprised 15 missed cases (seven with salt wasting, four of which were missed due to human error, and eight with mild late-onset disease) out of 6.29 million infants screened, compared with 409 cases of CAH detected.<sup>75</sup> The problem of false-positives with premature/low birth weight infants can successfully be overcome by using a different measurement range for these infants provided that birth weight/prematurity data are accurately entered on the blood-spot screening card. In cases of late-onset disease the 17OHP values overlap the normal range, but the ACTH stimulation test (60 minutes) appears successfully to identify patients with CAH.

Cost-effectiveness also crucially depends on the rapid and efficient return of results. Dhondt and colleagues<sup>20</sup> described changes in logistical and laboratory practices in France that made 96–99% of 17OHP results available by day 12 of life. These changes included sampling at day 3 rather than day 5 after birth. Since in the UK 75% of Guthrie cards are tested by day 10 (Department of Health statistic), with a regional median sampling time of between 6 and 11 days,<sup>7</sup> a universal acceptance of sampling at 6 days may well ensure a similar availability of results.

Wallace<sup>81,82</sup> was convinced of the cost-benefits of adding screening for CAH to the existing neonatal screening programme. However, further funding for the CAH screening programme in Scotland was not granted.

We identified in the literature two clinical reviews by UK endocrinologists on neonatal screening for CAH.<sup>83,84</sup> Hughes<sup>83</sup> recognised the morbidity and possible mortality associated with missed clinical diagnosis. Contrary to the view expressed by Hughes,<sup>83</sup> late-onset disease will be detected by neonatal screening,<sup>75</sup> except in mild cases. Whether patients with mild late-onset disease need or benefit from treatment is unclear.

Hughes<sup>83</sup> does not consider the benefits of earlier diagnosis but nevertheless recognises the need for pilot assessment programmes. Sardharwalla and Wraith<sup>85</sup> rejected neonatal screening stating that “the ‘non-salt-losing’ variety of CAH does not carry any long-term financial burden to the community” which is clearly not now true since somatrophin (biosynthetic growth hormone) and gonadotrophin analogues, which are both expensive, can be used to increase height. More recently, Kelnar<sup>84</sup> strongly argued for urgent prospective studies in the UK to assess the efficacy and cost-effectiveness of neonatal screening for CAH.

## Conclusions

CAH fulfils nearly all of the screening criteria used for critical appraisal (*Table 12*).

TABLE 12 CAH: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	✓
Period before onset during which intervention improves outcome	±
Ethical, safe, simple and robust screening test	✓

There is a growing body of world literature that indicates that neonatal screening for CAH can be effective in preventing acute adrenal crisis in a significant number of neonates, avoiding gender misassignment, and detecting severe cases of late-onset disease. The financial benefits of these improvements in care have not been calculated. CAH appears to be a spectrum of disease that extends from neonatal infant to adult. The cut-off values used in screening will determine the numbers of cases of less severe late-onset disease that are detected, but probably with a concomitantly increased false-positive rate. The ACTH stimulation test (two blood samples) appears to be an effective discriminant for borderline screening results and has been included in the financial costs of screening. The natural history of mild and therefore late-onset disease is still unclear. The false-positive rate for screening is acceptably low, and the cost implications are known.

Screening for CAH in other countries has been shown to be beneficial. Similar benefits should accrue from such a programme in the UK. As stated by Kelnar<sup>85</sup> the lack of interest in screening for CAH in the UK “may be due to an insufficient acknowledgement of the considerable potential morbidity associated with the condition and a concentration on (and satisfaction with) mortality statistics”. A national programme for neonatal screening for CAH would be justified on the evidence. If screening for CAH were to be introduced there would need to be structured, coordinated, on-going evaluation, and review after 5 years to ensure that the programme was cost-effective.

## Familial hypercholesterolaemia

### Introduction

Coronary artery disease is a major cause of morbidity and mortality, accounting for one-third of all deaths in middle life in Britain. In the USA it has been estimated that genetically determined lipid disorders account for 15% of all heart attacks and deaths from vascular disease in persons aged less than 60 years.<sup>86</sup> The best characterised of these disorders is familial hypercholesterolaemia (FH) which is an autosomal dominant condition with more marked expression in homozygotes than in heterozygotes. FH is due to a defect in the low-density lipoprotein (LDL) receptor which leads to the elevated levels of LDL cholesterol that characterise the condition. Five major mutations have been described.<sup>87</sup> In the more frequent heterozygous state the condition is associated with subcutaneous tendon lipid deposits, xanthomas, and the premature development of atherosclerosis, with myocardial infarction commonly occurring by the fourth or fifth decade of life. Homozygotes have similar symptoms much earlier, with death from arterial vascular disease usually before the age of 30 years. The presence of familial defective apolipoprotein B (apoB), which has recently been found, may complicate this picture.

It is now well accepted that the arterial vascular damage from hypercholesterolaemia (atherosclerosis) in FH starts in infancy and that treatment to decrease its rate of development should probably start in childhood.

### Incidence

Estimates of the incidence of the homozygous state in England and Wales give a frequency of 1:100,000.<sup>88</sup> The heterozygote incidence is therefore 1:500 (with a range of 1:200 to 1:1000),



which is higher than that of PKU (incidence approximately 1:12,000 live births) for which there is an established neonatal screening programme.

### Outcome

FH has been estimated to cause 5% of cases of premature coronary heart disease.<sup>89</sup> Approximately 50% of men with FH will develop coronary artery disease by the age of 50 years, and 50% of women by the age of 60 years. Mabuchi and colleagues,<sup>90</sup> in a study of 161 FH patients, concluded that coronary artery disease could not be detected angiographically until young adult life, indicating the uncertainty that surrounds the optimal timing of any treatment. Ohta and colleagues<sup>91</sup> showed that providing a diet free of saturated fat lowered LDL cholesterol by 15% in a group of 21-month-old infants and maintained this reduction over a period of 12 months. Vartiainen and co-workers<sup>92</sup> reported a slightly greater reduction in cholesterol by dietary intervention for persons aged 8–18 years which could be maintained for 3 months. In a group of children and adolescents with FH it was demonstrated that significant additional reductions in LDL cholesterol could be achieved when cholesterol binding resins, and the drugs niacin and statin, were added to dietary therapy.<sup>93</sup>

A double-blind crossover randomised controlled study in adult men<sup>94</sup> has shown that treatment with the cholesterol-lowering drug pravastatin decreased mortality from cardiovascular and cerebrovascular disease or stroke in asymptomatic individuals with hypercholesterolaemia irrespective of aetiology. This is in agreement with the results of previous trials<sup>94,95</sup> and the recent trial on lipid lowering after interventional surgery.<sup>96</sup>

In homozygous FH, ileal bypass, regular plasmapheresis, and liver transplantation have proven effectiveness in limiting the disease.

A recent joint publication in the UK,<sup>97</sup> and reports from the National Cholesterol Education Programme in the USA<sup>98</sup> and the WHO/UN Workshop on Childhood Prevention of Atherosclerosis,<sup>99</sup> clearly indicate that there are now appropriate treatments for dyslipidaemia in childhood.

### Screening options

Total or LDL cholesterol or apoB have all been proposed as specific screening tests for identifying hypercholesterolaemia in the neonatal period.

Asami and colleagues<sup>100</sup> used an auto analyser to measure total cholesterol from dried blood spots. Alternatively apoB, apolipoprotein A1 (apoA1) or the apoB:apoA1 ratio have been measured using a variety of methodological techniques including double rocket immunoelectrophoresis, nephelometry, radioimmunoassay (RIA), radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA). None of these techniques is easily applicable for large-scale screening. The storage time before analysis affected the apoB assay measured by RID and there was a 5.7% variability arising from gender, gestational age and birth weight of the infant.<sup>101</sup> To overcome variations in haematocrit, apoB:apoA1 ratios were measured by RIA<sup>102</sup> and showed apoB to correlate positively with male sex, whereas apoA1 measurements were influenced by age, gender, and length of gestation. The apoB:apoA1 ratio was stable for only 15 days after the samples were taken due to time-dependent instability of apoB. Double rocket immunoelectrophoresis for measurement of this ratio required storage of samples at  $-20^{\circ}\text{C}$  and assay within 7 days for the most reliable results.<sup>103</sup> ELISA was more robust than RID with samples stable for up to 20 days at  $4^{\circ}\text{C}$  but again the sex of the infant affected the result.<sup>104</sup>

Measurement of cholesterol or apoB in the neonatal period is only poorly predictive of later values. In a longitudinal study there was clear evidence of 'reverse' tracking (i.e. regression to the mean) of apoB:apoA1 ratios with high ratios in the neonatal period ( $n = 11$ ) subsequently identifying only two families with an FH profile.<sup>86</sup> This study concluded that the most propitious time to screen for FH was not in the neonatal period, but in the early years of schooling. This 'reverse' tracking where most, but not all, values measured in the neonatal period return to normal when rechecked 12 months later has been confirmed in other studies<sup>105</sup> and is independent of assay technique.<sup>106–108</sup> None of the studies excluded the possibility of the syndrome described by Kwiterovitch<sup>109</sup> of neonatal hyperapoB-lipoproteinaemia in which total circulating cholesterol levels are normal and for which the mechanism is unknown.

Barker<sup>110</sup> observed that poor foetal (low birth weight) and infant growth is followed by a high adult risk of ischaemic heart disease, and Wong and colleagues<sup>111</sup> have suggested that the age of weaning and method of infant feeding may also influence serum LDL concentrations.

DNA and mutation analysis for FH can be carried out<sup>112</sup> but these molecular techniques have not been fully addressed for neonatal screening (see chapter 4, page 39).

## Conclusions

Of the methods available there is no clearly preferred technique for measurement of apoB, apoA1, or apoB:apoA1 ratio. Tracking of values in infancy creates false-positives, and these may be further compounded by the syndrome in which neonates have hyperapoB-lipoproteinaemia and normal total cholesterol, with apoB values returning to normal by 1 year of age in 60% of cases. Treatment for FH is increasingly available and the epidemiological evidence is strong that early prevention should improve prognosis, but there is no evidence indicating an improved prognosis when therapy is started in childhood. The influence of intra-uterine events, postnatal factors and other risk factors such as hypertension, smoking and diet on the development of atherosclerosis suggest that neonatal screening for FH is unlikely to be the most effective way to reduce overall morbidity and mortality from arterial vascular disease.

The evidence shows that FH does not currently fulfil the criteria for a neonatal screening programme (*Table 13*).

TABLE 13 FH: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	±
Period before onset during which intervention improves outcome	±
Ethical, safe, simple and robust screening test	✗

## Peroxisomal disorders

### Introduction

Peroxisomes are subcellular organelles which occur in all nucleated human cells and are particularly abundant in cells that are active in lipid metabolism. They contain enzymes and pathways that are involved in a wide variety of essential catabolic and anabolic functions.

Catabolic functions include a hydrogen peroxide-based respiratory system, an oxidase-based  $\beta$ -oxidation system that is involved in the oxidation of very-long-chain fatty acids ( $> C_{24}$ ), branched-chain fatty acids, prostaglandins and xenobiotics, pathways of purine and polyamine catabolism, and ethanol and L-pipecolate oxidation. Anabolic functions include biosynthesis of cholesterol, plasmalogens, bile acids and dolichol, and glyoxylate transamination. Clinically severe metabolic disorders have been identified that result from the impairment of one or more peroxisomal functions and these are collectively called 'the peroxisomal disorders'.

Peroxisomal disorders exhibit considerable clinical heterogeneity and the nature of the diseases, the underlying primary defects, and the detail of peroxisomal function are still being elucidated. The range of peroxisomal disorders includes:

- disorders of biogenesis, for example, Zellweger syndrome, neonatal adrenoleukodystrophy
- disorders with loss of multiple peroxisomal functions, for example, rhizomelic chondrodysplasia punctata
- disorders with loss of only a single peroxisomal function and normal peroxisomal structure, for example, disorders of peroxisomal  $\beta$ -oxidation, X-linked adrenoleukodystrophy, acatalasaemia, primary hyperoxaluria type 1 (alanine-glyoxylate aminotransferase deficiency).

### Incidence

Peroxisomal disorders are perceived to be rare, but their incidence is generally unknown. However, as diagnostic methods and clinical awareness improve, the number of patients identified as having a peroxisomal disorder is increasing.

### Outcome

Most peroxisomal disorders present with multi-system involvement including severe neurological dysfunction, cranial dysmorphism, hearing loss, visual problems and gross psychomotor retardation. However, some disorders, for example, X-linked adrenoleukodystrophy, may show a wide spectrum of clinical presentations even among siblings, ranging from childhood cerebral dysfunction to (adult) adrenomyeloneuropathy or Addison's disease, while some affected individuals remain asymptomatic.

Most peroxisomal disorders are very difficult to treat, and clinical outcome is generally very poor, especially for disorders of biogenesis which are

clinically manifested at birth probably following damage *in utero*. Disorders associated with progressive neurological dysfunction also show universally poor response to treatment, with the early cerebral presentations showing poorest outcome. Other peroxisomal disorders, for example, primary hyperoxaluria type 1 and acatalasaemia, are more amenable to treatment and the long-term outcome and survival are good.

### Screening options

Initial diagnosis of peroxisomal disorders generally depends upon measurement of very-long-chain fatty acids in blood plasma. Differential biochemical diagnosis includes the measurement of plasma very-long-chain fatty acids, phytanic, pristanic and pipelicolic acids, plasmalogens and bile acids. These analyses involve extraction, derivative preparation and gas chromatography with mass spectrometry and usually isotope dilution analysis.<sup>113</sup> Such methods, applied to dried ('Guthrie') blood spots, are unsuitable for neonatal screening although they may have application for limited epidemiological studies.<sup>114,115</sup>

Underivatised bile acids can be measured in blood plasma using negative ion tandem MS<sup>116</sup> and this approach is currently being developed for screening blood spots from newborn infants (see chapter 4, page 43), although with the aim of detecting biliary atresia rather than peroxisomal disorders.

### Conclusions

In general, the criteria for neonatal screening are not fulfilled for peroxisomal disorders (*Table 14*): the incidence of most disorders is unknown, effective treatment is unavailable for virtually all peroxisomal disorders, and the natural history of the disorders is still being elucidated. In addition,

**TABLE 14** *Peroxisomal disorders: summary of conclusions*

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	X
Known incidence in populations relevant to UK	X
Disorder associated with significant morbidity or mortality	X
Effective treatment available	±
Period before onset during which intervention improves outcome	±
Ethical, safe, simple and robust screening test	X

except for tandem MS which can be applied as an adjunct to newborn screening for other disorders, most diagnostic methodologies would not be applicable or cost-effective.<sup>115</sup>

On the basis of the evidence available, there is no justification to consider neonatal screening for peroxisomal disorders at the present time.

## Disorders of the urea cycle

### Introduction

The urea cycle is the self-regenerating pathway or cycle by which all waste nitrogen is converted into urea for renal excretion. It is partly cytoplasmic and partly intra-mitochondrial and involves five distinct and separate enzymic steps which convert ammonia to urea. Two transport steps that join the cycle across the mitochondrial membrane are also recognised.

There are five urea cycle disorders, each representing a defect in one of the enzymes in the urea cycle:

- carbamoyl phosphate synthase deficiency
- ornithine carbamoyltransferase (transcarbamylase) deficiency
- argininosuccinate synthase deficiency (citrullinaemia)
- argininosuccinate lyase deficiency (argininosuccinic aciduria)
- arginase deficiency (argininaemia).

All of these disorders have signs and symptoms in common because deficiency at any step in the cycle results in the accumulation of ammonia. On the other hand, the accumulation of arginine occurs only in cases of arginine deficiency, and not in disorders of the other four steps. Arginine accumulation appears to lead to a specific neurotoxicity as evidenced by progressive spastic diplegia and psychomotor retardation. A defect in one of the two transporter steps for ornithine (hyperammonaemia, hyperornithinaemia with homocitrullinuria) presents with intermittent hyperammonaemia in infancy and early childhood, but later developmental delay and neurological signs occur that are similar to those of arginase deficiency which involves the immediately preceding enzymic step.

The urea cycle makes sequential use of two-, three-, and four-nitrogen-containing amino acid intermediates to build the urea structure. Direct diagnosis of defects in the cycle can be made by

identifying accumulation of the immediate precursor substrate, except in the case of argininosuccinate lyase when there is little accumulation of argininosuccinate in the blood because of its low renal threshold. Similarly, ornithine carbamoyltransferase deficiency is not associated with the accumulation of ornithine, which is rapidly metabolised by ornithine-D-aminotransferase. There is also no immediate amino acid substrate for carbamoylphosphate synthetase. Although hyperammonaemia is the cardinal feature of urea cycle defects, unfortunately it cannot be measured in a dried blood spot. However, indirect evidence of urea cycle deficiency can be provided by elevations of glutamine and alanine which are major sources of nitrogen for the urea cycle. However, glutamine is very difficult to measure and levels in hyperammonaemic and normal individuals overlap considerably, and there may be other causes of alanine elevation. Urinary levels of orotic acid and orotidine are very useful for diagnosing defects of the urea cycle, but these substances cannot at present be measured in a blood spot.

### Incidence

Msall and colleagues,<sup>117</sup> quote an overall incidence of 1:30,000 live births for urea cycle defects. In the Austrian Screening Programme Widhalm and colleagues<sup>118</sup> found an incidence of 1:121,000 for argininosuccinate lyase deficiency based on a microbiological assay system for capillary blood spots, but these cases appeared to be only partially deficient with an excellent prognosis and no severe hyperammonaemic crises. Most laboratories have stopped using this microbiological test because of problems with the assay, and the incidence data shown in *Table 15* are largely based on screening of urine using chromatography. Naylor<sup>119</sup> and Lemieux and co-workers<sup>120</sup>

summarised the known screening programmes up until 1981.

The neonatal screening programmes using urine have collected samples after the second week of life. Infants dying with severe neonatal onset of disease will therefore be missed. No cases of ornithine carbamoyltransferase deficiency were detected (88,000 infants screened) when urinary orotic acid was assayed by a non-specific photometric technique. Concurrent amino acid screening detected other cases of other urea cycle defects.<sup>120</sup> The true incidence of urea cycle defects, particularly ornithine carbamoyltransferase deficiency, is unknown. There is a marked geographical variation in incidence between populations of potentially similar ethnic origin (i.e. in Australia, the USA and Canada) which is currently unexplained but may be due to methodological problems. Also unexplained is the apparent predominance of partial defects of argininosuccinate lyase deficiency in Austria that was found by analysis of capillary blood spots, usually taken in the first week of life.<sup>118</sup>

### Outcome

The prognosis for urea cycle defects remains generally poor and uncertain even with the best available treatment. The best prospective results were reported by Maestri and colleagues<sup>121</sup> who used a protocol in infants at risk for urea cycle disorders which involved the provision of alternative pathways of nitrogen disposal, protein restriction, and haemodialysis. Even with this protocol, three out of eight neonatal infants with ornithine carbamoyltransferase deficiency died, and there were two further deaths in the subsequent 4 years of life. Each of the ornithine carbamoyltransferase-deficient

**TABLE 15** Incidence of urea cycle defects: data from newborn screening programmes (urine)

Deficiency	Country	Incidence
(1) Argininosuccinate lyase	New South Wales, Australia <sup>119</sup>	1:500,000
	Massachusetts, USA <sup>119</sup>	1:80,000
	British Columbia, Canada <sup>119</sup>	1:156,000
	Quebec, Canada <sup>120</sup>	1:90,000
(2) Arginase	Quebec, Canada <sup>120</sup>	1:990,000
(3) Argininosuccinate synthetase	Quebec, Canada <sup>120</sup>	1:500,000
Deficiencies (1), (2) and (3)	Quebec, Canada <sup>120</sup>	1:53,000
Deficiencies (1), (2) and (3)	Wales, UK <sup>119</sup> (135,295 screened)	No cases detected

survivors subsequently received a liver transplant. The treatment regimen was described as burdensome, and it did not prevent recurrent episodes of hyperammonaemia and neurodevelopmental delay. Unacceptable toxicity has arisen from one of the drugs used to enhance nitrogen excretion.

However, late-onset or partial argininosuccinate lyase deficiency appears to have an excellent prognosis. Widhalm and colleagues<sup>118</sup> described how early dietary treatment of infants with argininosuccinate lyase deficiency detected by neonatal blood-spot screening can result in normal development.

### Screening options

Neonatal screening is unlikely to be of benefit to infants with neonatal onset of urea cycle defects who will sustain hyperammonaemic brain damage before screening is performed at 6–11 days of age (regional range for medians for timing of neonatal screening in the UK). The relative proportion of neonatal onset to late-onset disease is not currently known for any of the urea cycle defects.

### Conclusions

Neonatal screening for urea cycle defects is not justified on the current evidence (*Table 16*).

**TABLE 16** Disorders of the urea cycle: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	±
Known incidence in populations relevant to UK	±
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	±
Period before onset during which intervention improves outcome	±
Ethical, safe, simple and robust screening test	✗

## Biotinidase deficiency

### Introduction

Biotinidase is an essential enzyme for the cleavage of biocytin (biotinyl-L-lysine) into free biotin and lysine. Biotin is a water-soluble B-complex vitamin that is the essential co-factor for three important mitochondrial carboxylase enzymes that are involved in amino acid degradation (3-methylcrotonyl CoA carboxylase), amino

acid and odd-chain fatty acid metabolism (propionyl CoA carboxylase), and pyruvate metabolism (pyruvate carboxylase) at the first step in gluconeogenesis. It is also the essential co-factor for one cytosolic carboxylase, acetyl CoA carboxylase which provides the first key step in fatty-acid biosynthesis. Biotin occurs in the free form in the diet but is primarily absorbed in the form of biotinylpeptides, for example, biocytin. Biocytin is also produced from endogenous carboxylase turnover.

Biotinidase deficiency, which is of autosomal recessive inheritance, presents in infancy and early childhood with a variety of symptoms including alopecia, skin rashes and a progressive neurological illness, with ataxia, seizures, neurosensory hearing loss and deafness, and developmental regression. Without treatment, death ensues in many cases. More than 80% of patients show moderate organic aciduria, although this is frequently slight, and there may be some accumulation of abnormal acylcarnitines in blood and urine. Absolute diagnosis depends upon measurement of biotinidase activity in blood plasma, serum or other tissues.

### Incidence

Estimations of the incidence of profound biotinidase deficiency vary depending upon the population concerned and have generally been derived from pilot and on-going neonatal screening programmes. Incidence data may be compromised by inclusion of subjects with partial deficiencies who do not require treatment and hence also would not normally require identification in a screening programme. Incidences of profound biotinidase deficiency between 1:109,000 and 1:211,000 live births have been reported, while rather higher incidences of the partial deficiency have been recorded (1:86,000 to 1:160,000).<sup>122</sup> The large majority of cases of biotinidase deficiency occur in Caucasian populations; very few black, and no Asian or Oriental, patients with the disorder have been reported.

The reported incidence of biotinidase deficiency from neonatal screening programmes from a number of countries is summarised in *Table 17*. The data show that there is an apparently high incidence in some populations, whereas in other areas, such as Scotland, there are apparently very low incidences. The quoted incidence for a particular country or region may also vary quite widely according to the source, as is the case for Austria. Furthermore, findings may be determined in part by chance and timing: for example, in the Madrid area of Spain 176,000 newborn infants

TABLE 17 Worldwide incidence of biotinidase deficiency: data from newborn screening programmes

Country or region	Profound deficiency	Partial deficiency
Worldwide (8,532,617 newborn infants screened in 14 countries)	1:112,300 95% CI: 1:85,000 to 1:145,000 <sup>124</sup>	1:129,300 95% CI: 1:112,700 to 1:177,000 <sup>124</sup>
Austria	1:83,000 <sup>124</sup> 1:107,500 <sup>122</sup> 1:88,600 <sup>125</sup>	–
Lower Saxony	1:73,000 <sup>124</sup>	–
Quebec	1:48,000 <sup>124</sup>	–
Australia	1:259,000 <sup>124</sup>	–
Scotland	0:160,000 <sup>124</sup>	–
Sweden	1:170,000 <sup>124</sup> 0:215,000 <sup>126</sup>	–
Madrid, Spain	0:176,000 (1988–96) <sup>123</sup> 1:88,000 (1996) <sup>127</sup>	–

were screened over 8 years without a single case being identified and then two cases appeared within 9 days, making the apparent ‘incidence’ 1:88,000 live births.<sup>123</sup>

There is essentially no reliable data on the incidence of profound biotinidase deficiency in England and Wales. Data for ethnically-similar populations from the most recent report of worldwide experience of screening and incidence<sup>124</sup> (i.e. for Australia, Canada excluding Quebec, New Zealand and all states of the USA), show an incidence of profound biotinidase deficiency of the order of 1:108,000 (52 cases of profound deficiency in 5,607,470 newborn infants screened) and an incidence of partial deficiency of 1:151,600. It would be reasonable to assume that the incidences in England and Wales are similar.

### Outcome

Treatment is relatively simple and cheap: pharmacological oral doses of biotin (5–20 mg/day) completely abolish the symptoms and permit normal development. Patients with partial biotinidase deficiency have also been identified but normally do not require treatment except rarely in cases of severe infections or gastroenteritis.<sup>128</sup> There is no information on the long-term outcome for patients treated early or on the possible toxicity of biocytin, which continues to accumulate.

### Screening options

The biochemical assay for biotinidase is relatively simple, and is based upon measurement of enzyme activity through the release of 4-aminobenzoate

(PABA) from biotinyl-4-aminobenzoate and the colorimetric determination of the diazo derivative of PABA. The assay, which is unaffected by the biotin status of the patient, has been adapted for the semi-quantitative analysis of blood spots<sup>129,130</sup> and applied to neonatal screening in a number of countries. The simplicity of the screening methodology, and the benefits of prevention of severe neurological disease in patients with profound biotinidase deficiency that would be achieved by an effective nationwide screening programme, may outweigh the relatively low incidence of the disorder when considering screening in UK and similar populations. However, screening has been discontinued in parts of Australia because of the low incidence of the disorder<sup>131</sup> and even in regions where the incidence is relatively high, some screening programmes have been discontinued on financial grounds, for example, in Quebec. In Austria, the value of screening from clinical outcome was said to be unclear<sup>125</sup> and although the disease is seen as meeting several of the criteria for whole-population neonatal screening, on incidence alone screening may not be justified. This must, however, be offset against the permanent developmental and neurological damage that may be associated with late diagnosis and treatment, in comparison with the normal development of patients who are detected through newborn screening and treated early.<sup>132,133</sup>

### Conclusions

The incidence of the disorder, as extrapolated to England and Wales from appropriate worldwide data together with the outcome of the pilot study

in Scotland, may be considered to be too low to justify whole population neonatal screening for biotinidase deficiency in the UK.

This must be balanced against the improved outcome associated with early treatment, and the prevention of neurological damage. In an annual neonatal population of about 550,000 in England and Wales, five cases of profound biotinidase deficiency might be expected. Early diagnosis of these cases and prevention of life-long neurological handicap by simple and cheap treatment might be considered cost-beneficial.

Thus careful evaluation of the cost-effectiveness of neonatal screening for profound biotinidase deficiency is required. It is recommended that this is conducted on a nationwide basis in order to obtain an adequate number of cases within a reasonable time period.

A nationwide newborn screening programme for profound biotinidase deficiency would meet the accepted criteria (*Table 18*). If screening is introduced it should be accompanied by effective CBA carried out from initiation of the programme, and reassessment should be carried out after 5 years to determine the validity of continuing the programme.

**TABLE 18** Biotinidase deficiency: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	✓
Period before onset during which intervention improves outcome	✓
Ethical, safe, simple and robust screening test	✓

## Trace metal disorders

A number of trace metals perform essential metabolic functions in a wide range of different types of reaction. However, relatively few specific inherited disorders have been described and these concern only two elements, copper and molybdenum.

### Disorders of copper metabolism

There are three genetic disorders of copper metabolism: Wilson disease, Menkes disease

and the related occipital horn syndrome. Wilson disease and Menkes disease are caused by defects in closely related proteins involved in copper transport that belong to the large family of ATP-dependent P-type cation transporters. The Wilson gene is expressed in the liver and the Menkes gene in the gut. Several mutations and deletions have been reported for both genes.

Menkes disease is rare (1:250,000 live births in Europe<sup>134</sup>) and untreatable. Neither Menkes disease nor occipital horn syndrome is considered to be a candidate for neonatal screening. Wilson disease is considered in more detail below.

## Wilson disease

### Introduction

Patients with Wilson disease rarely present before the age of 6 years and the majority of cases present in late childhood, adolescence and young adulthood.<sup>135,136</sup> Very rarely, cases have been reported in older persons (up to the age of 60 years). Children and younger patients normally present with symptoms of liver failure which can be of rapid onset and fatal. In later years a neuropsychiatric picture predominates. However, the primary presentation can be with haematological, endocrine or renal symptoms. Patients may die of fulminant liver failure or suffer a chronic course of neurological deterioration and liver disease.

The initial diagnosis of Wilson disease depends on the measurement of serum copper and caeruloplasmin concentration. Caeruloplasmin is the copper-transporting protein of plasma and is normally measured by an immunological method. Caeruloplasmin is an acute phase protein and the serum concentration is increased in infections. The serum concentration is also age related, being low in neonates and reaching adult values by 1 year. In the majority (95%) of patients with Wilson disease, serum caeruloplasmin concentration is low.

### Incidence

The incidence of this autosomal recessive disease is in the order of 1:50,000 to 1:100,000 live births. Two epidemiological studies give figures of 1:60,000 in the Irish Republic<sup>137</sup> and 3:100,000 in Sardinia<sup>138</sup> although there are wide CIs because of the small populations in these studies. Similar figures have been published for Japan.<sup>139</sup>

### Outcome

Treatment of both pre-symptomatic and established Wilson disease is with copper-chelating drugs to remove the excess copper from the body. Penicillamine is used most frequently,

but other chelating agents such as trientine and tetrathiomolybdate may be used where penicillamine proves to be toxic. Patients who present with fulminant liver failure are candidates for liver transplantation.

Recently, zinc acetate has been added to the treatment regimen with the objective of preventing copper absorption through the gut. This was first used as an adjunct to chelation but might be effective as a primary treatment. Brewer and colleagues<sup>140</sup> have reported on a series of 13 pre-symptomatic patients with Wilson disease who were identified in family studies and were treated for a number of years with zinc alone. None of the patients developed symptoms. However, no other studies have repeated this effect. Further long-term evaluation suggests that pre-symptomatic treatment for Wilson disease may be possible.

There is no information on treating patients from the neonatal period and the question of the timing of the introduction of pre-symptomatic treatment remains to be answered. The benefits of preventing the symptoms of the disease by drug therapy are considerable in view of the numbers of deaths, liver transplantations and serious neuropsychiatric disorders associated with symptomatic Wilson disease.

### Screening options

Screening for Wilson disease using an enzymatic measurement of caeruloplasmin was proposed in 1977.<sup>141</sup> More recently, preliminary data have been reported on an ELISA method which may be more practicable.<sup>142</sup> A clear difference in blood caeruloplasmin concentrations was demonstrated between normal neonatal infants and older patients with the disorder. However, the concentration of caeruloplasmin in neonatal infants with Wilson disease is not known and more information is required.

### Conclusions

Wilson disease is a serious, treatable medical condition with an incidence about three times less than that of PKU. A potential screening test has been suggested and there is the possibility of pre-symptomatic treatment with zinc which will prevent progression to the clinical disease.

However, although Wilson disease fulfils most of the criteria for neonatal screening (*Table 19*), a number of questions still need to be answered and screening for this disease is not advocated at present.

**TABLE 19** Wilson disease: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✓
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	✓
Period before onset during which intervention improves outcome	✓
Ethical, safe, simple and robust screening test	±

## Molybdenum co-factor deficiency

### Introduction

The role of molybdenum in metabolism has been reviewed by Sardesai.<sup>143</sup> *In vivo*, molybdenum exists as part of a complex – molybdenum co-factor – which is required for the three enzymes sulphite oxidase, xanthine oxidase and aldehyde oxidase. Deficiency of all three enzymes is a result of molybdenum co-factor deficiency.

### Incidence

Molybdenum co-factor deficiency is an extremely rare disorder: only about 50 cases have been reported worldwide, although it is believed that many cases could have been missed because of early deaths.

### Outcome

Patients present soon after birth with severe and intractable seizures. There are a number of pathognomonic changes in the brain, including neuronal loss, demyelination and gliosis. With this severe brain damage it is not surprising that all treatments tried so far have proved ineffective although recently it has been suggested that sulphur-containing amino acids may produce some amelioration of symptoms.<sup>144</sup> Patients usually die within a few months.

### Screening options

All three molybdenum-requiring enzymes are deficient and this is reflected in the biochemical findings of increased urinary sulphite, thiosulphate, S-sulphocysteine, xanthine and hypoxanthine and low plasma urate. Some of these biochemical findings offer potential screening markers for this condition but are as yet unevaluated.

### Conclusions

The extremely poor prognosis and lack of treatment make this disorder unsuitable for neonatal screening (*Table 20*).



**TABLE 20** Molybdenum co-factor deficiency: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	✗
Disorder associated with significant morbidity or mortality	✗
Effective treatment available	✗
Period before onset during which intervention improves outcome	✗
Ethical, safe, simple and robust screening test	✗

## Disorders of purine and pyrimidine metabolism

### Introduction

Purines and pyrimidines are not only the basic constituents of DNA and RNA which comprise the genetic material within each cell, but also act as fundamental components of many cellular high-energy molecules which are required to facilitate vital intracellular metabolic processes. There are now eight recognised inherited metabolic defects in the metabolic pathway of purines, and five in the pathway of pyrimidines.

Screening has been proposed for adenylosuccinase deficiency,<sup>139</sup> a defect in purine metabolism, primarily to provide epidemiological data. The disorder was first described in 1984 and by 1995 only 16 cases had been identified worldwide.

### Incidence

For most of these disorders there are no accurate incidence data. Hypoxanthine–guanine phosphoribosyltransferase deficiency (Lesch–Nyhan syndrome) has a quoted incidence of 1:380,000<sup>146</sup> and the other purine and pyrimidine disorders have a similar or lower incidence. The exceptions are hereditary xanthinuria which may well have a higher incidence (one case in 68,420 patients screened<sup>147</sup>), and adenylyate deaminase deficiency the incidence of which has been suggested to be as high as 1:100 to 1:200.<sup>148</sup>

### Outcome

The outcome varies depending on the deficiency:

- fatal in the neonatal period with immunodeficiency (adenosine deaminase deficiency)

- psychomotor retardation with either autistic features (adenylosuccinase deficiency) or self-mutilation (Lesch–Nyhan syndrome)
- renal calculus formation and renal failure at any age, 50% of cases being asymptomatic and 50% cases presenting before the age of 10 years (hereditary xanthinuria)
- presentation in adult life with muscle cramps/disease (adenylyate deaminase deficiency), although the contribution of the inherited enzyme deficiency to the pathology is variable and uncertain.

No effective treatment is yet available for most of the disorders, particularly adenylosuccinase deficiency or adenylyate deaminase deficiency. High fluid intake and reduced purine intake is advised for patients with hereditary xanthinuria.

### Screening options

No cases of adenylosuccinase deficiency have yet been discovered in the UK despite diagnostic screening (albeit on a small scale so far) of clinically suspected cases, namely those with psychomotor retardation. The screening test proposed<sup>145</sup> uses urine and has not yet been validated for use in the neonatal period.

Orotic acid which is elevated in certain disorders of pyrimidine metabolism as well as in disorders of the urea cycle has been proposed as a neonatal screening test. Examination of 88,000 urine samples revealed no positive cases and the false-positive rate was said to be unacceptable.<sup>120</sup> Column chromatography has been used to screen for purine and pyrimidine disorders, but when urine was used only 600 samples were examined. The methodology was labour-intensive, and expertise was required in visual interpretation of the chromatogram.<sup>149</sup>

### Conclusions

Neonatal screening is not justified for inherited disorders of purine and pyrimidine metabolism because of their unknown frequency, lack of effective therapy, and absence of an effective screening test (*Table 21*).

## Lysosomal disorders

### Introduction

Lysosomes are membrane-bound intracellular bodies that provide an acidic environment for enzymes that degrade materials no longer required by the cell. Inherited defects have been described for many of these enzymes, which are involved in the metabolism of glycogen (Pompe's disease), glycolipid (Tay–Sachs disease, Gaucher's disease,

TABLE 21 Disorders of purine and pyrimidine metabolism: summary of conclusions

Screening criteria	Criteria fulfilled	
	Purine	Pyrimidine
Clinically and biochemically well-defined disorder	±	±
Known incidence in populations relevant to UK	±	×
Disorder associated with significant morbidity or mortality	±	±
Effective treatment available	±	±
Period before onset during which intervention improves outcome	±	×
Ethical, safe, simple and robust screening test	×	×

etc.), glycosaminoglycans (mucopolysaccharidoses) and glycoprotein (aspartylglycosaminuria, mannosidosis, etc.). Screening to date has been confined to clinically suspected cases.<sup>150</sup>

### Incidence

Individually these defects are rare, although their incidence is relatively higher in distinct genetic communities, for example, Tay–Sachs and Gaucher’s disease in certain Jewish communities, and aspartylglycosaminuria in Finland.<sup>151</sup>

### Outcome

Most lysosomal disorders are untreatable. Gaucher’s disease type 1 is an exception.

### Screening options

Neonatal screening has been proposed for aspartylglycosaminuria in Finland<sup>151</sup> where umbilical cord serum is already used for neonatal screening. No work related to use of blood spots has been identified.

### Conclusions

Lysosomal disorders fulfil few of the criteria for neonatal screening (*Table 22*) and the evidence does not justify screening.

TABLE 22 Lysosomal disorders: summary of conclusions

Screening criteria	Criteria fulfilled
Clinically and biochemically well-defined disorder	✓
Known incidence in populations relevant to UK	±
Disorder associated with significant morbidity or mortality	✓
Effective treatment available	×
Period before onset during which intervention improves outcome	×
Ethical, safe, simple and robust screening test	×

## Chapter 4

# Review of new technologies for neonatal screening and screening automation

Several different techniques including bacteriological ('Guthrie') tests, immunoassay, chromatography and fluorometry are currently used in neonatal screening laboratories in the UK. A laboratory screening for more than one disorder will normally use more than one technique, and the methods used vary from being totally manual to partially automated. This chapter is concerned with new technologies for the automation of all or parts of the screening process and with completely new technologies that have application to newborn screening for inborn errors of metabolism. Review of current methodologies and of their relative efficacies has not been made although some comments on these have been included in the appropriate disease sections (chapter 3). Some of the current methodologies, for example, microbiological assays, are also not amenable to automation and might be expected to be phased out if automated approaches are introduced.

The sections below relate to review of automation of blood-spot card punching (which is applicable to any following methodology), to automation of chemical and immunoassay methods for neonatal screening, and to the only new and most promising technology, tandem MS.

### Automation of card punching

The first step in the laboratory testing process for neonatal screening is the punching of the appropriate number of blood spots from the screening card. Two manufacturers produce equipment that can take multiple blood spots from the card and dispense them to the correct analytical container (tubes or microtitre plates). These are very expensive, however, and UK laboratories normally either punch manually or use a semi-automated mechanical punch that cuts only one disc at a time. All of these methods allow visual inspection of the spots prior to punching and the rejection of unsuitable cards or spots.

Any fully automated punch would need to have two main attributes: the correct linking of each spot with the infant from whom it was taken and the

ability to assess spot quality. While the use of bar coding can resolve the first problem with relative ease, the latter is much more complicated because there are several reasons why a spot can be rejected, for example, it may be too small, or be composed of multiple small spots. The solution to the automation of the assessment of spot quality will either come from a rule-based computer program that can examine the image of the spot obtained using a video camera, or by training a neural network to recognise inadequate spots for rejection. The successful development of a fully automated puncher would have major implications for the automation of the remainder of the screening process but this ideal appears to be some way off at present.

### Automation of current screening methodologies

#### Introduction

The variety of measurement end-points in the different tests used in newborn screening makes the design of more automated, comprehensive and flexible systems difficult. In addition, any newly developed fully automated analyser must be able to interface directly with both laboratory computers and computers storing child-health records.

The variety of different analytes screened for and the different measurement end-points – fluorescence, luminescence and spectrophotometric – are a serious technical challenge to the development of a fully automated neonatal screening analyser. In addition to the different methodologies used, different procedures for elution of the blood from the punched out disc may be required for each analyte, and hence more than one disc may be needed for multiple analyses.

Two approaches are being tried to improve screening automation. The first is to attempt to standardise the measurement end-point and hence use a single detector. This means that an optimal method may not be possible for each analyte and a compromise may have to be reached. This is the

case with the fluorometric methods for the diagnosis of PKU which have a number of problems. An alternative approach is to develop a multi-functional optical detection system which is capable of being used as a luminometer, spectrophotometer or a fluorometer as required by the individual assays. In this case different assay techniques could be used on a single piece of equipment. However, this technique also requires some compromise in assay methodology such as in incubation times and temperatures.

### Equipment review

A number of modular systems are on the market which together with a suitable disc punch and assay kits can be easily adapted to screen for neonatal hypothyroidism, cystic fibrosis, CAH, galactosaemia and PKU. Additionally several automated immunoassay analysers designed for serum samples are marketed. At least three of these have been adapted in various screening laboratories for use with eluants from dried blood spots. However, these latter instruments lack versatility, particularly in the detector, and would not necessarily be adaptable for a large range of tests. The market for equipment for neonatal screening laboratories is small compared with that for pathology laboratories in general. It is not surprising therefore that few companies have chosen to specialise in this area and that the major effort is in the modification of existing equipment rather than in design from scratch.

Two major companies in the field internationally are Isolab (USA) and Wallac (Finland). Isolab market a modular system using a single fluorescent end-point for each assay and considerable manual input is required to transfer plates between the individual modules. Wallac have a similar modular system (DELFLIA®) but have in addition a more automated version, the AUTODELFIA®. Both Wallac systems use time-resolved fluorescence and are in general reliant on specific kits marketed by Wallac. Time-resolved fluorescence has the potential advantage that up to four tests could be carried out in a single tube using different fluorescent labels if a suitable fluorometer is used. DELFLIA® is a widely used technology in neonatal screening, especially in the UK and other parts of Europe. It is also used in many laboratories for antenatal screening for Down's syndrome and for a wide range of endocrinological tests. A visit to Wallac Oy in Turku, Finland was made as part of this Health Technology Assessment.

Wallac are currently devoting considerable effort to the development of a fully automated machine for

neonatal screening based on adapting the AUTODELFIA® concept. To this end they have proposed the international adoption of a standard neonatal screening card with agreed spot spacing to fit onto an automated punch with bar code reading. They are also developing such a punch which can be attached to the sampling side of the AUTODELFIA®. In order to offer to laboratories as wide a range of techniques as possible, Wallac have a new multi-purpose detector 'Victor™', which can measure in three modes, spectrophotometric, fluorometric and luminometric. Victor™ is already marketed as a stand-alone instrument.

Methods that are already suitable for use on the proposed analyser include those to detect thyroid stimulating hormone, immunoreactive trypsin (IRT), 17OHP, creatine kinase, and phenylalanine and galactose (both analysed by Shield Diagnostics Quantase™ methodology). Methods for other analyses are under development in collaboration with Shield Diagnostics (Scotland). With the current configuration of the system, there are limitations to the through-put because of the requirement to incubate the assays on the analyser itself. Calculations suggest that a single AUTODELFIA® could screen 112,500 infants for PKU and congenital hypothyroidism per year. This would mean that a large screening laboratory would require two analysers to cope with down time and maintenance. If cystic fibrosis and CAH were added to the screening programme, then the through-put in terms of numbers of patients would be reduced unless simultaneous multi-label assays were developed.

Of potential interest in the neonatal screening field is the fact that time-resolved fluorescence has been applied as a method for the detection of DNA probes. For example, it has been used successfully to look for the  $\Delta F508$  mutation in cystic fibrosis.

### Costs

The costs of a fully automated neonatal screening analyser are difficult to estimate at this stage because information is commercially sensitive. Based on the cost of a standard AUTODELFIA® the list price can be estimated as between £75,000 and £100,000. Each test would cost in the order of £0.35–0.50.

The modular Isolab system varies in price depending on the degree of sophistication required. The costs of a fully comprehensive system with punch, automated reagent dispensing, shaker, fluorometer and computer are approximately £70,000 and reagent costs are similar to those for the AUTODELFIA®. Through-put should

be higher with the Isolab instrument because incubation takes place 'off line', but this requires substantial manual intervention.

### Conclusions

If successfully developed, a fully automated immunoassay analyser would complement tandem MS, covering a different range of analytes, in particular larger polypeptide hormones and other substances which either cannot be measured by tandem MS or for which that technology is insufficiently sensitive. Such an analyser would then not need to perform assays for PKU. Alternatively, such an analyser could be used as a stand-alone analyser if for any reason it was decided not to screen for disorders other than PKU that could be detected by tandem MS. Of course, if that were the case, an assay for PKU would have to be included in the repertoire of the automated immunoassay analyser.

The major manual stage in neonatal screening is disc punching (see above) and almost all other stages are currently adequately performed by modular units. The actual benefits from a fully automated neonatal screening system remain to be demonstrated. These benefits will probably only be achieved if the range of tests is expanded to include those for disorders other than congenital hypothyroidism (and PKU) and this will in turn depend upon decisions about screening newborn infants for a wider range of disorders.

### Molecular (DNA) technologies

It might be expected that modern molecular techniques and in particular DNA analysis would be of great importance in the development of neonatal screening. As knowledge of the individual inborn errors of metabolism has developed over the past few years, a considerable body of information has been accumulated on the individual genetic defects or mutations that cause these diseases. Two basic patterns emerge. In the first, which includes MCAD deficiency in particular, there is a relatively frequent mutation which accounts for 80–90% of all disease alleles. Almost all of the remaining inborn errors of metabolism are represented by the second pattern, in which no individual mutation or deletion accounts for more than 10–15% of affected alleles. Even in the case of MCAD deficiency the total number of identified and potential alleles is large.

For screening using molecular techniques to be as efficient as phenotypic screening, methods will have to be developed that can screen for large

numbers of mutations simultaneously. Such technologies are in the early stages of development, and at the moment are being investigated for only a relatively small numbers of mutations. The cost of molecular screening technology is not known but it is likely to be very high, especially if it is applied to screening for a number of diseases. In addition, methods for the mass extraction of genetic material from dried blood spots would need to be developed.

In conclusion, the role of molecular techniques in neonatal screening is likely to be as a second-stage screening test. Alternatively, molecular technology could be used to confirm positive results and for phenotypic prognosis. At present there is no indication for newborn screening using molecular techniques.

### Tandem mass spectrometry

#### Introduction

Tandem mass spectrometry, often abbreviated to MS-MS (MS/MS) or MS<sup>2</sup> (or as in this report to tandem MS), can be considered as the most important of the emerging technologies for neonatal screening for inborn errors of metabolism. It has the potential for simultaneous and robust multi-disease screening using a single analytical technique and is complementary to immunoassay-based methods for detecting hypothyroidism, cystic fibrosis and CAH.

The pressure to develop and introduce such technology is particularly acute in countries such as the USA where the early discharge of newborn babies from hospital (often within 24 hours of delivery) and the lack of a structure or organisation for subsequent follow-up or sample collection require the acquisition of blood spots within 24–48 hours of birth. The methods that are currently used are not sensitive enough to detect PKU in blood samples obtained so soon after birth, and so there is an urgent need for more sensitive and discriminatory methods to fit in with the changes in neonatal management in such countries. These pressures do not apply to the UK, where the country-wide system of midwife-led follow-up and collection of blood samples from newborn infants at about 6–8 days of age, widely admired elsewhere, ensures that appropriate and suitable samples are available for screening.

However, the ability of tandem MS to simultaneously screen for many other inborn errors of metabolism of increasing importance, in addition

to PKU, makes consideration of this emerging technology most important in any assessment of the future development of screening strategies in the UK. The use of tandem MS may also enable the addition of new tests without incurring additional costs since the same instrumentation can be utilised, and efficiency will be increased if only one method is used to screen for multiple diseases.

As part of this systematic review of neonatal screening for inborn errors of metabolism, a detailed review of the current status and future of neonatal screening by tandem MS has been undertaken. Evidence was obtained from a literature review and visits to four laboratories that are currently using or developing the technology. The laboratories visited were the only ones worldwide that were known to be conducting or working actively towards neonatal screening for metabolic disorders using tandem MS, and were as follows.

1. The mass spectrometry facility of Duke University Mass Spectrometry Facility, Durham, North Carolina, USA, where the technique was initiated and where much of the methodological development, based upon FAB ionisation, has occurred.
2. Neogen Inc., Pittsburgh, Pennsylvania, USA where the technique has been extended to electrospray ionisation and where the only prospective screening applied to well-baby neonatal screening has been undertaken.
3. The Institute of Child Health of the University of London.
4. The Sir James Spence Institute of Child Health at the University of Newcastle upon Tyne.

The visits in the USA were conducted jointly with Dr RJ Pollitt, Director of the Neonatal Screening Laboratory at the Children's Hospital, Sheffield, who represented another group who were undertaking a Department of Health-commissioned review of aspects of neonatal screening.

Because tandem MS is a relatively new method, some detail has been given in Appendix 1 to provide the necessary technical background to this approach to neonatal screening. There is additionally a paucity of published literature in this field and the literature that is available comes primarily from the laboratories to which visits were made. Therefore the data from the literature review have been combined with the information obtained from the laboratory visits to provide a coherent overview of the current status of tandem MS for neonatal screening for metabolic disorders. In this section no opinion on the evidence to

justify screening for particular disorders has been included: that analysis has been confined to reviews of the individual diseases and this section is purely concerned with evaluation of the technology.

### **Basis of methodology**

The procedure used is similar in all of the laboratories developing or evaluating tandem MS for neonatal screening, although each laboratory has introduced variations to suit individual protocols. A punched sample from the dried ('Guthrie') blood-spot card is extracted with solvent containing appropriate standards labelled with stable isotopes. The extracted metabolites and standards are converted into butyl esters and these are identified and quantified using tandem MS either with FAB or electrospray ionisation. Full details are given in Appendix 1.

### **Duke University Mass Spectrometry Facility**

Dr DS Millington and Dr DH Chace and their colleagues have carried out most of the published developmental work on the analysis of blood spots for neonatal screening. Method development and evaluation are the primary interests of this laboratory and little or no actual prospective or routine neonatal screening were being undertaken at the time of the visit. All work is carried out using manual sample introduction and FAB ionisation using two instruments on a research basis. Facilities for electrospray ionisation were not available at the time of the visit although some studies have recently been reported using a loaned Sciex instrument.<sup>152</sup> Prospective and potentially routine neonatal screening programmes are being developed in collaboration with Neogen Inc., rather than in-house: it is understood that neonatal screening at Duke Medical Center by Neogen Inc. will commence during 1997.

The methodology used was initially developed for the analysis of acylcarnitines in urine and blood samples, and subsequently in blood spots, using methyl esters as analytes. It has been shown to be able reliably to detect and discriminate between many of the known disorders of organic acid and fatty acid metabolism in which acyl CoA esters and hence acylcarnitines accumulate. The suggestion that the methodology might be applicable to neonatal screening for disorders of organic acid and fatty acid metabolism<sup>63</sup> stimulated interest in extending the approach to disorders of amino acid metabolism and the use of n-butyl esters as analytes was introduced as more universally applicable to a wider range of metabolites.<sup>153</sup> The initial development towards neonatal screening at Duke

University was applied to PKU since neonatal screening for this disorder is carried out universally and therefore any new technology must be able to include PKU amongst the range of disorders covered. The methodologies developed at Duke University are applied in essence in all of the other laboratories that are developing tandem MS for neonatal screening.

### **Phenylketonuria**

The method for PKU screening is based upon determination of phenylalanine and tyrosine from the amino acid profile. The phenylalanine:tyrosine ratio is also determined in order to filter out secondary disorders in which concentrations of both amino acids are elevated. The method is highly specific by virtue of the mass spectrometry used, and was shown to be sensitive (estimated detection limits for phenylalanine and tyrosine are 3 and 10  $\mu\text{mol/l}$ , respectively – well below normal physiological ranges), accurate and precise. Because tandem MS allows determination of phenylalanine:tyrosine ratios it has been shown to have advantages over the conventional microbiological and fluorometric assays used for PKU screening by avoiding known false-positives (e.g. caused by parenteral feeding or chemical interference), and errors introduced by seasonal variations and inadequate or overloaded blood spots. The method should also avoid the potential false-negatives that can be introduced in the microbiological assays when the infants concerned are receiving antibiotics. Tandem MS also showed complete discrimination of samples from known patients with tyrosinaemia who would be detected as positives in a normal PKU screening programme, and the technique was considered sufficiently robust for application to routine neonatal screening for PKU.<sup>18</sup> This method thus provides simultaneous screening for both PKU and for tyrosinaemia.

The amino acid profile produced also permits the simultaneous quantification of a range of other amino acids after introduction of appropriate internal standards during the extraction phase. The method has been extended at the Duke University facility to the determination of branched-chain amino acids, suitable for screening for MSUD, and of methionine, suitable for screening for homocystinuria.

### **MSUD**

MSUD is extremely rare in the UK and similar populations, but several States in the USA still routinely screen for this disorder using methods other than tandem MS, primarily microbiological

inhibition assays similar to those used for PKU screening. Thus there was incentive for the American laboratories to include this disorder in the range for which screening was developed. Such screening could be performed simultaneously with that for PKU using tandem MS by simple switching of the masses monitored. Leucine and isoleucine are not determined separately by this method and alloisoleucine and hydroxyproline also may cause some interference. However, blood hydroxyproline concentrations in newborn infants are generally very low, and since alloisoleucine is also associated with MSUD its presence would enhance detection rather than causing adverse interference. The results obtained demonstrated high sensitivity with limits of detection, estimated at 2  $\mu\text{mol/l}$  for leucine + isoleucine and 6  $\mu\text{mol/l}$  for valine, well below the normal physiological ranges for these amino acids. Accuracy and precision were also good and the ability of the method to detect known MSUD patients from analysis of neonatal blood spots was demonstrated.<sup>154</sup>

At this stage of development the Duke University Mass Spectrometry Facility had analysed more than 10,000 neonatal blood spots obtained through the North Carolina State Screening Laboratory.

### **Homocystinuria**

Homocystinuria and isolated hypermethioninaemia are very rare disorders but again are screened for by a number of centres in the USA. Tandem MS was more recently<sup>39</sup> extended to screening for these disorders, on the basis of determination of blood-spot methionine concentrations in the amino acid profile. Screening is conventionally carried out using a microbiological inhibition assay and with this assay there is controversy about the level at which methionine concentrations should be considered as abnormal, with proposed levels ranging from > 67  $\mu\text{mol/l}$  to > 134  $\mu\text{mol/l}$ . Low levels of blood methionine are found in breast-fed infants and, as with PKU, early hospital discharge of newborn infants in the USA was leading to greatly reduced levels of methionine in neonatal blood spots. Consequently, there was an imperative need for a more sensitive and reliable screening method to overcome an increasing number of false-negative results. The limit of detection using tandem MS was 4  $\mu\text{mol/l}$ , well below the physiological range for blood methionine (literature, 10–90  $\mu\text{mol/l}$ ; determined by tandem MS,  $19 \pm 8.3 \mu\text{mol/l}$ ) and accuracy and precision were high. Discrimination of normal values from positive values was further improved by comparison of methionine concentrations with those of another quantified amino acid,

for example, phenylalanine or isoleucine + leucine. Known cases of homocystinuria and hypermethioninaemia were accurately detected in a series of blood spots in a blinded trial.

At this stage of development, the Duke University Mass Spectrometry Facility had analysed some 16,000 neonatal blood spots for amino acids.

In Dr Chace's opinion, for future development and transfer of tandem MS technology into routine neonatal screening laboratories, there is an absolute need for two instruments per screening laboratory, both operating at much less than full capacity, to allow for instrument down time, maintenance and development, and for follow-up of abnormal results and patient studies. Individual operators are required for each instrument to allow for holiday periods, sick leave and development.

### Neogen Inc., Pittsburgh

Neogen Inc. of Pittsburgh, Pennsylvania, USA was founded by Dr EW Naylor who has extensive experience of neonatal screening through working for many years with Dr R Guthrie in Buffalo, New York, USA. Dr Naylor also operates a metabolic diagnostic laboratory at the McGee Women and Children's Hospital in Pittsburgh and the University of Pittsburgh. Neogen Inc. is a commercial operation, devoted exclusively to neonatal screening, which operates a supplemental newborn screening programme at many hospitals through Pennsylvania, eastern Ohio, the District of Columbia and parts of North Carolina and uses its own blood-spot card in addition to that of the State Public Health Laboratory. It is the only laboratory in the world known to be currently undertaking prospective whole population neonatal screening for inborn errors of metabolism using tandem MS and as a result has acquired some unique experience.

Some of the developmental work on tandem MS at Duke University Mass Spectrometry Facility was carried out in collaboration with Dr Naylor, and Neogen Inc. have extended the tandem MS technology to include electrospray ionisation, although about 15% of the workload is still carried out at present using FAB ionisation. At present the methods used by Neogen Inc. are closely similar to those used at Duke University, but in the future the increased sensitivity of electrospray ionisation will permit the use of a single  $\frac{1}{8}$ -in. punched spot and 96-well microtitre plates, as a preliminary to full automation of the procedure. At present the only automation is in the sample introduction into the electrospray mass spectrometer and because

this uses a Hewlett-Packard robot arm system designed for high-pressure liquid chromatography applications it is rather slow. However, the workload at present is about 105,000 samples per annum, which is achieved through a 16-hour working day, split-shift operation by two technicians who work with both the electrospray instrument and with the manual FAB instrument. However, it seems that the optimum operation would be achieved by using two electrospray instruments with two operators working normal days and working weeks: this allows for some instrument down time for source cleaning and maintenance while keeping up a high through-put with some slack for potential down time caused by instrument failure.

Because of the availability of tandem MS for neonatal screening, Neogen Inc. offer screening by amino acid analysis for PKU, MSUD, homocystinuria and citrullinaemia, and by acylcarnitine analysis for a number of disorders of organic acid metabolism including MMA, PA, IVA, glutaric aciduria, and disorders of fatty acid metabolism including MCAD deficiency. In addition to these disorders, Neogen Inc. also offers screening, using other methods, for congenital hypothyroidism, CAH, cystic fibrosis, Duchenne and Becker muscular dystrophies, sickle cell disease, galactosaemia, biotinidase deficiency, glucose-6-phosphate dehydrogenase deficiency, adenosine deaminase deficiency, arginase deficiency, and pyroglutamic aciduria.

The results of newborn screening for inborn errors of metabolism using tandem MS are shown in *Table 23*. The figures for MSUD and possibly GAI may be compromised because the current screening programme covers some of the Amish population of Pennsylvania in which, because of founder effects and the high degree of consanguinity, these disorders have a relatively high incidence. However, if MSUD is discounted and only half of the GAI patients are included, the overall incidence of affected patients diagnosed by tandem MS screening is 1:4132. In comparison, in the same laboratory the incidence for PKU alone was 1:14,500, the incidence of CAH was 1:18,879 and the incidence of cystic fibrosis of 1:4741 (in an ethnically-mixed population). These data suggest that tandem MS screening could be very cost effective. Neogen Inc. charges \$17.50 per baby for the full repertoire offered, in comparison with \$25–65 per baby charged by other USA neonatal screening laboratories for a much more limited range of disorders, often only PKU and congenital hypothyroidism. The results for the first 80,371 newborn infants to be screened prospectively for



**TABLE 23** Newborn screening by Neogen Inc. (Pittsburgh, USA) for inborn errors of metabolism using tandem MS

Disorder	Number of affected newborn infants identified*	Incidence
PKU	12 (No false-positives)	1:14,500
HPA	5	1:35,000
MSUD	2	–
Tyrosinaemia	1	1:175,000
Hypermethioninaemia	2	1:87,000
MCAD deficiency	15	1:11,500
GAI	5	1:35,000
PA	2	1:87,000
MMA	1	1:175,000
SCAD deficiency	1	–
HMGA	1	–
* 173,537 newborn infants were screened.		

MCAD deficiency by Neogen Inc. using tandem MS have been published.<sup>68</sup>

### Institute of Child Health, University of London

Dr A Johnson and Dr P Clayton at the Institute of Child Health are presently evaluating and developing tandem MS towards neonatal screening. Currently working with a single VG (Micro-mass) Quattro instrument, they have adapted the methodology initiated at Duke University, with an emphasis on moving towards full automation of the blood-spot punching, extraction and derivatisation. This work is being carried out in conjunction with Quattro Biosystems, who are apparently at the prototype stage of development of an automated sample preparation instrument; this will operate through 96-well microtitre plates, with spot punching (6 mm spot), methanol extraction, butylation of the dried extract at room temperature using modified chemistry, drying of the butylated extract and solution of the butyl derivatives in acetonitrile–water, ready for automated injection into an electrospray tandem mass spectrometer. At the time of the visit final details of the sample preparation process were being evaluated and the automated apparatus was not available, and therefore manual sample preparation was being carried out. The aim is to achieve sample

through-put of 400 samples per day. Data handling will be automated with full profiles of amino acids and acylcarnitines being generated, together with quantitation via ratios of specific ions and calibration curves, and with determination of ratios of related metabolites for identification of abnormal samples. The latter aspect is still under development.

The Institute of Child Health group have gained considerable experience by analysis of blood spots from patients with known metabolic disorders and by using the methodology to investigate blood spots from children and infants with suspected metabolic disorders. In addition to developing routine screening for amino acids and acylcarnitines, the group have a number of research interests and have been examining ways of developing the methodology to encompass urea cycle defects, the use of constant neutral loss (CNL) scanning for acylcarnitines<sup>155</sup> and, more recently and importantly, the detection of bile acids as a method of neonatal screening for biliary atresia.<sup>156</sup> The group is aiming to operate a 3-month screening of PKU jointly with the routine neonatal screening laboratory based at Great Ormond Street Hospital (GOS) and then to go on to screening for PKU and other disorders using tandem MS alone. Ultimately, as the operation becomes routine, it is expected to be taken over by the GOS laboratory personnel, with the group at the Institute providing specialist mass spectrometry back-up and advice, training, and follow-up of abnormal samples and affected patients. Thus there is apparently no conflict between the development of tandem MS-based screening and the operation of the routine laboratory for North Thames Region. The routine laboratory currently processes some 110,000 blood spots per annum, and for a tandem MS operation it appears it would be essential to provide two instruments, or one routine instrument backed up by a research and follow-up instrument, with appropriate staffing, if allowance is to be made for instrument down time, servicing, etc.

### Sir James Spence Institute of Child Health, University of Newcastle upon Tyne

The biomedical mass spectrometry facility in Newcastle upon Tyne is operated by Professor K Bartlett and Dr M Pourfarzam. They use a single tandem mass spectrometer (Quattro II) with electrospray ionisation for developmental work. They have modified the Duke University methodology and use 96-well microtitre plates for extraction and derivatisation. The dried butylated residue is taken up into acetonitrile–water and a 96-well plate

autosampler is used for sample introduction into the electrospray source of the mass spectrometer. None of the sample preparation is automated but this was the only group who had achieved a working 96-well plate autosampler. The autosampler allows overnight operation of the mass spectrometer, and with one sample being introduced every 3 minutes, a through-put of 400 samples per day can be achieved. Profiles are obtained for amino acids, basic amino acids and acylcarnitines. The computer software required for automated data analysis and output is being produced in conjunction with Micromass Ltd. The Newcastle upon Tyne group also have an interest in bile acids, with a view to screening for biliary atresia.

The group have accumulated considerable experience through analysis of about 30,000 blood spots from patients with known metabolic disorders and from acutely sick infants and children with suspected metabolic disorders. They are working towards introducing tandem MS into neonatal screening as soon as is practicable, and plan initially to cover the Northern Region in which there are some 40,000 births per annum. However to achieve this they will need to rationalise this objective with staff at the current Northern neonatal screening laboratories in Durham and Newcastle upon Tyne who appear highly resistant to such development. The team at the Sir James Spence Institute of Child Health plan to have two electrospray instruments within a practicable time-scale. They believe that a two-instrument operation would be essential and would give the capacity to cover a much wider area of Northern Britain.

It was notable that to purchase their first instrument the group had to obtain tenders: of the companies that were approached one (Sciex) did not reply, a second (Finnigan) was too expensive and only VG (now Micromass Ltd), who manufacture the Quattro instruments, provided a viable tender. It is also noteworthy that worldwide all of the laboratories currently using tandem MS for diagnosis of metabolic disorders, including those performing or working towards neonatal screening for these disorders, use VG or Micromass instruments and it is understood that this British company may be developing a bench-top instrument aimed at this application which would be considerably cheaper than the instruments currently available.

### **Financial and operational considerations**

Discussion with leading personnel at the laboratories visited provided a concept of

a neonatal screening laboratory that used tandem MS operating through a two-instrument facility. Laboratory requirements include some 50 m<sup>2</sup> floor space, high efficiency fume cupboards, appropriate sample preparation benching and services, possibly including three-phase power supplies, as well as water, etc. Equipment additional to the mass spectrometers could include an automated card punch (possibly shared with screening for congenital hypothyroidism and other disorders) capable of transfer to multi-well plates and appropriate equipment (automated or manual) for sample extraction and derivative preparation. Staffing requirements for a two-instrument facility would include one trained and experienced mass spectrometry technician/operator and one further technician/operator, both with at least a first degree, and one junior technician/trainee to help with card punching and sample preparation. Overview of the operation and results together with initiation of follow-up of abnormal results and liaison with clinical colleagues would be by a consultant biochemist or chemical pathologist, preferably with experience of screening and mass spectrometry. Ideally, the mass spectrometry screening laboratory should be adjacent to, or integral with, the metabolic investigation unit laboratories for follow-up and patient monitoring, with the instrumentation supplementing the facilities of the latter as required.

On the above basis, with allowance for the salaries of the three operating staff, running costs and instrument costs on a 7-year lease or purchase, and inclusive of commissioning costs and overheads, the cost of screening can be estimated at £3–4 per baby screened, depending on the size of the unit (the above cost range relates to screening of 100,000 to 45,000 newborn infants per annum). For optimal cost-effectiveness with such technology, larger units are essential and the operational sizes of units would be for screening of at least 80,000 newborn infants per year. This still allows for flexibility in operation, including down time for servicing, unscheduled staff absences, diagnostic investigations in acutely presenting patients, and research and development. The latter would be essential in any developing and progressive unit.

It can be estimated that for about 50% more than the present average costs of neonatal screening for PKU and hypothyroidism, a screening laboratory that used both tandem MS and automated immunoassay based methods (e.g. AUTODELFIA<sup>®</sup>) could provide screening for PKU, congenital hypothyroidism, cystic fibrosis,

CAH, tyrosinaemia (and possibly other selected disorders of amino acid metabolism), MCAD deficiency (and other selected disorders of fatty acid metabolism), GAI, MMA and IVA (and possibly other selected disorders of organic acid metabolism). For no additional cost, screening for biliary atresia and some other disorders might be included as methods evolve. In comparison with the current programme, tandem MS/automated immunoassay-based screening would be much more efficient and have greater avoidance of false-positives. It would also allow screening to be suitably expanded to include the groups of clinically significant and preventable (treatable) disorders that have been identified since nationwide neonatal screening was originally introduced.

## Conclusions

Evidence provided by the literature survey and, in particular, the laboratory visits and associated discussions indicates that robust (i.e. accurate, sensitive, lack of false-positives) tandem MS screening technology and methodology are now available. Tandem MS methodology appears reasonably well established for newborn screening for PKU although to date it has been applied to prospective neonatal screening in only one centre worldwide (Neogen Inc., Pittsburgh, USA). Tandem MS has also been applied successfully by Neogen Inc. to prospective screening for a wide range of other inborn errors of metabolism.

The introduction and application of new methods, tests and technologies must be determined by:

- the perception of and evidence for the need to screen in the newborn for each disorder or group of related disorders
- the perception of and evidence for the need for the introduction of a new method or technology into existing screening programmes.

### Disorders that could be screened for

The disorders to which tandem MS has potential application for newborn screening include, in particular, PKU for which newborn screening is already universally accepted and applied. Taken alone, screening for PKU using tandem MS would not appear to be immediately cost-competitive with conventional methods. However, because tandem MS generates highly accurate quantitative measurements of the phenylalanine:tyrosine ratio it can avoid false-positive results and other interferences (e.g. effects of antibiotics, parenteral feeding, prematurity, seasonal variations, inadequate or overloaded blood spots) that affect conventional screening methods and it may therefore be

considered to offer advantages and potential cost-benefits for PKU screening. Tandem MS also provides, with the same instrument and resources, the means for accurate monitoring of blood phenylalanine levels during follow-up and treatment, which is an integral part of any screening service. If a newborn screening programme were to be set up *de novo* for a previously unscreened population, tandem MS could be the method of choice today. Thus a good case could be made for the introduction of tandem MS into the UK neonatal screening programmes, even for PKU screening alone.

Review of the diseases and disease categories in chapter 3 has identified GAI and MCAD deficiency as disorders for which there is evidence to justify the introduction of neonatal screening, albeit with monitoring and review after 10 years. Newborn screening for both of these disorders is dependent upon tandem MS. However, the evidence for the introduction of a nationwide neonatal screening for these disorders is incomplete and a more staged approach might be indicated.

### The need for new technology

Evidence for the utility of tandem MS in prospective newborn screening has come from a single source and visits to laboratories have shown that developmental work is still continuing both on aspects of the instrumentation and automation and, perhaps more importantly, on the required operational computer software. Thus, as for the disorders, a case could be made for a more cautious approach to the widespread introduction of this expensive technology into neonatal screening in the UK.

### Options for action

There are therefore two options available if tandem MS were to be introduced.

1. Nationwide introduction of tandem MS into newborn screening in the UK for PKU (with abandonment of other screening methods for this disorder), and for GAI and MCAD deficiency, with the option to detect other (selected) disorders of amino acid, organic acid and fatty acid metabolism.
2. A staged introduction of tandem MS technology through primary research into its application to newborn screening in the UK.

### Option 1

The first option requires an absolute 'leap of faith' in tandem MS as the technology for the future. It would also necessitate considerable reorganisation

of the existing Regional neonatal screening services. At present there are some 22 laboratories in England and Wales which undertake newborn screening for PKU. Their workload ranges from less than 3000 to more than 100,000 newborn infants screened per year. It is clear that for many of the smaller laboratories, it will be uneconomic and impracticable to introduce tandem MS screening. Thus, if tandem MS-based neonatal screening were to be introduced, rationalisation of the screening laboratories and their catchment areas would be essential so that each laboratory performed an adequate number of tests each year. This is likely to reduce the number of neonatal screening laboratories to a maximum of one per new Health Region. With good postal/courier services and electronic communications, logistics and communications with such laboratories would not be a problem. The optimal locations of such screening laboratories could be identified relatively easily.

The cost and logistical implications of simultaneous introduction of this relatively expensive technology into multiple centres (including the potential strain on manufacturers in the supply of essentially custom-built instruments), together with the need for operator training, commissioning and initial evaluation periods (estimated at 1 year in each centre) must be considered. Also, as discussed in the latter part of this report (page 56), no new technology or screening for previously unscreened disorders should be initiated or undertaken unless the essential required infrastructure of clinical paediatric metabolic (and scientific), paediatric dietetic and clinical genetic services are established. High-quality infrastructure of this nature exists in very few centres in the UK – Manchester, London (Great Ormond Street Hospitals Trust and Institute of Child Health; St George's Hospital Medical School and Healthcare Trust), Newcastle upon Tyne and Glasgow. Elsewhere, infrastructures are much less well-established, particularly for clinical paediatric metabolism. Therefore this first option seems somewhat premature and ill-considered at the present time and, on balance, the second option (discussed below) is greatly preferred.

### Option 2

The second option, of primary research, would include a parallel comparison of the efficacy and cost-effectiveness of PKU screening using tandem MS with established methodologies, combined with monitoring of the cost-effectiveness and cost-benefit of screening for GAI and MCAD deficiency and other disorders using tandem MS. This primary research would need to be conducted

over a period of 5 years in order to provide definitive answers on the utility of tandem MS for neonatal screening and, in particular, on the incidence of the relatively rare disorders concerned. The research programme would need additional support and funding for instrumentation and for staffing and running expenses for these centres and must include a defined timetable. An external and independent group should also be established for progressing the research and for health economic, statistical and scientific monitoring and cost-effective evaluation of the technology and programmes.

Such research would clearly best be carried out in the UK at the two centres that already have equipment, expertise and experience in tandem MS and are currently researching in this area. However, in both instances, practical problems exist and for the research to progress efficiently and to achieve the required objectives within the indicated timescale, introduction of tandem MS for newborn screening will probably also be required in other selected laboratories.

The two centres already researching in this area have been identified in this report as the Institute of Child Health in London and the Sir James Spence Institute of Child Health in Newcastle upon Tyne. The laboratories at the London Institute of Child Health are associated with the North Thames Regional Newborn Screening programme at GOS which currently screens more than 110,000 newborn infants each year and is thus well placed to progress this research with the essential required collaboration. The Institute also covers a defined population (North Thames Region) and has the essential high-quality clinical paediatric metabolic and supportive infrastructure. However, the Institute laboratories have a primary interest in the development of new technologies and methodologies, and progress towards prospective screening appears to have been slow. Thus, for this primary research to progress satisfactorily and with the required momentum at GOS, it would probably be necessary for a separate single-instrument facility to be established in the Neonatal Screening Laboratory, supported by back-up and expertise from the Institute research laboratory.

The Sir James Spence Institute of Child Health in Newcastle upon Tyne would need to develop appropriate collaborations to progress to prospective newborn screening in the Northern Region. For the work carried out so far, supplies of neonatal blood spots have been obtained through informal collaboration with the Regional Screening Centres

in Manchester and Sheffield. This collaboration should be encouraged on a formal basis and, for practical and logistical purposes, single-instrument facilities should be established in both Manchester and Sheffield in collaboration with Newcastle upon Tyne, which would provide both back-up and expertise while continuing to try to develop tandem MS screening in the Northern Region. The Regional Screening Centres in Manchester (North Western Region) and Sheffield (Trent Region) annually screen about 55,000 and 70,000 newborn infants, respectively, making the combined operation (screening of 125,000 newborn infants) very similar to that in North Thames. In addition, the essential, high-quality clinical paediatric metabolic and supporting infrastructure exists in Manchester, Newcastle upon Tyne and to a lesser extent in Sheffield. The only other centre in England and Wales in which the required support infrastructure exists and which also has extensive mass spectrometry experience is based at St George's Hospital Medical School. This centre is closely linked with the South West Thames Regional Newborn Screening Service (which screens some 45,000 newborn infants each year) and could also collaborate with the South East Thames Newborn Screening Service (which screens 40,000 newborn infants per year).

These four centres – GOS/North Thames Region, Manchester/North Western Region, Sheffield/Trent Region and St George's/South Thames Region – collectively screen some 320,000 newborn infants each year (360,000 including Newcastle upon Tyne/Northern Region). This number represents about 40% of live births in the UK (some 793,000 per year) and the multi-ethnic populations covered would be representative of the UK population as a whole.

Over a 5-year period, the four centres would screen about 1,600,000 newborn infants. The proposed research programme should therefore provide the required information on:

- the utility of tandem MS in newborn screening in the UK
- the efficacy of screening for PKU using tandem MS in comparison with traditional techniques (the laboratories concerned use both Guthrie microbiological and chromatographic methods)
- the true incidence of MCAD deficiency, of GAL and of many of the other disorders that can be detected by tandem MS, together with some information on short-term outcome.

Further information on outcome (see chapter 3, pages 18 and 21) would be acquired during the

5 years after completion of an initial research programme through follow-up by metabolic paediatricians in the supporting infrastructures.

While the primary research is on-going, and until the results are reported and further decisions taken, there should be an embargo on the introduction of tandem MS into any other newborn screening laboratories in the UK. However, pilot studies would be appropriate to assess the potential role of new methods based on tandem MS (e.g. detection of bile acids) and their extension to newborn screening for other disorders (e.g. biliary atresia). Thus the embargo on tandem MS in newborn screening laboratories should not compromise the introduction of such technology into biomedical research laboratories.

### **Tandem MS: the way forward**

There is insufficient evidence at present to justify the widespread introduction of tandem MS technology into newborn screening programmes in the UK. However, the evidence does justify the further investigation of the value of tandem MS for newborn screening, and that this should be carried out by primary research conducted over 5 years with a defined timetable and external and independent statistical, health economic and scientific monitoring and evaluation of the technology and programmes.

This research should be conducted at the four UK centres that have the required support infrastructure and associated expertise in (tandem) mass spectrometry. These are as follows.

1. The Institute of Child Health in London in collaboration with the North Thames Region Newborn Screening Service (GOS) in London.
2. The North Western Region Newborn Screening Service in Manchester in collaboration as necessary with the Sir James Spence Institute of Child Health in Newcastle upon Tyne.
3. The Trent Region Newborn Screening Service in Sheffield in collaboration as necessary with the Sir James Spence Institute of Child Health in Newcastle upon Tyne.
4. St George's Hospital Medical School, London in collaboration with the South Thames Region Newborn Screening Services.

During this primary research and until reports are presented and evaluations made there should be an embargo on the introduction of tandem MS technology into other newborn screening laboratories in the UK.



## Chapter 5

### Review of economic evidence

#### Secondary analysis of economic evidence

In economic evaluation it is helpful to distinguish three types of costs and benefits:

- **direct** use of resources to provide health and social care
- **indirect** loss of productivity due to time off work
- **intangible** effects of anxiety, pain, grief or suffering.

This basic framework is illustrated for the case of PKU in *Table 24*. There are the direct costs of screening and providing follow-up care. There are also indirect costs, as parents, and later patients themselves, take time off work to attend follow-up appointments. In addition there will be intangible costs due to parental anxiety over false-positive results and due to the problems of dietary and other management. However, on the other side, there are the avoided costs that would have been incurred if the patient had not been detected and treated early. These include the extra direct costs of health and social care, indirect costs of lost productivity for the individual and for their parents, and, of course, the intrinsic value of lost years of life and quality of life.

Papers containing data on the costs or benefits of neonatal screening for inborn errors of metabolism were included in the economic review. As far as possible economic data from the literature were standardised to aid comparison.

Firstly, all costs and benefits were converted to 1995 pounds sterling, by applying the appropriate exchange rate\* and then up-rating for inflation†. In some cases the results were then re-calculated to fit the above framework. For example, the costs of education and health care for a healthy individual were subtracted from the costs of special education and health care for an untreated individual on the benefit side of the balance sheet, rather than added to the cost side. Clearly, this has no effect on net-benefits, but it does change the benefit–cost ratio, hence some of the figures quoted below are different from those given in the original papers. Wherever possible, a societal viewpoint was taken, including costs and benefits accruing to both public and private sectors, but excluding taxes and social security benefits (as transfers within society). Finally, the net benefit per 100,000 babies screened and per case detected were calculated.

Full standardisation was not always possible, as some papers did not include sufficient basic data.

**TABLE 24** Framework for assessing the costs and benefits of neonatal screening for PKU

Costs	Benefits (avoided costs)
<b>Direct</b> <ul style="list-style-type: none"> <li>• Screening and diagnosis</li> <li>• Follow-up and monitoring</li> <li>• Diet therapy (childhood only?)</li> <li>• Care for PKU mothers and babies</li> </ul>	<b>Direct</b> <ul style="list-style-type: none"> <li>• Health and social care for untreated individual for lifetime (net of costs for healthy individual)</li> </ul>
<b>Indirect</b> <ul style="list-style-type: none"> <li>• Time to attend follow-up appointments</li> </ul>	<b>Indirect</b> <ul style="list-style-type: none"> <li>• Production losses while parents look after child</li> <li>• Production losses for untreated individual throughout lifetime</li> </ul>
<b>Intangible</b> <ul style="list-style-type: none"> <li>• Parental anxiety over false-positive results</li> <li>• Problems of dietary treatment and monitoring</li> </ul>	<b>Intangible</b> <ul style="list-style-type: none"> <li>• Value of avoided loss of life and quality of life</li> </ul>

\* Average of daily telegraphic rates in London for the study year (Economic Trends, Annual Supplement, 1996/97 Edition, HMSO: London)

† Baseline analysis used UK general index of retail prices (Economic Trends, Annual Supplement, 1996/97). Calculations also done using GDP deflator (op cit).

For example, studies used different discount rates to allow for differential timing of costs and benefits, and it was not possible to recalculate results on the basis of the UK standard 6%. Papers also differed considerably in the costing methods used and in their underlying assumptions. Because of this heterogeneity no attempt was made to calculate average net benefits or benefit–cost ratios.

Since PKU is the most well-established screening test, the costs and benefits of PKU screening alone were compared to no screening (i.e. all of the costs of sample collection, where quoted, were included in the costs for PKU screening). So, if the net benefits of PKU screening are positive, then this alone justifies the collection of samples. The marginal costs and benefits of technology changes or the addition of other diseases can then be considered separately.

## Number and type of economic evaluation papers identified

Twelve published economic evaluation studies were identified (*Table 25*).<sup>132,157–167</sup> Ten of these<sup>157–166</sup> were CBAs, with quantification of monetary costs and benefits. Two were simpler cost-effectiveness analyses (CEAs)<sup>132,167</sup> which calculated only the cost of screening per case detected. There was only one cost–utility analysis (CUA)<sup>160</sup>, which gave an estimate of the number of quality adjusted life-years (QALYs) saved by screening.

The papers covered a wide range of disorders, but only PKU was included in more than one study. The costs and benefits of whole screening programmes including several disorders were assessed in three papers,<sup>158,162,165</sup> in two of which<sup>162,165</sup> there were insufficient raw data to enable disaggregation into separate costs and benefits for the various diseases included. For the other paper<sup>158</sup> it was possible to separate the costs and benefits for PKU from other disorders, but not to isolate the cost and benefits for each of those other disorders. No studies have been published that analyse the economic implications of introducing new multi-disease screening techniques.

## The economics of PKU screening

In all there were eight studies<sup>157–161,163,164,166</sup> in which the costs and benefits of neonatal screening for PKU were estimated (see *Table 26*). These covered very different populations and the number of cases detected varied considerably, from 1.2 to 17.9 per 100,000 babies screened. Much of this variation would be expected because of the different ethnic mix of the populations, but part might be due to the use of different cut-off points for PKU and HPAs, and to the failure of some studies to distinguish between them.

In all of the studies it was concluded that PKU screening is worthwhile in terms of the monetary

**TABLE 25** Papers containing economic data on neonatal screening for inborn errors of metabolism

Reference	Date	Country	Type of economic analysis	Diseases covered
Bush <i>et al</i> <sup>160</sup>	1973	USA	CBA/CUA	PKU
MDPH <sup>158</sup>	1974	USA	CBA	Whole programme
Komrower <i>et al</i> <sup>166</sup>	1979	England	CBA	PKU
Alm <i>et al</i> <sup>157</sup>	1982	Sweden	CBA	PKU, histidinaemia, homocystinuria, tyrosinaemia, galactosaemia
Holtzman <sup>165</sup>	1983	USA	CBA	Whole programme
Barden <i>et al</i> <sup>159</sup>	1984	USA	CBA	PKU
Dagenais <i>et al</i> <sup>162</sup>	1985	Canada	CBA	Whole programme
Dunkel <i>et al</i> <sup>132</sup>	1989	Canada	CEA	Biotinidase deficiency
Dhondt <i>et al</i> <sup>163</sup>	1991	France	CBA	PKU, congenital hypothyroidism
Cockburn <i>et al</i> <sup>161</sup>	1992	Scotland	CBA	PKU
Hisashige <sup>164</sup>	1994	Japan	CBA	PKU
Sprinkle <i>et al</i> <sup>167</sup>	1994	USA	CEA	PKU, haemoglobinopathies



TABLE 26 Papers containing CBAs of neonatal screening for PKU

Reference	Area	Screening period	Babies screened (000s)	Percentage of live births screened	Cases detected per 100,000 screened	
					PKU	HPA
Bush <i>et al</i> <sup>160</sup>	USA	1965–70	1893	–	6.3	(0.6)
MDPH <sup>158</sup>	USA	1972–73	77	–	9.1	*
Komrower <i>et al</i> <sup>166</sup>	UK	1968–78	507	98.5	13.6	*
Alm <i>et al</i> <sup>157</sup>	Sweden	1965–79	1326	98.0	3.2	(1.7)
Barden <i>et al</i> <sup>159</sup>	USA	1978–82	295	–	5.8	(6.4)
Dhondt <i>et al</i> <sup>163</sup>	France	1970–90	–	99.8	6.3	*
Cockburn <i>et al</i> <sup>161</sup>	Scotland	1988	67	–	17.9	*
Hisashige <sup>164</sup>	Japan	1977–90	18,700	–	1.2	

\* PKU and HPA (non-PKU hyperphenylalaninaemia) not distinguished.

costs and benefits – screening and treatment costs are outweighed by the costs that would have been incurred in caring for untreated individuals throughout their lifetimes. There was more than a 15-fold variation in estimates of the net benefit of PKU screening (Table 27), from £278,000 to £4.3 million for every 100,000 babies screened (in 1995 pounds sterling). Much of this variation was due to differing incidence: the cost per case detected varied from £42,000 to £356,000, an eight-fold difference (Table 28). Bush and colleagues<sup>160</sup> also estimated that screening based on paper chromatography would be cheaper than Guthrie testing, with a small decrease in the number of

HPA cases detected. However, their figures are now very out of date.

The quality of the CBAs of PKU screening varies considerably. Three studies<sup>158,160,166</sup> did not include estimates of the indirect benefits of screening and in two<sup>158,166</sup> of these future costs and benefits were not discounted. In another study<sup>161</sup> a discounted overall net benefit was quoted, but the discount rate that had been used was not specified. The discount rate used in the other studies ranged from 4 to 7%. Very different assumptions were made across the different studies about outcomes and service use with and without screening. For

TABLE 27 Costs and benefits of neonatal screening for PKU per 100,000 babies screened

Reference	Discount rate (%)	Costs (1995 £000s)			Benefits* (1995 £000s)			Net benefit (2–1, 1995 £000s)	Benefit/cost ratio (2/1)
		Screening + diagnosis	Treatment + follow-up	Total (1)	Health + social care	Productivity	Total (2)		
Bush <i>et al</i> <sup>160</sup>	4	853	38	891	1181	–	1181	290	1.3
MDPH <sup>158</sup>	0	654	243	896	2327	–	2327	1431	2.6
Komrower <i>et al</i> <sup>166</sup>	0	98	697	795	3688	–	3688	2892	4.6
Alm <i>et al</i> <sup>157</sup>	6	796	405	1201	1170	930	2100	899	1.8
Barden <i>et al</i> <sup>159</sup>	7	627	247	874	1312	453	1765	891	2.0
Dhondt <i>et al</i> <sup>163</sup>	4.5	212	111	323	1746	809	2555	2232	7.9
Cockburn <i>et al</i> <sup>161</sup>	0	117	1594	1711	14,447	8962	23,408	4351 <sup>†</sup>	13.7
Hisashige <sup>164</sup>	7	115	69	184	222	240	462	278	2.5

\* Avoided costs.  
<sup>†</sup> Overall discounted net benefit quoted, but the discount rate used is not specified.

TABLE 28 Costs and benefits of neonatal screening for PKU per case detected

Reference	Discount rate (%)	Costs (1995 £000s)			Benefits* (1995 £000s)			Net benefit (2-1, 1995 £000s)	Benefit/cost ratio (2/1)
		Screening + diagnosis	Treatment + follow-up	Total (1)	Health + social care	Productivity	Total (2)		
Bush et al <sup>160</sup>	4	122	6	128	169	–	169	42	1.3
MDPH <sup>158</sup>	0	72	27	99	256	–	256	157	2.6
Komrower et al <sup>166</sup>	0	7	51	58	271	–	271	212	4.6
Alm et al <sup>157</sup>	6	246	125	370	361	287	648	277	1.8
Barden et al <sup>159</sup>	7	109	43	152	228	79	306	155	2.0
Dhondt et al <sup>163</sup>	4.5	34	18	52	279	129	408	356	7.9
Cockburn et al <sup>161</sup>	0	7	89	96	806	500	1306	243 <sup>†</sup>	13.7
Hisashige <sup>164</sup>	7	92	56	148	179	193	372	224	2.5

\* Avoided costs.  
<sup>†</sup> Overall discounted net benefit quoted, but the discount rate used is not specified.

example, estimates of the duration of dietary treatment ranged from 2 years<sup>160</sup> to 20 years.<sup>159</sup> Current thinking is that the latter is more appropriate. Only two papers<sup>157,161</sup> included the costs of treatment during pregnancy for mothers with PKU. In only three papers<sup>157,159,164</sup> was any serious attempt made at sensitivity analysis, and these results showed that PKU screening remained cost-beneficial under plausible variations in incidence, sensitivity, specificity, service use and discount rates. Overall, the CBAs by Alm and colleagues<sup>157</sup> and Barden and co-workers<sup>159</sup> appear to be the most credible.

Bush and co-workers<sup>160</sup> are the only group who have attempted to quantify the intrinsic value of the saved years of life and health improvements due to PKU screening. They estimated that 47 QALYs could be saved for each person if classic PKU was detected early through screening and was treated (undiscounted figure based on consultant preference weightings).

Bush and colleagues<sup>160</sup> estimated that selective ethnic screening for PKU (testing only white non-Jewish and Puerto Rican babies) would be more cost-effective than universal screening in New York State. However, they did not make any allowance for missed cases due to ethnic misclassification or for babies of mixed ethnic background. Sprinkle and colleagues<sup>167</sup> present an interesting analysis of how the cost-effectiveness of universal and selective screening for PKU and haemoglobinopathies (including sickle cell

varies between the US states with the ethnic mix of the population.

## The economics of screening for other inborn errors of metabolism

There is some, limited, evidence on the economic implications of screening for other disorders which we have included in our review.

### Galactosaemia, tyrosinaemia, homocystinuria and histidinaemia

Alm and colleagues<sup>157</sup> assessed the marginal costs and benefits of adding screening for four other inborn errors of metabolism to PKU screening. For galactosaemia they found a positive net benefit of £257,000 per 100,000 babies screened, or £209,000 per case detected. The net benefits for tyrosinaemia, homocystinuria and histidinaemia were all negative. It is difficult to reach firm conclusions from this one study.

In addition Harris<sup>56</sup> quotes a cost of £0.19 per test or £11,248 per case detected for galactosaemia screening, and Kirkby and co-workers<sup>168</sup> quotes a cost of about £1 per test (converted to 1995 pounds sterling). These figures do not come from full economic analyses and it is not clear what they include.

### Biotinidase deficiency

Dunkel and colleagues<sup>132</sup> estimated that the cost of screening per case of biotinidase (complete)

deficiency was £11,000. This estimate did not include follow-up or treatment costs, and no details of the costing methodology were reported.

### **Congenital adrenal hyperplasia**

There are no full economic analyses of CAH screening in the published literature. In an abstract, Brosnan, Riley and Brosnan<sup>169</sup> reported that nine out of 16 cases of CAH in Texas in 1994 were detected through screening (611,980 tests performed), and that this cost £190,465 per 100,000 tests or £129,512 per case detected (converted to 1995 pounds sterling). This figure includes the costs of sample collection, laboratory screening tests, follow-up tests and diagnostic evaluation.

In another abstract, Nordenstrom and colleagues<sup>76</sup> report that between 1986 and 1994 CAH screening in Sweden had identified 66 true-positive cases (out of 1.01 million children tested), 35 of whom were suspected before screening, and that 11 false-negative cases had been reported. They quote a cost of £1.58 per test (1995 pounds sterling). No details are given of how this cost estimate was reached.

### **The economic justification for new screening technologies**

The questions of whether laboratories should change to the new multi-disease screening technologies can be addressed in a sequential manner.

For tandem MS, we can first consider whether such a change is justified for PKU alone. If we assume that there will be no change in sample collection or follow-up methods (and therefore costs), the economic decision rests on laboratory costs alone. There are three possibilities.

1. If tandem MS screening for PKU alone is cheaper than existing methods (under realistic assumptions about the number and size of UK laboratories) then this suggests that the change should be made – although the extra set-up costs in the first year of operation must also be considered. The decision on whether to use the mass spectrometer to extend screening to other disorders, which meet all of the other screening criteria, then rests on the balance of **extra** costs, benefits and health improvements for each disorder.
2. If tandem MS screening is more expensive for PKU alone, it could still be justified if other

disorders are screened in addition to PKU. This will only be the case if the extra laboratory costs of tandem MS screening compared with conventional screening for PKU can be offset by monetary and health gains from detecting other disorders. Additionally, the improved accuracy of tandem MS screening for PKU must be considered.

3. The third possibility is that tandem MS technology is not justified at all. If this is the case, then we need to estimate the marginal costs, benefits and health effects of screening for the other disorders using other technologies where possible.

The cost of laboratory tests for PKU using conventional methods as estimated in the literature varied from £1.10 per test in Japan<sup>164</sup> to £4.20 per test in Sweden.<sup>157</sup> We have estimated the cost of tandem MS screening at between £3 and £4 per test, depending on the scale of the operation (see chapter 4, page 44). It is not possible to conclude whether tandem MS screening for PKU alone would be cheaper or more expensive than existing methods given the wide variation in costing methodologies used in these estimates, and in the absence of detailed and credible cost studies in contemporary UK newborn screening laboratories.

Technologies for automated immunoassay testing are not sufficiently developed for routine application. If and when they do become available, the economic justification for their introduction for routine neonatal screening in the UK will depend on:

- whether they are more or less expensive or accurate than conventional technologies for congenital hyperthyroid screening
- whether they are more or less expensive or accurate for PKU screening, if tandem MS has not been adopted by that time
- the marginal net benefit of introducing screening for other disorders, such as CAH and possibly cystic fibrosis.

### **Conclusions**

The literature provides good evidence that PKU screening provides a positive net monetary benefit for society, and that PKU screening alone justifies the collection of blood samples from neonates. This finding would almost certainly be strengthened if the intangible costs and benefits were included, since for PKU a dramatic and lasting health improvement results from relatively simple treatment.

However, dietary management is not without complications. Recent guidance<sup>14</sup> suggests that the diet should be maintained for life, and that great care is needed in the follow-up and management of women with PKU because of the risks to the foetus during pregnancy. The cost–benefit estimates in the literature do not allow for this stricter management. A level of uncertainty also remains as to the balance of costs and benefits for PKU screening because of the very variable quality of the literature, because estimates of health outcomes and service use without treatment are necessarily based on old and poor data (since untreated PKU is now very rare), and because most estimates are based on countries where PKU incidence, healthcare systems, and cost structures are very different from those in the UK.

There is not sufficient evidence in the literature to support a change to tandem MS-based screening for PKU alone on economic grounds.

Detailed estimates are needed of the laboratory costs of screening for PKU in the UK using existing methods and using tandem MS screening (under realistic assumptions on laboratory sizes).

There is insufficient evidence in the literature to assess the economic value of screening for any other inborn errors of metabolism. More information is needed on the cost-effectiveness of extending screening to the other disorders suggested in this report (MCAD deficiency, GAI, biotinidase deficiency and CAH). Such information would be obtained from the primary research on tandem MS and from the evaluative screening programmes proposed. Economic modelling based on this information should include extensive sensitivity analysis to allow for the very high level of uncertainty about long-term treatment and health outcome for these rare disorders.

## Chapter 6

### General discussion

This Health Technology Assessment of newborn screening for inborn errors of metabolism has included a systematic review of the literature, laboratory visits to assess new technologies, and a questionnaire to all directors of UK newborn screening laboratories. An analysis has also been made of the available economic evidence on newborn screening for inborn errors of metabolism. Evidence has been obtained for the need or otherwise to screen in newborn infants for a wide range of inborn errors of metabolism.

The analysis of the evidence shows firstly that newborn screening for PKU alone justifies the infrastructure and costs of sample collection and testing of newborn blood spots. This is an important observation and although some inadequacies in the system for the follow-up and care of diagnosed patients with PKU have been identified, newborn screening for PKU can clearly still be taken as the standard against which to assess any other current or putative newborn screening programme.

A further important observation is that review of the other 12 categories of inborn errors of metabolism covered by this assessment has identified only four (biotinidase deficiency, CAH, MCAD deficiency and GAI) as disorders for which neonatal screening could be justified on the evidence available. Even for these disorders, structured, coordinated on-going evaluation with CEA would be required if a programme were implemented. Additionally, screening for biotinidase deficiency and CAH would require reassessment after 5 years and screening for MCAD deficiency and GAI, because of the need to obtain outcome data, would require reassessment after 10 years. Newborn screening for MCAD deficiency and GAI would also be dependent upon introduction of tandem MS technology into the UK programme.

Considerable progress in newborn screening technologies and automation has been made but further progress followed by primary research into their application is required before universal adoption. Molecular (DNA) technologies, which superficially appear very attractive, are unsuitable for newborn screening for inborn errors of metabolism because of the very large number of different mutations associated with each disorder and the

inability of current molecular techniques to cope with this multiplicity. Fully automated immunoassay technology still appears to be some way off and the real benefits that might accrue from application of such technology remain to be demonstrated.

The utility and application of tandem MS, which can be considered as the most promising of the new technologies for newborn screening, has been demonstrated only in one centre in the USA and for only a relatively short period of time, with only a limited number of newborn infants screened so far. Some aspects of instrumentation and automation, and especially of data analysis computer software, are still under development. This technology requires further assessment through primary research in the UK environment, and on a larger scale than the American operation, before consideration for universal introduction. This conclusion was somewhat unexpected but has highlighted the value of systematic literature review and evidence gathering under a Health Technology Assessment before introduction of a new technology or system into health care in the UK.

One disappointing aspect of the present assessment exercise was the poor response to the questionnaire sent to directors of UK newborn screening laboratories and the poor quality of the information provided in many of the replies that were received. Most of the information received was essentially unusable, particularly for any economic analysis of the current screening programmes. Perhaps this should have been expected. Many questionnaires have been directed to newborn screening laboratories during recent years, often for purely academic reasons, and a degree of saturation may have occurred.<sup>170</sup> A poor response might also have arisen because the questionnaire was considered to be too complex or asked for unattainable information. However, this seems unlikely since the present consortium and the SCHARR team, with which the questionnaire was jointly conducted, included four current directors of UK newborn screening laboratories. Despite this, even one of those directors' laboratories failed to return a completed questionnaire. Postal questionnaires perceived by the recipients to encroach on, be irrelevant to, interfere with or perhaps to threaten

established practice are other reasons for lack of response.<sup>170</sup> Indeed, two UK laboratory directors declined to return the questionnaire on the grounds that the Health Technology Assessment exercise was not seen to be of any potential benefit to their own local screening service. This view and the poor response is perhaps a disappointing reflection on the lack of perception by some laboratory directors of the need for review and evaluation, not only of new technologies and potential changes in organisation and practice, but also of the current screening programmes themselves.

All newborn screening programmes should be evaluated on a regular basis by a body independent of the laboratories that are performing the testing. Such evaluation should assess not only technical matters but also issues such as whether early detection of affected children actually improves their long-term prognosis and the effect of false-positive test results on children and their families. No new tests should be incorporated into newborn screening programmes without a similar evaluation.<sup>2</sup> This need for regular review, and other aspects revealed by the present assessment, for example, the great variation in size of catchment populations and laboratory workloads and of methodologies used, serve to highlight the requirement for a coordinated national policy and structure for newborn screening in the UK or at least in England and Wales.

The local expansion of neonatal screening in the UK to disorders other than PKU and congenital hypothyroidism without review of effectiveness and cost-effectiveness has been questioned.<sup>3</sup> Of equal importance is the inadvisability of expanding such programmes without consideration of the infrastructure necessary to deal with the infants identified by newborn screening. Review of PKU screening indicated variations in outcome that could be due to inadequate structures for notification and long-term follow-up of affected cases. In many areas of the UK, there is relatively poor liaison between the screening laboratories, midwives and other healthcare personnel and although positive results are notified to general practitioners, no provision is made for coordinated follow-up or management of these cases.<sup>23</sup> There is clearly a need for a better infrastructure for the notification (preferably to a regional consultant in paediatric metabolism) and continued care and management of patients with an inborn error of metabolism identified through newborn screening. This infrastructure (clinical consultants and scientists in paediatric metabolism with the necessary associated

metabolic investigation laboratories, paediatric dietitians and clinical genetic services) should be in place **before** any expansion of newborn screening programmes is implemented. There are possibly only four UK centres with all these facilities (see chapter 4, page 47) and while elsewhere laboratory, scientific and probably paediatric dietetic facilities may be good, the essential clinical specialists in paediatric metabolism are generally lacking.

There is a suggestion from evidence obtained in this review that for both PKU and CAH screening, the collection of newborn blood spots in the UK at slightly earlier times (i.e. at 5–6 days of age rather than at the current 6–11 days) might facilitate earlier and clinically more effective therapy. However, earlier collection and reporting of results would underline the need for a better infrastructure for clinical follow-up and management of identified cases.

Few areas in the UK offer adequate information to parents and few require informed parental consent to newborn screening, even for PKU and congenital hypothyroidism. However, parents are entitled to be informed and to give consent, before samples are taken, for screening for each of the disorders that will be included.<sup>2</sup> This is particularly pertinent when there is pressure (and current practice) to use the dried blood spots for expanded screening programmes or for purposes for which the blood spots have not been originally obtained. Expansion of screening programmes simply for the intrinsic value of genetic information itself is highly questionable, even if coupled with genetic counselling: if unrequested, genetic counselling itself may be seen as an intrusion by the families concerned, and may provide information that is unwanted and could lead to considerable distress in the future. There is a need for a coordinated national policy for newborn screening in the UK, for any expansion in the generally accepted national practice. Adequate and informed consent must be obtained if such screening is introduced.

The review of the literature and technologies for newborn screening have suggested minimum sizes for efficient operation of a newborn screening laboratory. Some years ago, Naylor<sup>171</sup> suggested that the minimum workload of any screening laboratory should be 25,000 samples per year with a maximum of around 300,000 samples per year. The present assessment of new technologies such as automated immunoassays and tandem MS indicates optimal workloads of at least 80,000 newborn infants screened each year. Nationwide introduction of

these new technologies, coupled with the review of existing practices noted above, would necessitate considerable rationalisation and reorganisation of neonatal screening in the UK. If the primary research programme outlined in this report were

to be carried out, it would allow plenty of time for reorganisation to be initiated. On the basis of the evidence presented in this report purchasers should be encouraged through a national policy to move towards such rationalisation.







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# Appendix I

## Tandem MS: technical details

Tandem MS coupled with a suitable 'soft' ionisation technique, for example, FAB (liquid secondary ionisation) or electrospray, is very suitable for the rapid assay of selected and specific components in complex mixtures, for example, unseparated biological samples. The use of a 'soft' ionisation technique at the sample introduction stage results in the production of mostly intact but ionised molecular species of solutes present in the complex mixture. The initial separation of the ionised parent molecular species from un-ionised material takes place in the first quadrupole. These parent molecular ions may then be introduced into a second quadrupole operated under radio frequency only (collision cell) where, by interaction with neutral gas molecules, further fragmentation occurs (collision-induced decomposition or CID). The 'daughter ions' produced from the parent molecular species can then be further separated and analysed using the third quadrupole of the instrument. Use of appropriate scanning functions allows detection and measurement of specific daughter ions of particular molecular parent ions, or of all parent ions producing a common daughter fragment, or, by scanning both mass analysers (first and third sectors of the triple quadrupole instrument, MS1 and MS2) over a selected mass range simultaneously and with a constant mass difference (constant neutral loss (CNL) scan), a profile of product ions corresponding to  $(M + H - X)^+$  can be obtained; the mass scale of the final data is then adjusted to show the masses of the molecular ions that correspond to each product ion mass. With the introduction of deuterium-labelled internal standards of the analytes of interest, the analytes may also be quantified. Thus, by use of computer-controlled scan functions and rapid, simple mass switching, a very wide variety of compounds of different chemical classes can be determined simultaneously. It is this analytical approach that forms the basis for the neonatal screening for inborn errors of metabolism using tandem MS.

### Principle of the method

The principle of the method used is similar in all the laboratories developing or evaluating tandem MS for neonatal screening, although

each laboratory has introduced variations to suit individual particular protocols. A sample of the blood spot is punched from the conventional 'Guthrie' card and placed in a vial or a well of a multi-titre plate. Currently two  $\frac{3}{16}$ -in. punches are used, corresponding to approximately 15  $\mu$ l blood. A solution of methanol containing weighed amounts of the deuterated standards of amino acids and acylcarnitines is added (400  $\mu$ l) and the metabolites of interest are extracted from the blood spot into the methanol in an orbital shaker. The supernatant solutions, now containing the extracted metabolites and deuterated standards, are evaporated to dryness at 50 °C under a stream of nitrogen. A solution (50  $\mu$ l) of hydrochloric acid in anhydrous n-butanol is added to each vial or well, mixed and sealed and the metabolites and deuterated standards are converted into the corresponding n-butyl esters by heating at 50 °C for 15 minutes. The butanol solutions are then dried under nitrogen as above and the dry residues are reconstituted in an appropriate solution (35  $\mu$ l) for mass spectrometric analysis. The solutions used vary: for example, methanol-glycerol containing sodium octyl sulphate for FAB ionisation; acetonitrile-water for electrospray ionisation. Samples are prepared in batches (of 60-96) with extraction and derivatisation taking about 2.5 hours per batch without automation.

The complete mass spectrometric analysis for all metabolites including switching of scan function takes about 3 minutes using FAB ionisation and about 1 minute using electrospray ionisation. In all laboratories visited it was envisaged that electrospray ionisation would be used for routine screening because of the advantages of more rapid and sensitive analyses, ease of sample introduction, simplicity of daily instrument maintenance and ability to automate sample introduction. The mass spectrometric analysis and subsequent data analysis and production of results are all under computer control and may thus be fully automated.

### Amino acidopathies

Amino acids are determined by using CNL scans of 102 (loss of elements of butyl formate) by scanning both mass analysers (first and third sectors of the

triple quadrupole instrument, MS1 and MS2) over a mass range appropriate for the molecular masses of butylated amino acids simultaneously with a constant mass difference of 102 Da (i.e. MS1 is scanned over a mass range between  $m/z$  125 and 300 while simultaneously scanning MS2 at a mass range 102 Da lower, from  $m/z$  23 to  $m/z$  198). This results in a profile of product ions corresponding to  $(M + H - 102)^+$ ; the mass scale of the final data is then adjusted to show the masses of the molecular ions that correspond to each product ion mass. Sensitivity for different amino acids varies considerably depending partly on the efficiencies of ionisation and of CID and some signals are composites of more than one amino acid sharing the same molecular mass (e.g. leucine, isoleucine and hydroxyproline, see below) or a common fragment ion (e.g. glutamine and lysine). A CNL scan of 119 may be used for more specific determination of basic amino acids where necessary. Results are obtained as a profile of amino acids, which may be quantified by determining the ratio of ion abundance for any undeuterated (sample) amino acid against the deuterated standard (essentially isotope dilution analysis); for diagnosis of specific disorders, metabolite concentrations may also be compared as ratios where required.<sup>153,172</sup>

The aromatic amino acids have unique molecular masses and for PKU screening phenylalanine is quantified by determination of the abundance of ions at  $m/z$  222 and 227, corresponding to undeuterated (sample) and deuterated phenylalanine respectively and by reference to standard curves; tyrosine is also quantified, by determination of the abundances at  $m/z$  238 and 242, and the phenylalanine:tyrosine ratios are also determined. Thus concentrations of phenylalanine and tyrosine are obtained together with the ratio of these metabolically-associated amino acids. The method is highly specific, and was shown to be sensitive (estimated detection limits based upon signal-to-noise ratios for phenylalanine and tyrosine are 3 and 10  $\mu\text{mol/l}$  respectively, well below normal physiological ranges), accurate and precise.

The amino acid profile produced also permits the simultaneous quantification of a range of other amino acids after introduction of appropriate deuterated internal standards.

MSUD (branched-chain ketoaciduria) may be screened for by determination of the branched-chain amino acids, leucine, isoleucine and valine, in blood spots. Leucine and isoleucine are not determined separately by tandem MS since they

have identical masses: the molecular masses of protonated  $[M + H]^+$  butyl esters of leucine and isoleucine are at  $m/z$  188 and that of valine is at  $m/z$  174. Alloisoleucine and hydroxyproline also have masses at  $m/z$  188 and may cause some interference but blood hydroxyproline concentrations in newborn infants are generally very low and alloisoleucine is also associated with MSUD and thus its presence enhances detection rather than causing adverse interference. Results demonstrated high sensitivity with limits of detection, based upon signal-to-noise ratios, estimated at 2  $\mu\text{mol/l}$  for leucine + isoleucine and 6  $\mu\text{mol/l}$  for valine, well below the normal physiological ranges for these amino acids. Accuracy and precision were also good and the ability of the method to detect known MSUD patients from analysis of neonatal blood spots has been demonstrated.<sup>154</sup>

Screening for homocystinuria (and isolated hypermethioninaemia) is based upon determination of blood spot methionine concentrations. Again, the CNL 102 profile is utilised, with introduction of deuterated methionine as internal standard in the initial methanol extraction solution. The protonated molecular ion of butylated methionine has a unique mass of 206; thus scanning for ions at  $m/z$  206 and 209 (deuterated standard), and use of appropriate calibration curves, allows accurate quantification of methionine in the blood spots. The limit of detection, based upon signal-to-noise ratio, was 4  $\mu\text{mol/l}$ , well below the physiological range for blood methionine (literature 10–90  $\mu\text{mol/l}$ ; determined by this method  $19 \pm 8.3 \mu\text{mol/l}$ ), and accuracy and precision are high. Discrimination of normal values from positives was further improved by comparison of methionine concentrations with those of another quantified amino acid, for example, phenylalanine or isoleucine + leucine. Known cases of homocystinuria and hypermethioninaemia were accurately detected in a series of blood spots in a blinded trial.

## Disorders of organic acid and fatty acid metabolism

Disorders of organic acid metabolism in which acyl CoA esters accumulate are characterised by the accumulation of corresponding and characteristic acylcarnitines<sup>173</sup> (see chapter 3, page 16). Mass spectrometry using FAB ionisation and double focusing instruments with magnetic and electrostatic sectors with linked scan techniques were first used to identify these acylcarnitines in urine from patients with a variety of organic acidurias.<sup>174–179</sup>

The use of CNL scanning and isobutyl esters of acylcarnitines<sup>180</sup> and of tandem MS,<sup>181</sup> was soon applied to similar measurements in blood plasma and thence to dried blood spots with a view to application to neonatal screening for organic acidurias, including the newly identified and

important disorders of fatty acid  $\beta$ -oxidation.<sup>63,182</sup> Acylcarnitines are determined separately using tandem MS by switching to a scan for parent ions of the specific daughter ion at  $m/z$  85. A profile of acylcarnitines is obtained and quantified in a similar manner to that used for the amino acids.



# Appendix 2

## Critical appraisal checklist

**REFERENCE**

Study Name: \_\_\_\_\_

Study ID: \_\_\_\_\_

Paper Refs: \_\_\_\_\_

**DISEASE** (tick as many as apply)

- Disorders of carbohydrate metabolism
- Trace metal disorders
- Lysosomal disorders
- Respiratory chain/TCA cycle disorders
- Purine/pyrimidine disorders
- Phenylketonuria
- Aminoacidopathies
- Organic acidurias

- Disorders of the urea cycle
- Fatty acid oxidation defects (MCAD)
- Fatty acid oxidation defects (other)
- Peroxisomal disorders
- Lipoprotein disorders
- Disorders of adrenal steroidogenesis
- Haemochromatosis
- Other (specify) \_\_\_\_\_

**SCREENING TEST** (tick as many as apply)

**Sample used**

- Heel-prick blood
- Capillary blood
- Plasma
- Serum
- Urine
- Other (specify) \_\_\_\_\_

**Method used**

- Guthrie (bacterial inhibition assay)
- Chromatography
- DELFA<sup>®</sup>
- Mass spectrometry (MS-MS)
- Enzymology
- DNA
- Radioimmunoassay
- Other (specify) \_\_\_\_\_

**SUBJECT** (tick as many as apply)

- Incidence
- Sensitivity and specificity
- Health outcome without screening
- Health outcome with screening
- Costs of the screening programme
- Costs of care and treatment without screening
- Costs of care and treatment with screening
- Outcomes and costs of false-positives and false-negatives
- Intangible costs and benefits (psychological effects)
- Methodology

**REVIEW**

Review ID \_\_\_\_\_

Status                      1. Pending                      2. In review                      3. Awaiting summary                      4. Complete

Reviewer 1 \_\_\_\_\_ Date sent \_\_\_\_\_ Date returned \_\_\_\_\_

Reviewer 2 \_\_\_\_\_ Date sent \_\_\_\_\_ Date returned \_\_\_\_\_

Date of summary meeting \_\_\_\_\_ Date returned \_\_\_\_\_

**STUDY POPULATION****Relevance**

1. Location:
2. a) Was the population: 1. General 2. Targeted (see b) 3. Not stated  
b) If targeted: what was the method of selection?
3. a) Was the sample relevant to the general UK population?  
1. Yes (see b) 2. No 3. Not stated 4. Don't know  
b) If Yes, which sections of the UK population was the sample relevant to?

**Size**

4. a) Was the sample size clearly stated? 1. Yes (see b) 2. No  
b) If Yes, what was the sample size?
5. a) Was the sample size adequate for the purposes of the study?  
1. Yes 2. No 3. Don't know  
b) Was the sample size adequate for relevance to neonatal screening?  
1. Yes 2. No 3. Don't know

**Completeness**

6. Compliance (% of target population screened):
7. What was the extent of missing data?  
1. Minimal 2. Moderate 3. Extensive 4. Don't know

**Comments****SCREENING METHOD****Timing of test**

8. Age (days)
9. a) In your opinion is this timing clinically appropriate? 1. Yes 2. No (see b)  
b) If No, why not?
10. a) Does this timing affect the accuracy of test? 1. Yes (see b) 2. No  
b) If Yes, how?

**Accuracy of test**

11. a) What was the estimated sensitivity of the analytical test?  
b) In your opinion, was this level of sensitivity adequate? 1. Yes 2. No (see c)  
c) If No, why not?
12. a) What was the estimated specificity of the test?  
b) In your opinion, was this level of specificity adequate? 1. Yes 2. No (see c)  
c) If No, why not?

**Comments****METHODOLOGY**

13. What was the basis of the methodology?
14. What was the occurrence of false positives?
15. What was the occurrence of false negatives?
16. In your opinion what was the adequacy of the method for population screening?  
1. Good 2. Fair 3. Poor 4. Don't know
17. How did this method compare to other established methods?  
1. Good 2. Fair 3. Poor 4. Don't know

18. Was the method reproducible? 1. Yes 2. No  
 19. How was the reliability of the method measured?  
 20. Were adequate calibrations performed?  
 21. How was quality control assessed?

**Comments**

**METHOD RESOURCES**

22. List below the resources required for the methodology, putting in the units (year and currency) as appropriate:  
 a) Equipment costs  
 b) Personnel – numbers, grades, full-time equivalents  
 c) Time taken to do assay

**Comments**

**INCIDENCE**

23. What was the incidence rate (number:100,000) ?  
 24. What was the overall applicability of the incidence rate to the general UK population (allowing for population characteristics and study methodology)?  
 1. Good 2. Fair 3. Poor 4. Don't know

**Comments**

**CLINICAL FOLLOW-UP PROCEDURE**

**Short-term follow-up**

25. What short-term procedures were taken to follow up a positive result?

**Length**

26. a) What was the length of clinical follow-up?  
 b) In your opinion, was this length adequate? 1. Yes 2. No (see c)  
 c) If No, why not?

**Frequency**

27. a) How frequent were the follow-up assessments?  
 b) In your opinion, was this frequency adequate? 1. Yes 2. No (see c)  
 c) If No, why not?

**Retention**

28. a) What was the retention rate (% of study population remaining at final follow-up) ?  
 b) In your opinion, was this retention adequate ? 1. Yes 2. No (see c)  
 c) If No, why not?

**Treatment**

29. a) What therapeutic intervention(s) were offered?  
 b) In your opinion, was this therapy clinically appropriate? 1. Yes 2. No (see c)  
 c) If No, why not?

**Comments**

**OUTCOMES**

30. List in the table below outcomes, e.g. consequences of adverse events, evidence of significant effect of disease on foetus during pregnancy, length of life measures, number of cases detected, quality of life measures.

<b>OUTCOME MEASURES (units)</b>	<b>With screening</b>	<b>Without screening</b>
a.		
b.		
c.		
d.		
e.		

31. In your opinion how good is the evidence from this study of outcome gain from screening?  
 1. Good                      2. Fair                      3. Poor                      4. Don't know

**Comments****STUDY COSTS**

32. List below the monetary costs and benefits from the study, putting in the units (year and currency) as appropriate.

<b>COSTS</b>	<b>With screening</b>	<b>Without screening</b>
a. Screening		
b. Diagnosis		
c. On-going care and treatment: Public  Private		
d. Indirect costs (e.g. time off work)		
e. Value of life		



33. a) Was the cost-effectiveness ratio given? 1. Yes (see b) 2. No  
b) If Yes, what was it?
34. a) Was a cost-benefit ratio given? 1. Yes (see b) 2. No  
b) If Yes, what was it?
35. What discount rate was used (%)?
36. In your opinion how good is the evidence from this study of cost-effectiveness of screening?  
1. Good 2. Fair 3. Poor 4. Don't know

**Comments**

**OVERALL COMMENTS**

37. Please give any additional information which you consider to be relevant to the assessment of the study.



# Appendix 3

## Screening questionnaire

CONFIDENTIAL

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### NEONATAL SCREENING LABS QUESTIONNAIRE

This questionnaire is being carried out in complete confidentiality. This sheet will be detached on receipt to ensure confidentiality.

**PLEASE ANSWER ALL THE QUESTIONS IN ALL FOUR SECTIONS A TO D.**

Please return your completed questionnaire to:

Dr. G. M. Addison  
Department of Biochemistry  
Royal Manchester Children's Hospital  
Pendlebury  
Manchester  
M27 1HA

#### **Personal information**

Name \_\_\_\_\_

Your job title \_\_\_\_\_

Your work address \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Telephone Number \_\_\_\_\_

Fax. Number \_\_\_\_\_

**PLEASE DO NOT DETACH THIS PAGE, THIS WILL BE DONE BY THE RESEARCHERS  
AT ST. GEORGE'S & SCHARR**

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## NEONATAL SCREENING LABS QUESTIONNAIRE

### Section A: ACTIVITY

- 1) What diseases are screened for by your laboratory? Please **include** both the primary disorders screened for and those disorders detected as by-products (*e.g. Galactosaemia from PKU screening*).

*(Please tick and add to)*

CODE*	DISORDER	AS PRIMARY PURPOSE OF SCREEN <sup>#</sup>	AS BY-PRODUCT
01	PHENYLKETONURIA		
02	CONGENITAL HYPOTHYROIDISM		
03			
04			
05			
08			
07			

\* This code will be used as a disorder identifier throughout sections B and C.

<sup>#</sup> Please tick the appropriate box.

- 2) How many neonates were screened by your laboratory in the Calendar/Financial year of 1995  
*(delete as appropriate)?*
- 

### Section B: MANPOWER COSTS

- 3) Please list, in table a) the posts of **ALL** staff undertaking work on Neonatal Screening in your laboratory. (*e.g. M.L.S.O. 1, Clerical officer grade 2, etc.*)

Also indicate, in tables b), c) and d), how the time of **EACH** employee is divided between the 'specific' stages of Neonatal Screening process and the different screens performed by your laboratory.

*(Please give the answers in whole time equivalents (WTE – e.g. PKU 0.5; CHT 0.5) **including** the proportion of staff from other sections for holiday and sick cover but **excluding** time for the HIV surveillance scheme.)*

Table a) A list of all employees undertaking work on neonatal screening in your laboratory

POSTS (type & grade)	WTE

Table b) Specimen Receipt &amp; Result Reporting\*\*

POSTS (type & grade)	WTE

\*\* Do NOT include time spent performing detailed checking against birth lists or similar records.

Table c) Testing and Analysis

POSTS (type & grade)	DIVISION OF TIME (WTE <sup>+</sup> )							TOTAL WTE
	DISORDER CODE <sup>##</sup>							
	01	02	03	04	05	06	07	

<sup>+</sup> Please give your answer in whole time equivalents – *e.g. if an employee works 0.5 WTE and 50% of his time is spent on 'testing & analysing disorder 01' then WTE = 0.25.*

<sup>##</sup> The disorder codes are as defined in question 1).

Table d) Confirmation, Referral, Advise for positive cases\*\*\*

POSTS (type & grade)	DIVISION OF TIME (WTE <sup>+</sup> )							TOTAL WTE
	DISORDER CODE <sup>##</sup>							
	01	02	03	04	05	06	07	

\*\*\* Please ignore long term follow-up, teaching and training time

<sup>+</sup> Please give your answer in whole time equivalents – *e.g. if an employee works 0.5 WTE and 50% of his time is spent on 'confirmation, referral, advise for positive cases of disorder 01' then WTE = 0.25.*

<sup>##</sup> The disorder codes are as defined in question 1).

- 4) Which additional service components does your laboratory provide?  
(e.g. liaison nurse, attendants at metabolic clinics, etc.)

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- 5) What is the floor area of your laboratory devoted to each of the primary screens identified in question 1), **including** office and storage areas but **excluding** Guthrie card storage space? Where areas are shared with other procedures, please proportion the floor area accordingly?  
(Please give the answer to the nearest m<sup>2</sup>)

DISORDER CODES		FLOOR AREA (m <sup>2</sup> )
N/A	SPECIMEN RECEPTION (for all disorders collectively)	
01	PHENYLKETONURIA	
02	CONGENITAL HYPOTHYROIDISM	
03		
04		
05		
06		
07		

### Section C

The questions in this section are presented as a form and relate to the primary disorders screened for by your laboratory as listed in question 1. Separate forms are attached for Phenylketonuria and Congenital Hypothyroidism. For each additional primary disorder screened for by your laboratory, please complete one of the **Disorder Unspecified** forms.

When completing the enclosed forms **please** include the costs of the primary screen **plus** 'by-product' disorders (e.g. Galactosaemia from PKU screen).

DISORDER CODE: **01: PHENYLKETONURIA (& by-products)**

- i) What technique do you use as your first line screen for phenylketonuria?  
(Please tick or add to)

TECHNIQUE	please tick
Chromatography	
Fluorometry	

TECHNIQUE	please tick
Guthrie	

ii) Please list below the capital equipment required for screening and initial confirmation of Phenylketonuria (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to Phenylketonuria.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation of Phenylketonuria (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) Any further comments

**DISORDER CODE: 02: CONGENITAL HYPOTHYROIDISM**

i) a) What method do you use to screen for Congenital Hypothyroidism?

\_\_\_\_\_

b) What manufacturer and kit do you use?

Manufacturer: \_\_\_\_\_

Kit: \_\_\_\_\_

ii) Please list below the capital equipment required for screening and initial confirmation of Congenital Hypothyroidism (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to Congenital Hypothyroidism.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation of Congenital Hypothyroidism (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) Any further comments

DISORDER UNSPECIFIED: **DISORDER CODE: 03**

i) What technique do you use as your first line screen for this disease?

\_\_\_\_\_

ii) Please list below the capital equipment required for screening and initial confirmation of this disease (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to this specific disease.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation for this specific disease (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery.)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) What is the incidence for this disease in the population screened for by your laboratory (per 100,000 births)? (*Please state on how many years data this incidence is calculated*)

\_\_\_\_\_ per 100,000 births      \_\_\_\_\_ years

vi) Any further comments



DISORDER UNSPECIFIED: **DISORDER CODE: 04**

i) What technique do you use as your first line screen for this disease?

\_\_\_\_\_

ii) Please list below the capital equipment required for screening and initial confirmation of this disease (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to this specific disease.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation for this specific disease (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery.)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) What is the incidence for this disease in the population screened for by your laboratory (per 100,000 births)? (*Please state on how many years data this incidence is calculated*)

\_\_\_\_\_ per 100,000 births                      \_\_\_\_\_ years

vi) Any further comments

DISORDER UNSPECIFIED: **DISORDER CODE: 05**

i) What technique do you use as your first line screen for this disease?

\_\_\_\_\_

ii) Please list below the capital equipment required for screening and initial confirmation of this disease (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to this specific disease.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation for this specific disease (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery.)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) What is the incidence for this disease in the population screened for by your laboratory (per 100,000 births)? (*Please state on how many years data this incidence is calculated*)

\_\_\_\_\_ per 100,000 births                      \_\_\_\_\_ years

vi) Any further comments

DISORDER UNSPECIFIED: **DISORDER CODE: 06**

i) What technique do you use as your first line screen for this disease?

\_\_\_\_\_

ii) Please list below the capital equipment required for screening and initial confirmation of this disease (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to this specific disease.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation for this specific disease (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery.)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) What is the incidence for this disease in the population screened for by your laboratory (per 100,000 births)? (*Please state on how many years data this incidence is calculated*)

\_\_\_\_\_ per 100,000 births                      \_\_\_\_\_ years

vi) Any further comments

DISORDER UNSPECIFIED: **DISORDER CODE: 07**

i) What technique do you use as your first line screen for this disease?

\_\_\_\_\_

ii) Please list below the capital equipment required for screening and initial confirmation of this disease (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to this specific disease.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation for this specific disease (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery.)?

\_\_\_\_\_

iv) How many repeat blood samples per year are required due to abnormal results?

\_\_\_\_\_

v) What is the incidence for this disease in the population screened for by your laboratory (per 100,000 births)? (*Please state on how many years data this incidence is calculated*)

\_\_\_\_\_ per 100,000 births                      \_\_\_\_\_ years

vi) Any further comments

**Section D: RESEARCH AND DEVELOPMENT**

6) Please give details of any pilot neonatal screening studies carried out in your region over the last ten years, **including** both published and unpublished data such as local reports.

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7) a) Have you any plans to extend the neonatal screening programme in your region during the next five years?

YES/NO (*delete as appropriate*)

b) If yes,

i) What disorders (**NOT** just inborn errors of metabolism) do you plan to screen for?

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ii) How easily would this expansion fit in with your existing programme?

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iii) What tests are proposed?

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iv) What are the resource implications? (*e.g. equipment, staff*)

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8) Any further comments



## Acute Sector Panel

Chair: Professor John Farndon, University of Bristol †

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