Expanded Newborn Screening Study

July 2012 to July 2013

Report to National Screening Committee

21st November 2013

Contents

Executive Summary

i. Newborn screening for five conditions was conducted in six centres: Leeds Teaching Hospitals NHS Trust, Central Manchester University Hospitals NHS Foundation Trust, Sheffield Children's NHS Foundation Trust, Birmingham Children's Hospital NHS Foundation Trust, Great Ormond Street Hospital NHS Foundation Trust and Guys and St Thomas' NHS Foundation Trust from 16 July 2012 to 19 July 2013. The conditions included were maple syrup urine disease (MSUD), glutaric aciduria type 1 (GA1), isovaleric acidaemia (IVA), long chain hyroxyacyl CoA dehydrogenase deficiency/MTP (LCHADD/MTP) and homocystinuria (pyridoxine unresponsive) (HCU).

ii. 437,187 samples were analysed and 30 screen positive cases identified. The findings (see Table 1) were broadly in line with the original expectations based upon a survey of the international literature[1]:

Table 1 – Expected and observed prevalence of the five conditions

Condition	Prevalence		True positives		False positives		PPV%	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
MSUD	1:116,000	1:437,000	4	1	4	1	50	50
HCU	1:144,000	1:219,000	3	2	5	1	38	67
GA1	1:109,191	1:110,000	4	4	6	0	40	100
IVA	1:155,396	1:110,000	3	4	7	14	30	18
LCHADD/MTP	1:218,564	1:437,000	2	1	3	2	40	33
Total	**1:28,000**	**1:36,500**	**16**	**12**	**25**	**18**	**39**	**40**

iii. It is likely that the GA1 (n=4), MSUD (n=1) and HCU (n=2) patients identified during the study gained significant benefit. The LCHADD/MTP patient had been recognised clinically and the positive screening result did not offer additional benefit. The patients diagnosed with IVA were mostly (3 of 4) mild from a biochemical perspective, and the remaining patient sadly died.

iv. Patients identified clinically during the study period were monitored and no false negative cases were reported.

v. Two diagnoses were made at autopsy in children who died prior to screening (both LCHADD/MTP). Three cases (two cases of IVA and one case of LCHADD/MTP) were identified within the project's geographical area by testing siblings of known cases prior to screening. These have not been included in the results shown above.

vi. The mean age at referral in screen positive cases was 11 days.

vii. Confirmatory or diagnostic testing was conducted in line with the diagnostic protocols and average response time was 4 days from referral (range 0 – 14).

viii. External Quality Assurance (EQA) of the assays used in screening was arranged and the assays performed satisfactorily during the study period as assessed by EQA returns and population monitoring.

ix. The clinical staff, metabolic physicians and dietitians managed the activity within existing resources and did not report significant practical difficulties.

x. The study did not appear to cause disquiet among the families involved nor health professionals including midwives and other involved in the logistics so far as can be gauged. Babies whose parents declined testing on their behalf were <0.05% of those offered testing.

xi. The website www.expandedscreening.org was an integral part of the study organisation and provided a means of offering information to the public and health professionals, and a route by which queries could be received. There were approximately 6000 unique visitors to the site during the study, and 43 queries from the public and health professionals were received.

xii. The health economics report prepared by ScHARR indicates that newborn screening for each of the five disorders included in the study was cost saving.

1. Background to the study

a. The role of CLAHRC

In 2008 the NIHR funded nine Collaborations for Leadership in Applied Health Research and Care (CLAHRCs) in England. They brought together health service providers, universities, commissioners and the private sector to attempt to evaluate, and where necessary overcome the block described in the Cooksey report[2] as the second gap in translation; the operational implementation of proven research findings into widespread practice. In this context, technological developments in the 1990s had resulted in the development of "expanded" newborn screening programmes in many countries and, at least in the view of those who operated these programmes, had demonstrated their effectiveness. The Genetics theme within the CLAHRC for South Yorkshire provided the opportunity to evaluate screening for at least some of these disorders in England.

b. The international picture in relation to screening for inherited metabolic disorders at the study design stage

While many countries included additional conditions, there appeared to be widespread agreement that these five disorders (see Table 2) should be included as part of the newborn screening programme. Table 2 - Five conditions and countries that offer newborn screening for these conditions

Country	MSUD	Hcys	GA1	IVA	LCHADD/MTP
USA	√	√	√	√	√
Australia	√	√	√	√	√
New Zealand	√	√	√	√	√
Denmark*	√	X	√	X	√
Canada	√	√	√	√	√
Portugal	√	√	√	√	√
Germany	√	X	√	√	√
Austria	√	√	√	√	√
Netherlands	√	√	√	√	√
Belgium	√	X	√	√	√
Poland	√	X	√	√	√
Spain (Galicia)	√	X	√	√	√
Qatar	√	√	√	√	√
Chile	√	√	√	√	√
Costa Rica	√	X	√	√	√

** Denmark considered that the case for IVA was strong but declined screening because of the use of pivaloyl antibiotics in that country.*

c. The proposed pilot disorders

After careful consideration of the international experience and discussion within the UK among health professionals involved in the care of patients with inherited metabolic disorders it was concluded that the case for universal screening for these conditions seemed strong but that some additional information concerning the practical logistics of screening in a UK context needed to be provided by the conduct of a limited pilot study.

d. The aims and status of the study

A proposed study outline was prepared and submitted to Dr Anne Mackie, Director of the UK National Screening Committee (NSC) on 7 April 2010 and subsequently discussed at the NSC and at the Fetal, Maternal and Child Health (FMCH) sub-committee meetings. While the funding and responsibility for the study would remain within the remit of CLAHRC SY it was recognised that active collaboration of the whole screening programme would be needed if a large study were to be undertaken. Following discussion at the NSC, Dr Mackie recommended a number of pre-requisites in a letter of 22 November 2011, and these were addressed in a response on 30 December 2011. On 10 January 2012, Dr Mackie agreed that the NSC would endorse the conduct of the proposed evaluation subject to a jointly appointed project manager and the establishment of a formal oversight committee.

In line with the original aims outlined in April 2010, it was agreed that the study would:

- Help determine disease incidence more closely in the screened areas
- Determine the appropriate cut-offs and screening algorithms to achieve acceptable specificity
- Define algorithms for confirmatory testing suitable for practical use
- Define the analytical performance of the assays in a multi-centre trial monitored using recognised EQA performance criteria
- Offer guidance about the practical issues of parental information and consent in this context

- Assess the logistic problems with positive case referral and confirmatory testing
- Gather data to be used subsequently in outcome studies
- Help define the costs of screening and undertake cost effectiveness assessment

e. The centres to be involved, timescales, expected number of births

It was agreed that the study would take place in the areas covered by six screening laboratories at Leeds, Manchester, Sheffield, Birmingham, Great Ormond Street Hospital and Guy's and St Thomas. These centres had formed the study group for the implementation of newborn screening for medium chain acylCoA dehydrogenase deficiency (MCADD) in 2004 to 2006, and had ready access to clinical metabolic referral and advice. It was agreed that the conditions to be included would be: maple syrup urine disease (MSUD), homocystinuria (pyridoxine unresponsive) (HCU), glutaric aciduria type 1 (GA1), isovaleric acidaemia (IVA), long chain hydroxyl acyl CoA dehydrogenase deficiency (LCHADD) and it was forecast that a one year study (16 July 2012 – 19 July 2013) in these centres would provide information on approximately 430,000 births. It was accepted that the duration of the study would preclude meaningful assessment of patient outcomes except by the most crude means such as deaths avoided.

From a careful analysis of relevant international experience it was forecast that the following number cases might be identified (Table 3):

Table 3 - Predicted prevalence of the five conditions.

Condition	Prevalence	Screen positives	True positives	False positives	PPV%
MSUD	1:116,000	8	4	4	50
HCU	1:144,000	8	3	5	38
GA1	1:109.191	10	4	6	40
IVA	1:155,396	10	3	7	30
LCHADD/MTP	1:218,564	5	2	3	40
TOTAL	**1:28,000**	**41**	**16**	**25**	**39**

f. Organisation and governance arrangements

The pilot was conducted in six screening centres in England: Guys and St Thomas', Great Ormond Street Hospital (GOSH), Birmingham, Leeds, Manchester and Sheffield.

The project initiation Board included:

- Dr J Bonham, Sheffield (Clinical scientist)
- Dr M Sharrard, Sheffield (Metabolic physician)
- Mr J Sowter, Sheffield (Project manager)
- Dr A Chakrapani, Birmingham (Metabolic physician)
- Dr A Morris, Manchester (Metabolic physician)
- Ms Melissa Hewitt, Genetics Interest Group
- Mr Steve Hannigan, CLIMB patients group
- Ms F White, Manchester (Metabolic dietitian)
- Dr M Henderson, Leeds (Clinical scientist)
- Ms F White, Manchester (Metabolic dietitian)
- Ms M Dixon, London (Metabolic dietitian)
- Dr M Champion, London (Metabolic physician)
- Dr S Grünewald, London (Metabolic physician)
- Professor Simon Heales, London (Clinical scientist)
- Dr F Carragher, London, (Clinical scientist)
- Professor Georg Hoffmann, Heidelberg (Metabolic physician)
- Dr H Burton, Cambridge (Public health)
- Dr S Dixon, Sheffield (Health economist)

The project was run as part of the Genetics theme within the CLAHRC for South Yorkshire and was hosted by Sheffield Children's NHS FT. The theme lead was Dr J Bonham, the project manager was Dr C Gibson followed by Dr K Jeays-Ward, and the project co-ordinator was J Sowter.

The practical activity was co-ordinated by a combined project group comprising clinicians, laboratory scientists, dietitians, members of the regional teams and health economists. This project group met quarterly.

Three work groups were organised into:

i. Laboratory screening workgroup – Led by Dr Bonham (Sheffield)
ii. Clinical working group – Led by Dr Andrew Morris (Manchester)
iii. Dietetic working group – led by Marjorie Dixon (GOSH) and Fiona White (Manchester)

In order to ensure that the output from the project addressed the key issues of interest to the NSC, an Advisory & Monitoring Committee comprising those listed below received reports on the project progress:

Dr Anne Mackie	National Screening Lead
Prof James Leonard	Chair, Diagnostic review panel
Dr David Elliman	Co-Director UKNSPC
Dr Stuart Moat	Lab Director, Cardiff
Ali Cryer	Regional Team for East Midlands
Pam Tarn	Regional Team for Yorkshire
Prof Sue Mawson	Lead CLAHRC SY
Jim Chilcott	Health Economist ScHARR
Steve Hannigan	Patient representative CLIMB
Tim McAree	Midwifery team manager, Nottingham
Dr Jim Bonham	Project Lead
Dr Clare Gibson/ Dr Katherine Jeays-Ward	Project Manager
Jason Sowter	Project co-ordinator

Screen positive and true positive cases were be reviewed by a Diagnostic Review panel chaired by Professor James Leonard.

The follow-up of screen positive cases was undertaken as a research activity with Research Ethics Committee (REC) consent. This will also supply information for the cost effectiveness study. The Chief Investigator is Dr J Bonham. The Principal Investigators are the paediatric metabolic consultants based at each of the sites. The economic analysis was undertaken by Jim Chilcott and Professor Simon Dixon at the School of Health and Related Research (ScHARR), University of Sheffield.

2. The preparatory activities

a. Determining the information to be given to parents

A study was undertaken to explore perceptions and attitudes of parents and future parents to an expanded newborn screening programme in the UK, and the necessary information provision and consent processes[3]. The study was conducted by psychologists employed at the University of Coventry employing a mixed methods study involving focus groups (n=29) and a web-survey (n=142) undertaken with parents and future parents.

It concluded that parents wanted guaranteed information provision with clear decision making powers and an awareness of the choices available to them. The difference between routine screening provision and expanded screening was not considered to be significant enough by participants to warrant formal written, informed consent for expanded screening. It is argued that the ethical review processes needs to be more flexible towards the provision of information.

Participants agreed that they would initially like to receive basic information on screening, with access to more detailed resources. The importance of being able to individually select the additional information that was meaningful to them was emphasised. A more detailed and lengthy leaflet was not required, instead brief paper-based reading material with the option to consult more detailed written or web-based information would be welcomed. This approach is analogous to the approach used in the Netherlands. Those participating indicated that processes needed to be more flexible towards the provision of information and consent for service developments within this context. The agreed leaflet is attached as Appendix A.

b. A detailed literature search

This was designed to update the 2004 Health Technology Assessment (HTA) (which drew upon references up to 2002) in relation to the five candidate disorders. This work was commissioned from the Public Health Genetics (PHG)

Foundation and was conducted by Dr Hilary Burton and Dr Sowmiya Moorthie[1]. Articles were eligible for inclusion if they were published after January 2002 and reported on the target population of interest (neonates or newborns), screened for any one of the five conditions of interest (MSUD, HCU, GA1, IVA and LCHADD), and involved primary screening by tandem mass spectrometry (MS/MS) in the neonatal or newborn period or secondary screening by other methods such as the Guthrie (neonatal heel prick) test, thin layer chromatography (TLC) et cetera. Study outcomes such as incidence and/or birth prevalence, natural history, prognosis, analytical and clinical validity of MS/MS screening (including sensitivity, specificity, predictive values and receiver operating characteristic or ROC curves), effectiveness of treatment, effectiveness, and cost-effectiveness of screening were all eligible for inclusion. Studies using the following designs: primary randomised controlled trials, cohort studies, case-control studies, other non-randomised evaluations of treatment effectiveness, and cross-sectional epidemiological study designs were all included. Any study published before 2002, involving non-human participants, or involving fewer than 5 subjects were excluded. An electronic and paper-based, pre-piloted extraction form was used by independent reviewers to extract data. A random sample of 10% of articles was subjected to a second, independent extraction to determine the reliability of the abstraction process and provide a quality control measure. Any disagreements or uncertainties were resolved in conference or by a third reviewer.

The report concluded that:

- A pilot should be undertaken to address gaps in our knowledge relevant to the expansion of newborn screening in the UK[1].

An updated systematic review[4] was produced by the PHG Foundation in which the authors provided updated estimates for birth prevalence of the five metabolic disorders under consideration. The updated estimated prevalence of each disorder in western populations is shown below (Table 4), though the earlier estimation was used throughout the ENBS project.

Table 4 – estimates of prevalence of each disorder in Western populations according to PHG Foundation reviews

Condition	Estimated prevalence in Western population[1] (PHG Foundation, 2010)	Estimated prevalence in Western population[4] (PHG Foundation, 2013)
MSUD	1:116,000	1:140,845
HCU	1:144,000	1:204,081
GA1	1:109,191	1:96,153
IVA	1:155,396	1:123,457
LCHADD	1:218,564	1:149,254
Total	1:28,000	1:27,000

c. A study to examine the likely impact of false positive results

The generation of false positive results is widely perceived as a major dysbenefit in all forms of screening. Before considering a study we wanted to gauge the likely public reaction in this specific context. We used a well-established economic model of assessment. The work, conducted by researchers from ScHARR, used a contingent valuation method – which asks parents to give their maximum willingness to pay for an extension in a screening programme and the degree to which the potential for false positive results diminishes their valuations.

160 parents of a child or children under the age of 16 years were surveyed and given descriptions of the current screening programme in the UK, an extended programme and an extended programme with no false positives. 148 (92.5%) respondents said they would accept the screen for the 5 extra conditions in an expanded screening programme whilst 10 (6.3%) said they would not and two were unsure. When asked to indicate if they would choose to be screened under an expanded screening programme with no false positive results, 152 (95%) said they would, five (3.1%) said they would not, two were unsure, and there was one non-response[5].

The mean willingness to pay for the expanded programme was £178 compared to £219 for the hypothetical expanded programme without false positives ($p>0.05$)[5].

The results suggest that there is widespread parental support for extended screening in the UK and that the number of false-positives is a relatively small issue.

3. The study design and operation

a. Screening protocols

Screening protocols for the five conditions are shown below. The cut off value for C16OH acyl carnitine used in the detection of LCHADD was lowered from 0.20 to 0.15 µmol/L following a “missed case” in Birmingham. By the time this change was made the study had progressed far enough that we were confidents the change would not drastically increase the false positive rate.

Figure 1

Maple Syrup Urine Disease (MSUD) screening algorithm

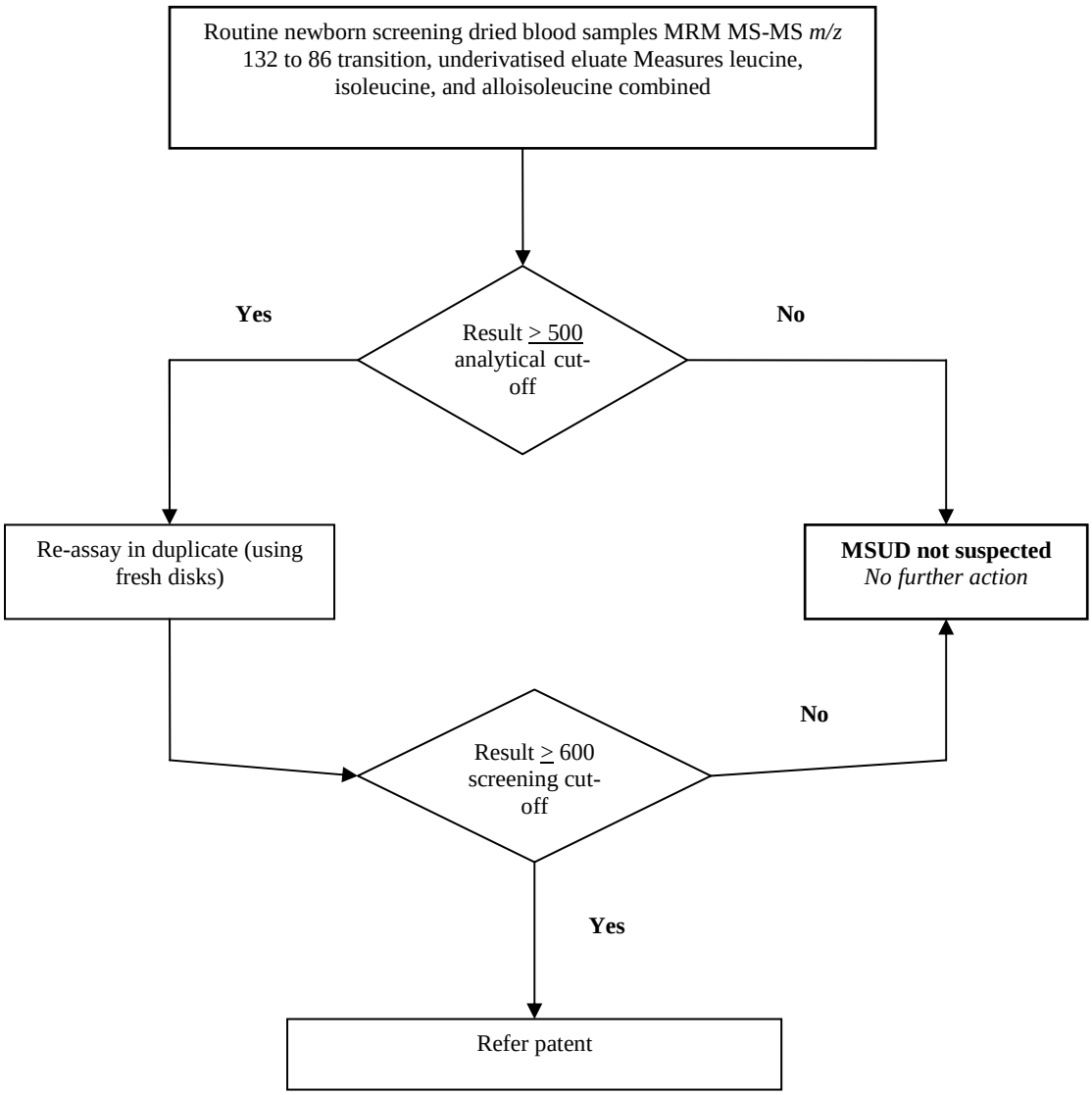


Figure 2

Pyridoxine unresponsive homocystinuria (HCU) screening algorithm

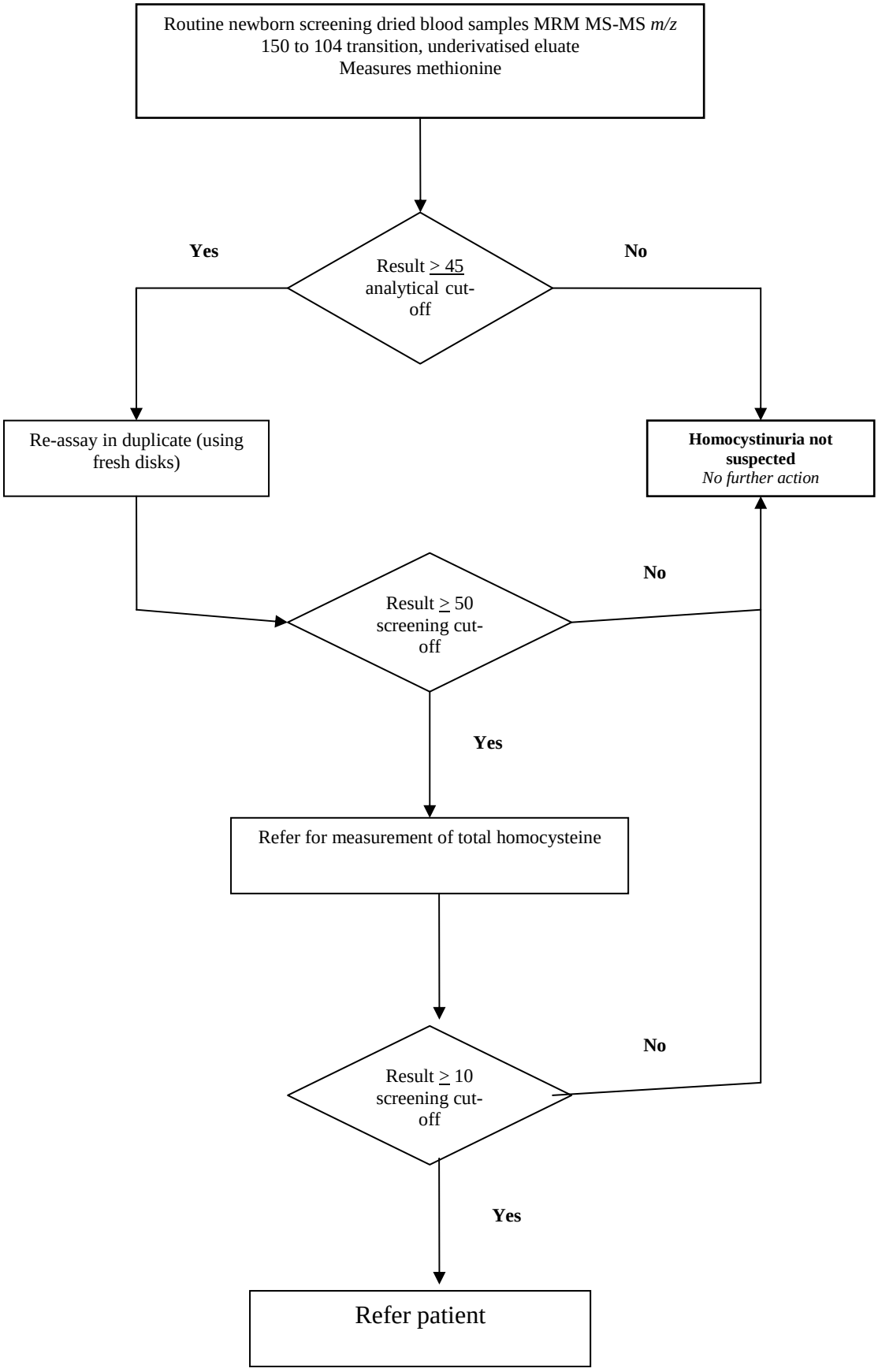


Figure 3

Isovaleric Acidaemia (IVA) screening algorithm

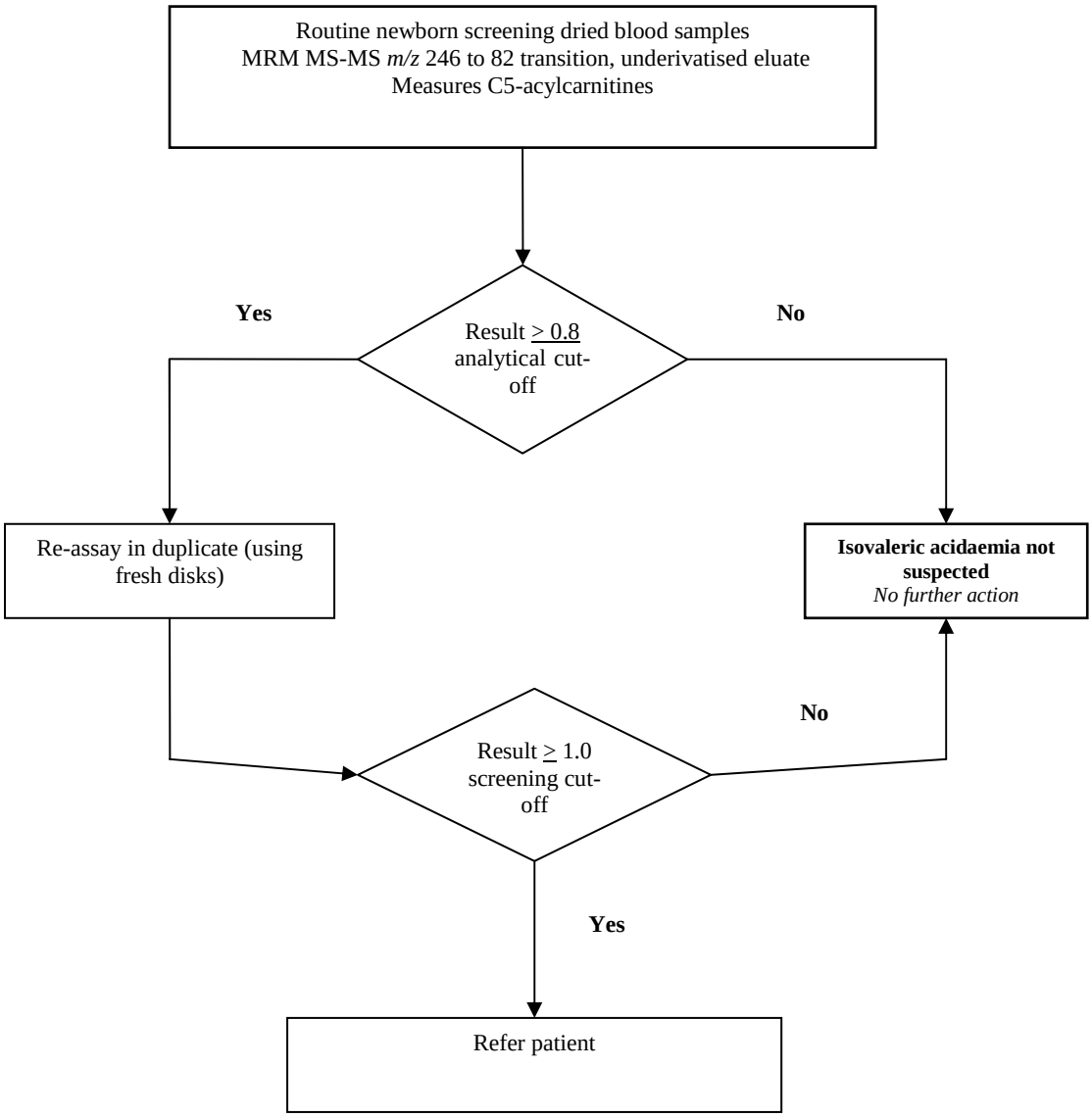


Figure 4

Glutaric aciduria type 1 (GA1) screening algorithm

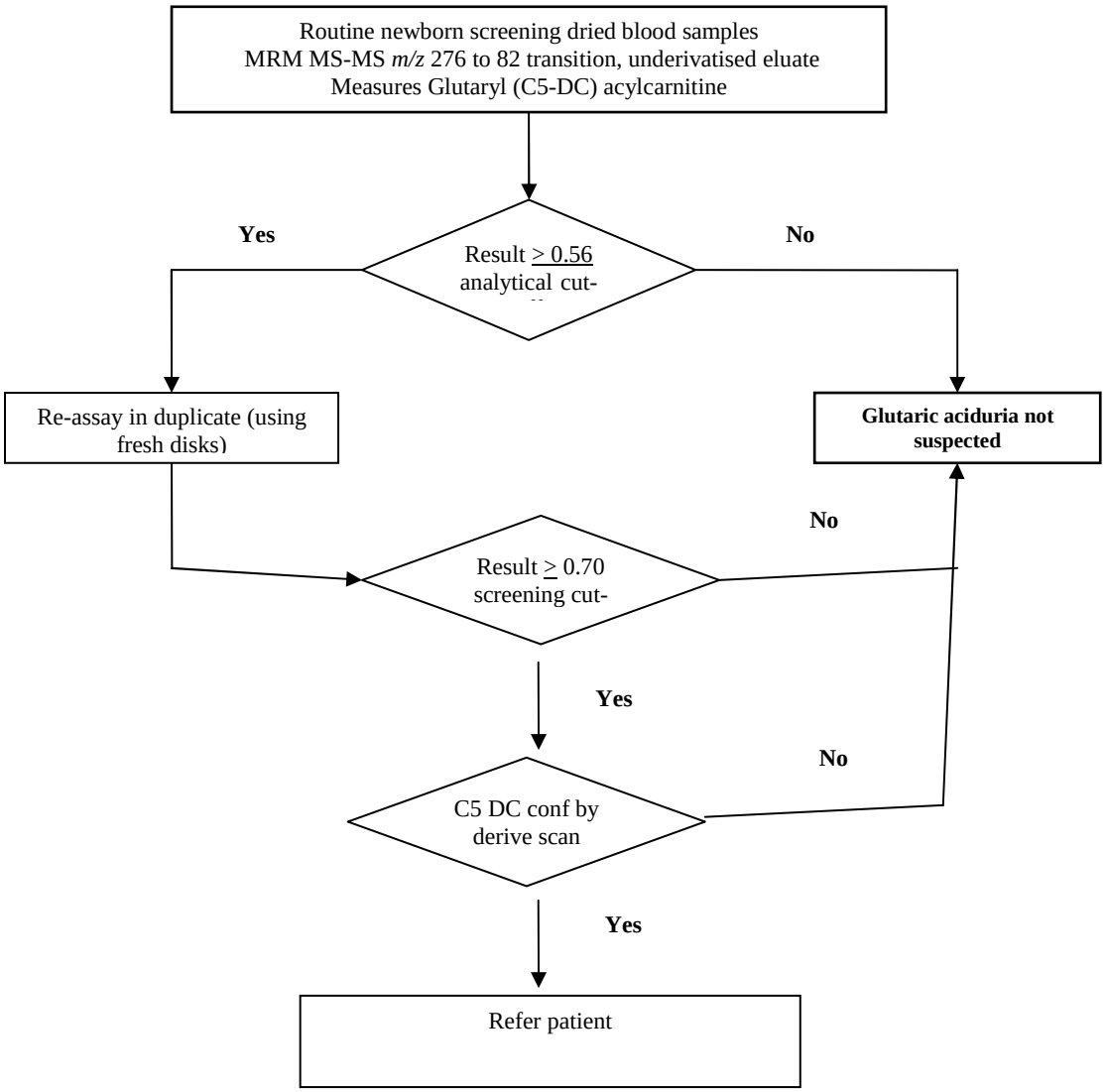
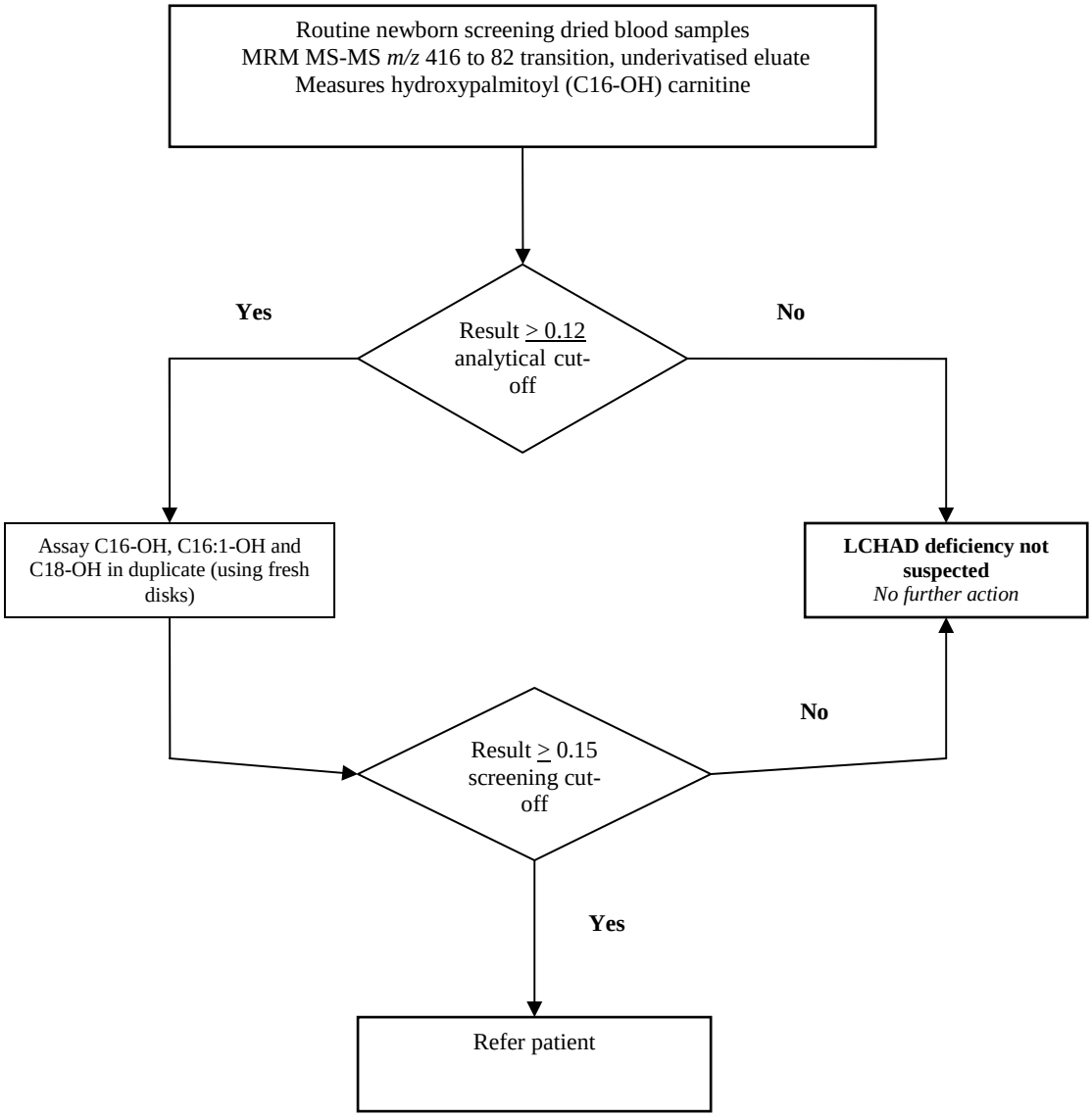


Figure 5

Long Chain 3-Hydroxyacyl-CoA Dehydronenase Deficiency/MTP (LCHADD/MTP) screening algorithm



b. Diagnostic protocols

Figure 6

Maple Syrup Urine Disease (MSUD) diagnostic algorithm

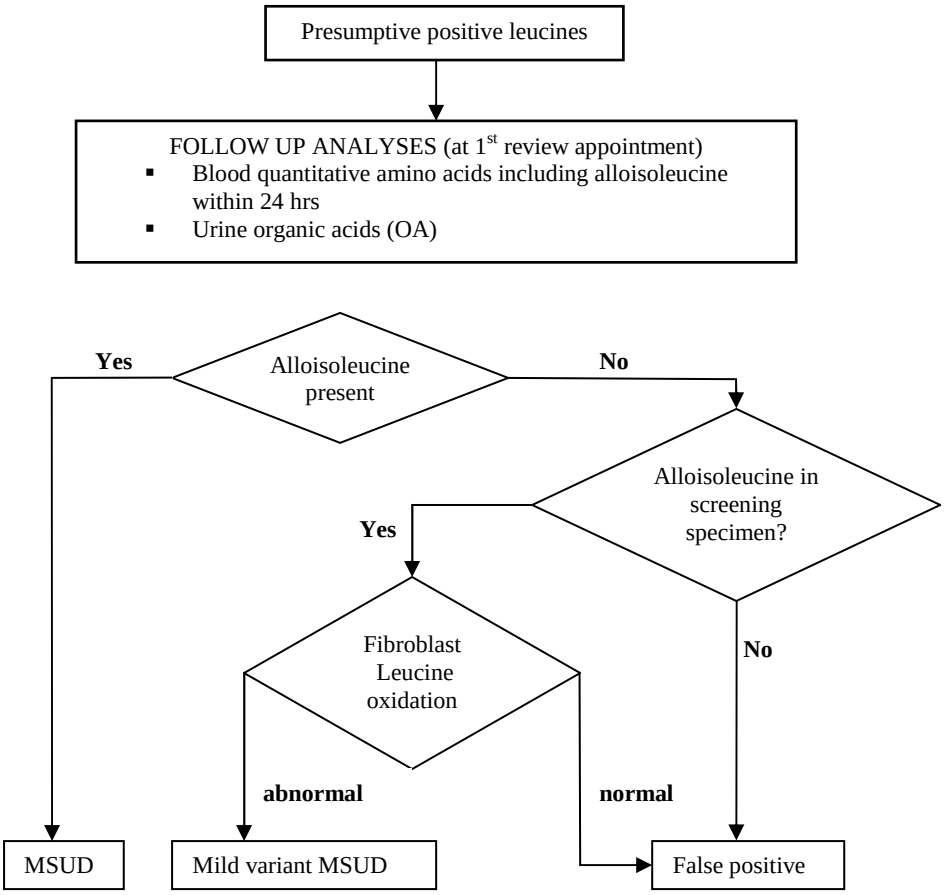


Figure 7

Homocystinuria (non-pyridoxine dependent) (HCU) diagnostic algorithm

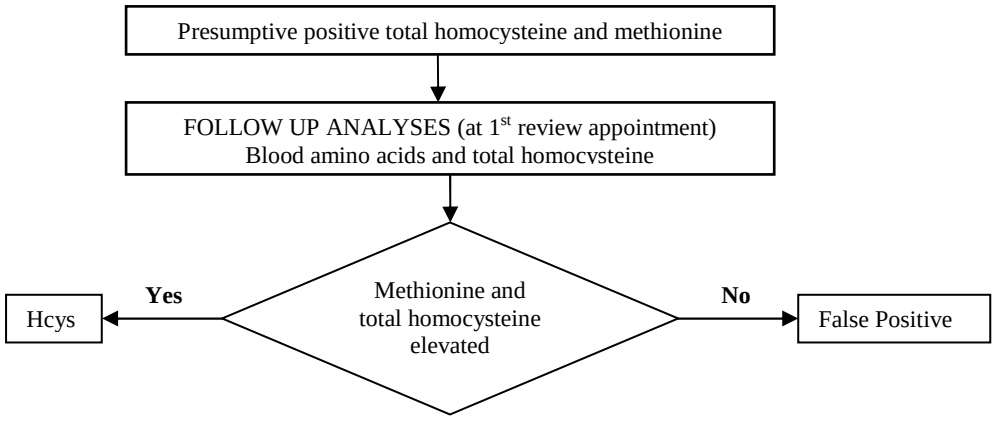


Figure 8

Isovaleric Acidaemia (IVA) diagnostic algorithm

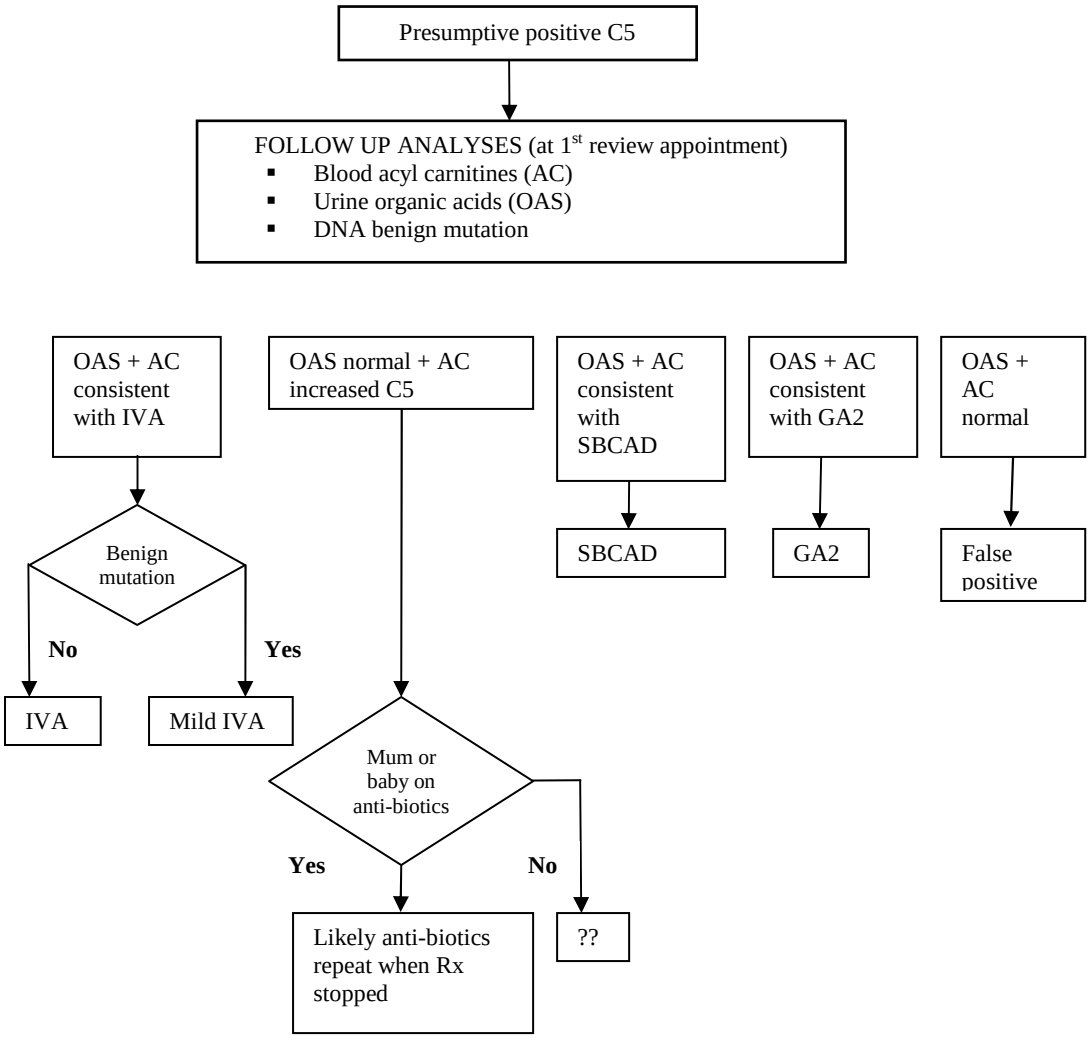


Figure 9

Glutaric aciduria type 1 (GA1) diagnostic algorithm

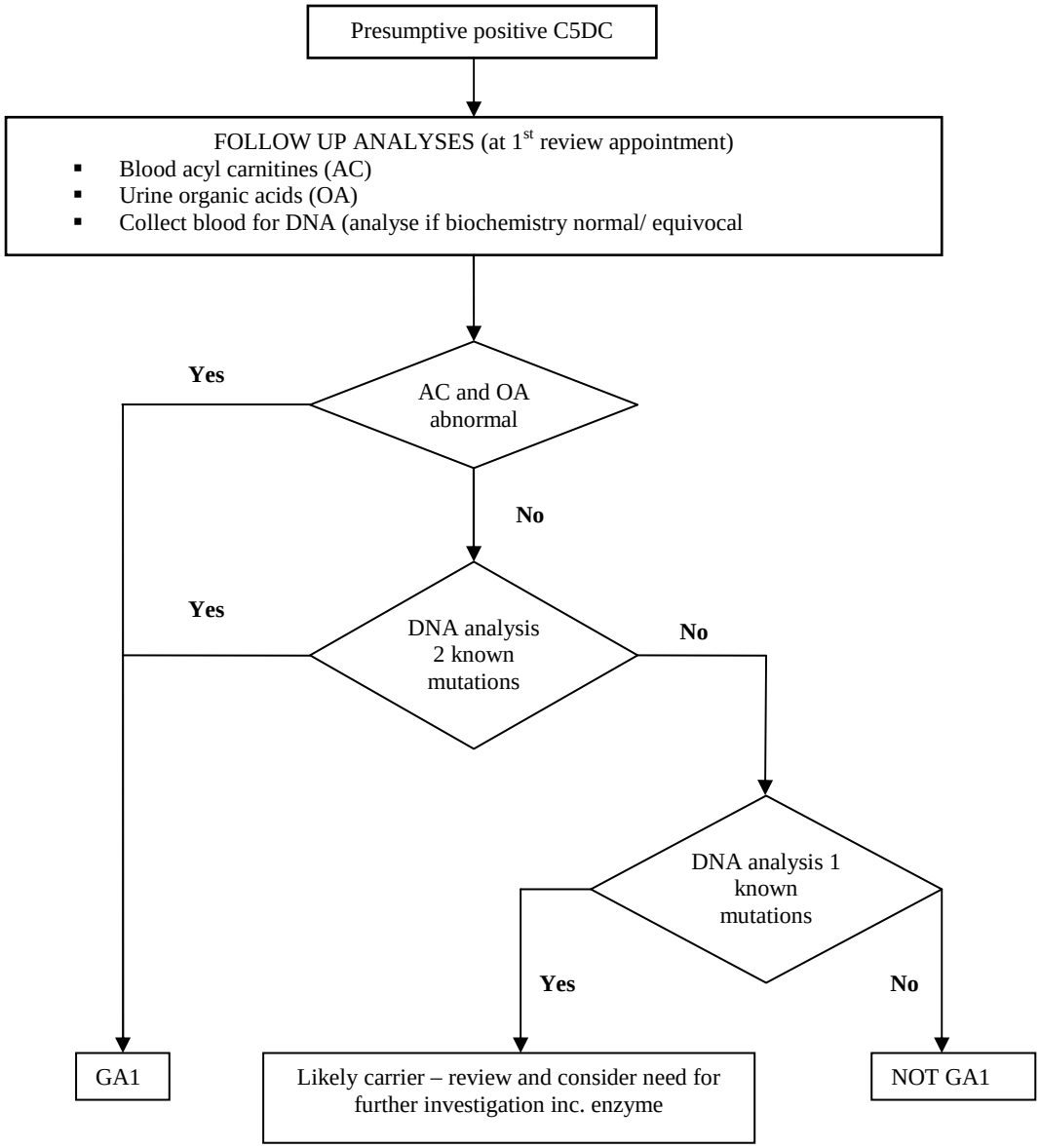
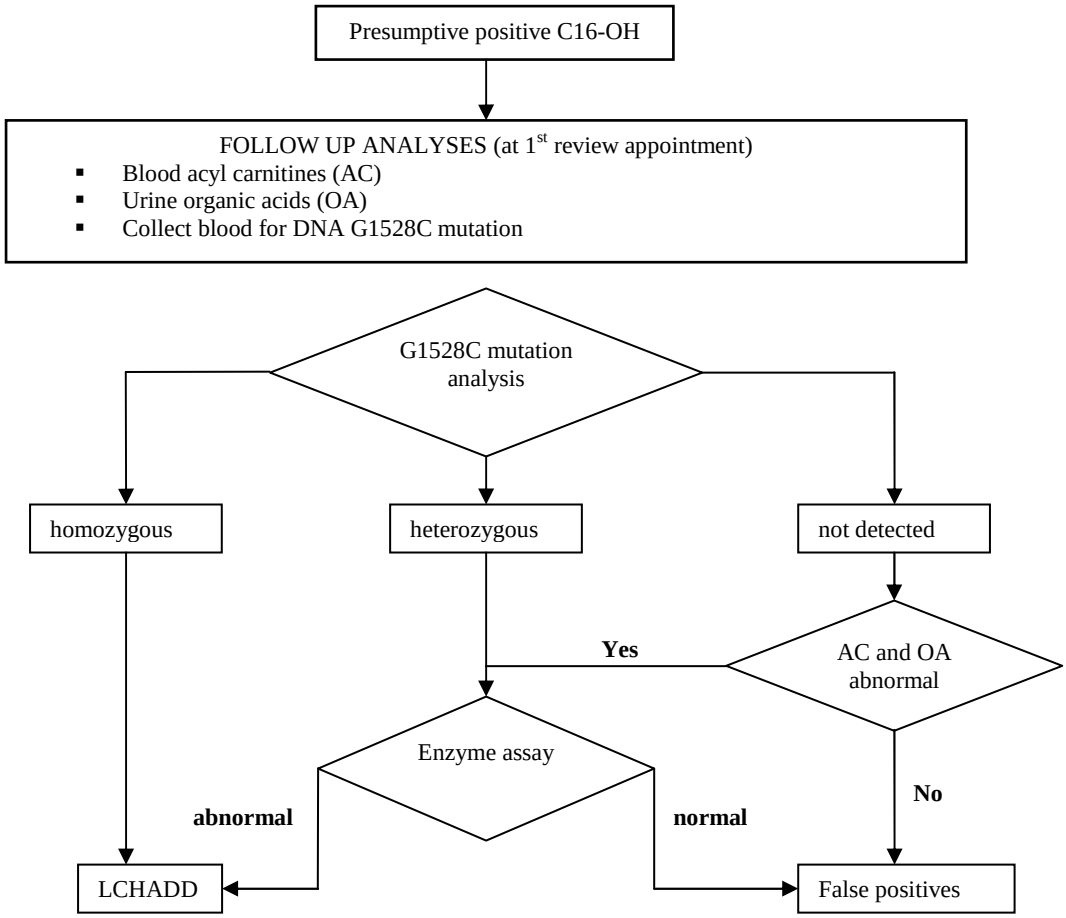


Figure 10

Long-Chain 3-hydroxyl acyl CoA dehydrogenase deficiency/MTP (LCHADD/MTP) diagnostic algorithm



c. Treatment protocols

Detailed clinical and dietetic treatment protocols for each condition were developed and presented at a Steering Group Meeting on 23 April 2012 prior to commencement of the study. These have not been reproduced in detail here. It was recognised that these may need to be modified according to the particular case and local considerations. In an acute situation within a General District Hospital the standard British Inherited Metabolic Disease Group (BIMDG) emergency management guidelines would be followed.

The impact on both the metabolic physician service and the dietetic service was discussed in detail both within the study area and with the national representatives of both groups of staff. They indicated that they could accommodate the workload involved and the study agreed to reimburse the departments involved for the time spent dealing with these cases.

d. The design of information for parents of screen positive and confirmed positive cases

"Condition suspected" and "Condition confirmed" leaflets for parents and accompanying suggested form letters to GPs were prepared and agreed at the monitoring committee meeting in August 2012. These are not all reproduced here but we have included the Homocystinuria (HCU) leaflets and letters as examples.

Results of the Newborn Blood Spot Screening

Homocystinuria (HCU) is Suspected

What is my baby's screening result?

When your baby was about a week old, your midwife took some blood from your baby's heel, the 'heel prick test'. The blood was used to test for some rare conditions, including Homocystinuria (HCU) pronounced as ho-mo-sis-tin-ur-ee-a.

The outcome of the screening test suggests that your baby may have HCU although this result needs to be confirmed through further blood and urine tests.

This leaflet gives some information about HCU and what happens next.

What is Homocystinuria?

Homocystinuria is a rare disorder that prevents the normal breakdown of protein. In order for the body to use protein from the food we eat, it is broken down into smaller parts called amino acids. In Homocystinuria one of these amino acids does not break down in the usual way and a chemical called homocysteine builds up in the blood. Without early treatment this can lead to long term health problems, including learning difficulties. However, these problems can be prevented by following the treatment advised by your Specialist Metabolic Team and your child will grow normally and have a normal life expectancy. The early detection provided by screening offers a very significant advantage to the outcome in the majority of cases.

What treatment is available for HCU?

In a few babies with HCU, the level of homocysteine can be controlled by giving Vitamin B6 (Pyridoxine). If this does not work, HCU can be treated effectively with a special low-protein diet and extra supplements. Other medications may also be given. These treatments prevent a build up of homocysteine in the blood.

What happens next?

You have been given an appointment to see a Specialist Metabolic Team as soon as possible. They will:

- Discuss the screening test result with you.
- Arrange for your baby to have a blood and urine test.
- Explain how the tests can confirm HCU.
- If diagnosis of HCU is confirmed, your baby will be given a trial of Pyroxidine for a week to see if it this decreases the homocysteine to safe levels. If the level remains high, your baby will need a special low-protein diet.
- Give you written information about HCU for you to share with your family, your GP and your local hospital,
- Answer any questions you may have.
- Arrange a follow-up appointment to discuss the results.

Parents of babies with HCU often ask the following questions:

Why do some children have HCU?

HCU is an inherited condition. Everyone has two copies of the gene for HCU. A baby with HCU has inherited two faulty copies of the gene. The parents have one normal copy and one faulty copy and are said to be 'carriers'. When two HCU carriers have a baby, they have a 1 in 4 (25 %) chance in each pregnancy of having a child with HCU. There is nothing the parents could have done to prevent their child having HCU.

What is life like for children with HCU?

Children with HCU can live full and active lives, but it is important for them to be diagnosed early by screening. Children with HCU need to follow special treatment and will be regularly seen by their Specialist Metabolic Team for review and monitoring.

Contact Details for the Specialist Metabolic Team:

Specialist Centre		
Consultant		☎
Metabolic Dietitian		☎
Clinical Specialist Nurse		☎
Ward (if applicable)		☎

Where can I find more information or support?

More information can be found at the Expanded Screening Programme website:

www.expandedscreening.org

CLIMB (The National Information Centre for Metabolic Diseases) provides information and support for people with HCU and their families.

Climb Building
176 Nantwich Road
Crewe
CW2 6BG

Telephone helpline: 0800 652 3181 (Freephone) or 0845 241 2172

Website: www.climb.org.uk

Email: info@climb.org.uk

The Expanded Newborn Screening evaluation project is funded by the National Institute for Health Research Collaborations for Leadership in Applied Health Research and Care (NIHR CLAHRC) for South Yorkshire.

We thank the UK Newborn Screening Programme Centre for their support and co-operation with the development of this booklet.

HCU - Parent Information - suspected
V1.0 <12/07/12>
Not to be used after August 31st 2013
Approved by Dr Andrew Morris,
Manchester Children's Hospital.

NHS
National Institute for Health Research

Results of the Newborn Blood Spot Screening

Parent Information Sheet Homocystinuria (HCU) - Confirmed Diagnosis

Your baby has been seen by the Specialist Metabolic Team and a diagnosis of HCU has been confirmed. However, each child with HCU is different and there may be some treatment differences from child to child.

This leaflet will help you to understand the condition and its treatment.

What is HCU?

HCU stands for Homocystinuria, pronounced ho-mo-sis-tin-ur-ee-a. Homocystinuria is a rare disorder that prevents the normal breakdown of protein. In order for the body to use protein from the food we eat, it is broken down into smaller parts called amino acids. In Homocystinuria one of these amino acids (methionine) does not break down in the usual way and methionine and a chemical called homocysteine builds up in the blood.

Without early treatment this can lead to long term health problems, including learning difficulties. These problems can be prevented by following the treatment advised by your Specialist Metabolic Team and your child will grow and develop normally and have a normal life expectancy.

What are the symptoms of HCU?

HCU may affect different babies in different ways. Babies with HCU are usually well in early life, although symptoms may develop later if untreated. Some children develop problems with their eyes, including severe short sightedness and dislocation of the lens.

Without early diagnosis and early start of treatment, children can develop damage to the brain, including learning difficulties. Children may also have thin bones (osteoporosis), bone and joint problems and may develop blood clots or strokes.

How is HCU Treated?

Treatment is given in children with HCU to prevent the build up of homocysteine. In a few babies with HCU, the level of homocysteine can be controlled by giving Vitamin B6 (Pyridoxine). If this does not work, HCU can be treated effectively with a special low-protein diet and extra supplements and medications. The low protein diet is controlled by the Specialist Metabolic Dietitian. The aim of the diet and other medications is to prevent the build up of Homocysteine in the blood.

Foods that provide the body with protein include milk, meat, fish, cheese, eggs, pulses and nuts. All baby milks (including breast milk) contain protein. Breast feeding is encouraged for babies. If you are breast feeding your baby who has HCU, you will be supported in continuing this as part of your baby's special diet. The amount of normal baby milk or breast milk a baby with HCU

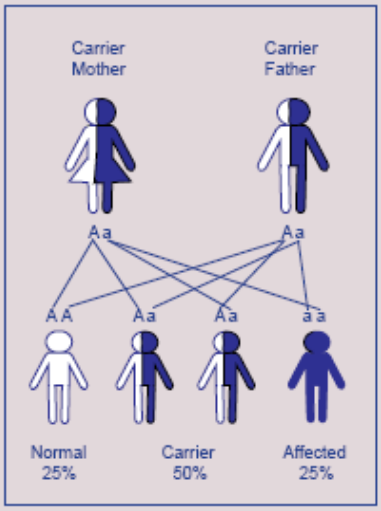
is given has to be limited. Your Specialist Metabolic Dietitian will advise you on this.

As breast feeds or normal baby milks have to be limited, a special baby milk consisting of amino acids without methionine is given to meet requirements for protein, calories, vitamins and minerals. This supplement is very important because it allows normal growth and development. Your Specialist Metabolic Dietitian will provide you with detailed information about the special baby milk and will explain how much your baby should have. Before your child starts weaning onto solids your Specialist Metabolic Dietitian will explain which foods can be used including special low protein foods such as low protein rusk, milk substitute, and low protein pasta which are available on prescription.

Your child will require regular blood tests to monitor the level of homocysteine in their blood. It is important to follow the advice regarding the low protein diet and supplements, in order to keep the levels in an acceptable range. Your child's diet will be adjusted as needed by the Specialist Metabolic Dietitian.

How will HCU affect my child's future?

Children with HCU can live full and active lives just like any other children, as long as they keep to the special low protein diet and/or medications or supplements as advised by their Specialist Metabolic Team. Children with HCU will be regularly monitored throughout their lives to check that homocysteine levels are well controlled.



Why does my baby have HCU?

HCU is an inherited condition. Everyone has two copies of the gene for HCU. A baby with HCU has inherited two faulty copies of the gene. The parents have one normal copy and one faulty copy and are said to be 'carriers'. When two HCU carriers have a baby, they have a 1 in 4 (25 %) chance in each pregnancy of having a child with

HCU. There is nothing the parents could have done to prevent their child having HCU. The diagram shows how this happens.

Will my other children need to be tested?

Your other children may be at risk of HCU even though they may not have shown any symptoms to date. It is therefore very important that they are tested if they have not been previously screened for HCU.

Who can I ask for advice and support?

The paediatric or metabolic clinician who is responsible for your child's care will be happy to discuss any queries you may have. Your Specialist Metabolic Dietitian will also be able to advise you.

Contact Details for the Specialist Metabolic Team:

Specialist Centre		
Consultant		☎
Metabolic Dietitian		☎
Clinical Specialist Nurse		☎
Ward (if applicable)		☎

My notes:

Where can I find more information or support?

More information can be found at the Expanded Screening Programme website:

www.expandedscreening.org

CLIMB (The National Information Centre for Metabolic Diseases) provides information and support for people with HCU and their families.

Climb Building
176 Nantwich Road
Crewe
CW2 6BG

Telephone helpline: 0800 652 3181 (Freephone) or 0845 241 2172

Website: www.climb.org.uk

Email: info@climb.org.uk

The Expanded Newborn Screening evaluation project is funded by the National Institute for Health Research Collaborations for Leadership in Applied Health Research and Care (NIHR CLAHRC) for South Yorkshire.

We thank the UK Newborn Screening Programme Centre for their support and co-operation with the development of this booklet.

HCU - Parent Information - Confirmed
V1.0 <16/07/12>
Not to be used after August 31st 2013
Approved by Dr Andrew Morris,
Manchester Children's Hospital.

NHS
National Institute for Health Research

Homocystinuria (non-pyridoxine dependent) GP Letter Disorder Suspected

Dear Doctor

Re: Positive Newborn Screening for HCU
[*name of child*], [date of birth]

[*name of child*] has had a positive test for Homocystinuria (HCU) on dried blood spot analysis (as part of the Expanded Newborn Screening Pilot).

As yet, we only have the result of a screening test. The Specialist Metabolic Team will review [name of child] to confirm the diagnosis, explain the condition to the family and start treatment.

Without treatment, HCU leads to long-term problems including learning difficulties, dislocation of the lens in the eye, skeletal abnormalities and a predisposition to blood clots. Fortunately, these problems can be prevented by bringing the blood homocysteine level back down towards normal. Treatment needs to be started early and continued throughout life.

Patients on treatment for homocystinuria do not become acutely unwell. Immunisations should be undertaken as normal, and general care is unaltered. The condition is inherited in an autosomal recessive fashion, with a 1:4 risk of recurrence in each pregnancy.

If you have any further questions, please do not hesitate to contact [*name of clinician*]. A further letter will be sent to you following review by the Specialist Metabolic Team.

Further information can be found about the Expanded Newborn Screening Pilot and HCU at www.expandedscreening.org

With kind regards
Yours sincerely

Homocystinuria (non-pyridoxine dependent) GP Letter Disorder Confirmed

Dear Doctor,

Re: Confirmed Diagnosis of HCU
[*name of child*], [date of birth]

[*name of child*] had a positive test for Homocystinuria (HCU) on dried blood spot analysis (as part of the Expanded Newborn Screening Pilot). We have, therefore, met the family and undertaken further tests that have confirmed the diagnosis. We have explained the nature of the condition and its management to the family.

HCU is a rare inherited disorder in which homocysteine cannot be broken down. Without treatment, this leads to long-term problems including learning difficulties, dislocation of the lens in the eye, skeletal abnormalities and a predisposition to blood clots. Fortunately, these problems can be prevented by bringing the blood homocysteine level back down towards normal. Treatment needs to be started early and continued throughout life.

There are 3 forms of treatment.

(i) A few patients respond to large doses of vitamin B6 (pyridoxine 50mg bd); we have already started [*name of child*] on this to see if he/she responds. We have also started folic acid 5mg daily.

(ii) Most patients require treatment with a special diet. The diet works by limiting the intake of methionine, from which homocysteine is made. The diet is extremely low in natural protein with supplements of all the amino acids except methionine. Babies are given some ordinary milk and some special formula that contains no methionine. If [*name of child*] does not respond to pyridoxine, we will write and ask you to prescribe this special methionine-free formula milk.

(iii) As patients get older, we sometimes add a drug called betaine, if it is difficult to control the homocysteine levels with the diet.

Patients on treatment for HCU do not become acutely unwell. Further guidelines for the treatment of HCU are available at BIMDG website (www.bimdg.org.uk) and at the Expanded Newborn Screening website (www.expandedscreening.org).

Immunisations should be undertaken as normal, and general care is unaltered. The condition is inherited in an autosomal recessive fashion, with a 1 in 4 risk of recurrence in each pregnancy

If you have any further questions, please do not hesitate to contact [*name of clinician*].

With kind regards
Yours sincerely

e. Routes for reporting and recording patient results

Screen Positive cases

It was agreed at the outset that all screen positive patients would be referred directly to a specialist metabolic physician. This worked well within the six centres and no difficulties with the availability of referral physicians or logistic problems with patient appointments were reported during the study.

Recording results on Child Health Record systems

Normal results were reported to the Child Health Record systems using the existing screening status codes. This was discussed briefly at the South Yorkshire/East Midlands "Blood Spot Forum meeting" and in greater detail at a meeting arranged with a Child Health Records Department (CHRD) manager, the Regional teams and representatives from the proposed study.

It was agreed that screen positive results were likely to be very rare, typically six instances per year with a screening workload of 70,000 pa with a true positive incidence of 1:28,000. The numbers are therefore similar to the cases of galactosaemia which is reported using an "07" *(Condition screened for) Not suspected Other Disorders Follow Up)* qualifier code against phenylketonuria (PKU).

The Child Health Records manager agreed that this approach was robust for galactosaemia and could be handled using the existing codes safely. By analogy it was therefore proposed by the group that for these disorders the "07" (Condition screened for) Not suspected Other Disorders Follow Up) qualifier code against MCAD as the major MS/MS detected disorder.

It was noted that it would not be appropriate to modify the text of the "normal letter for parents" to include the pilot conditions so the group agreed that this should be covered by adding a line to the parent information leaflet insert explaining that if the they receive a letter reporting existing conditions screened for are normal, then the conditions included within the pilot will also be normal.

f. External Quality Assurance and population monitoring

External quality assessment (EQA) was based on the circulation of dried blood spot cards arranged by the United Kingdom National External Quality Assessment Service (UK NEQAS) on a monthly basis to each of the Screening Centres involved. Samples were distributed containing all five metabolites forming part of the screening protocol. The samples were sent in replicate over this period to assess each Centre's long-term reproducibility of results. The concentrations reflected a range of "high", "borderline" and "normal" values. Reports were prepared by UK NEQAS and circulated on a monthly basis in the usual way. The samples were made available by the Centres for Disease Control and Prevention (CDC) and their support is acknowledged.

These results were discussed with the organiser, Finlay MacKenzie, on behalf of UK NEQAS at the "Expanded Newborn Screening Lab Group" meetings held throughout the project, and were circulated to members of the monitoring committee. The intra- and inter-laboratory variation was in-line with expectations and was considered by UK NEQAS, in discussion with the project team, to be acceptable.

In addition to these EQA arrangements, population data for four of the five metabolites (Leu, Met, C5 and C5DC) were collected and assembled into centile charts enabling direct comparison of distribution by laboratory. Results for C16OH were difficult to collect and record as the 10 – 90th centile range were effectively baseline values. While there was variation both within and between laboratories, the variation in the 90th centile remained significantly distinct from the cut off values and consequently did not prejudice the classification of "screen positive" cases.

g. Research consent for follow-up of screen positive cases

The National Research Ethics Service (NRES) considered the purpose of the organisation of the pilot to be service evaluation rather than research on the basis that such programmes were operating elsewhere and "the generation of new knowledge" was not the primary purpose of the work. Nevertheless, the named patient follow-up needed to inform the cost effectiveness study was submitted as

a multicentre research project and approved by the NRES committee: Yorkshire and the Humber, South Yorkshire on 9 July 2012 following a meeting on 28 June 2012. It was granted Multicentre Research Ethics Committee (MREC) approval: 12/YH/0320

h. Website

An important part of the communication strategy with the public and health professionals was fulfilled by the creation of a website: www.expandedscreening.org. This website contains detailed information about each of the five conditions, the screening and diagnostic protocols, treatment guidelines and the various leaflets: pre-screening, condition suspected and condition confirmed.

The website also provided a means of:

- Providing pre-screening leaflets as downloads in a variety of languages
- Providing translations of pre-screening leaflets as audio podcasts in a variety of languages
- Receiving queries from the public and professionals that could be responded to by email
- Allowing patients with each of the conditions to recount their own experience of living with the disorders as short videos for the benefit of families with a newly diagnosed child. These videos were professionally produced following contact and consent

i. Midwifery Education and Health Professional awareness

No additional sample was required to conduct the study and consequently no additional demands were placed on the midwifery services to collect a separate sample or an increased quantity of blood. Nevertheless, it was recognised that there needed to be midwifery training and this was been discussed with representatives of the Regional teams in East Midlands in Yorkshire and East Midlands together with a Head of midwifery services, community midwife team leader and screening lead in a midwifery unit. The Regional team were strongly represented in the steering group and the project group. They offered valuable advice about how this may be achieved. The communication remained the

responsibility of the study with some input from the appropriate regional teams. All group members agreed that the most appropriate way to arrange midwife awareness was using existing cascade mechanism within units following an educational event for those midwives and other health care professionals with screening educational lead responsibilities. Five issues were discussed and the following solutions agreed:

The nature of the information that should be provided to parents in relation to the proposed pilot study

The group agreed with the conclusions of the survey/focus group work undertaken by the Psychology Department at Coventry. In general they felt that outline information was to be much preferred to detailed information and that if this could be supplemented by additional web based information, also available in hard copy form for those without internet access, this would offer the best combination.

The form in which this information should be provided

It was felt that the most flexible solution would be a one page A5 insert issued separately from the NHS screening booklet "Screening tests for you and your baby or Screening Tests for your baby". This would be based on the information shown in the modified screening leaflet and offer access to web-based information and a route to obtain hard copies if needed.

The web-based resource would be provided by the study and would include additional information by disease for health professionals and the public, and a section of FAQs. There would also be a facility to receive and deal with enquiries via email.

The timing and delivery of this information

The most straightforward way to deliver this information, both verbally and by way of the A5 information sheet, was either at discharge or at the primary visit made by the community midwife following the discharge of the mother and baby from hospital. Both of these alternatives would allow a gap of at least one day before the screening sample was due to be taken. It was considered better not to include this in the antenatal pack as ending the study could then prove difficult because of the lead times involved particularly at the close of the study.

The training needed for midwives must ensure that they understood the project and the information to be provided to participants.

The most appropriate way to arrange midwife awareness was using existing cascade mechanism via email. Materials were accessible using the web resources.

During the MCADD pilot all midwives were able to attend regional meetings. This was seen as excessive in the current pilot due to the rarity of these conditions and the limited amount of demand these conditions will place on current services.

The means by which information and awareness of the study would be cascaded

It was important to engage all stakeholders in the study areas. Those who needed to be informed included:

- The Regional teams
- Strategic Health Authority (SHA) screening leads
- Primary Care Trust (PCT) screening leads
- Regional Directors of Public Health
- Heads of midwifery services
- Trust-based screening Antenatal and Newborn Screening Co-ordinators
- Child Health Record Department managers
- Health Visitor leads and Health Visitors
- GPs via PCT electronic links to GP and GP Notebook Newsletter
- Local Supervising Authority (LSA) officer for midwifery, who would also cover independent midwives
- Commissioners – for information only

j. Financial arrangements

Funding was provided to the six centres involved:

- Direct funding for laboratory testing @ £0.50 per baby screened
- Funding to aid the follow-up of screen positive cases @ £500/case
- Funding for dietetic time in relation to preparation and follow-up, £2k/centre

k. Mechanisms to identify missed cases

It was agreed that any clinically identified cases would have testing in one of the 15 laboratories that make up “MetBioNet” within the UK. Mary Anne Preece from Birmingham kindly agreed to collect date on all diagnoses made between July 2012 and July 2013 for the five conditions to be included in the study. This was gathered on a quarterly basis.

4. The findings

a. Original predictions and summary outcome data

Table 1 – Expected and observed prevalence of the five conditions

Condition	Prevalence		True positives		False positives		PPV%	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
MSUD	1:116,000	1:437,000	4	1	4	1	50	50
HCU	1:144,000	1:219,000	3	2	5	1	38	67
GA1	1:109,191	1:110,000	4	4	6	0	40	100
IVA	1:155,396	1:110,000	3	4	7	14	30	18
LCHADD	1:218,564	1:437,000	2	1	3	2	40	33
Total	**1:28,000**	**1:36,500**	**16**	**12**	**25**	**18**	**39**	**40**

b. Overview of screening results

Table 5 – IVA True and False Positive cases

	Centre	Date of birth (DOB)	DOB – Date of referral (days)	Diagnostic test date – Date of referral (days)	Screen C5 µmol/L (COV = 1.0)	IVG µmol /mmol cr	Diagnostic C5 µmol/L	Benign mutation	Comments
IVA True Positives									
1	Sheffield	31/07/2012	8	6	1.02	54.6 (ref<2.6)	1.56 (ref <0.5)	Not detected	
2	Sheffield	05/10/2012	10	2	1.36	4.9 (ref<2.6)	2.00 (ref <0.5)	Not detected	
3	Birmingham	26/07/2012	11	1	6.73	823 (ref<2.6)	7.4 (ref <0.3)	Heterozygous	
4	GOSH/GSTS	19/06/2013	8	4	11.85	Increased	8.38 (ref <0.3)	Not detected	RIP Homozygous for previously undescribed mutation predicted to be pathogenic.
IVA False Positives									
1	Birmingham	23/09/2012	10	9	1.32	Not increased (ref <2.6)	0.42	Not detected	
2	Birmingham	28/02/2013	11	3	3.6	0.3 (ref <2.6)	0.96	Not detected	
3	Manchester	04/06/2013	9	6	1.75	0.4 (ref <2.6)	0.79 (ref <0.64)	Not tested	Maternal pivampicillin. C5 within

									normal range upon follow up.
4	GOSH	04/12/2012	15	0	1.96	Trace (ref 0-30)	1.42 (ref 0-1.2)	Not tested	
5	GOSH	26/03/2012	8	2	1.49	2.0 (ref 0-30)	0.92 (ref 0-1.2)	Not detected	
6	GOSH	19/04/2013	7	3	1.25	Trace (ref 0-30)	1.54 (ref 0-1.2)	Not detected	
7	GOSH	25/04/2013	8	0	1.10	0	2.31 (ref 0-1.2)	Not detected	
8	GSTS	30/06/2012	24	1	1.33	Trace	0.15 (ref <0.3)	Not detected	
9	GSTS	18/08/2011	381	1	0.97	Trace	0.24 (ref <0.3)	Not detected	
10	Leeds	28/10/2012	12	3	1.25	0	1.11 (ref <0.3)	Not detected	SBCAD deficiency
11	Sheffield	02/10/2012	8	6	1.12	1.2 (ref <2.6)	0.45 (ref <0.5)	Not detected	Born at 30 weeks
12	Sheffield	20/01/2013	9	10	1.0	1.8 (ref <2.6)	0.46 (ref <0.5)	Not detected	Born at 30 weeks
13	Sheffield	16/05/2013	7	6	1.25	0.7 (ref <2.6)	0.49 (ref <0.5)	Not detected	Born at 31 weeks
14	Sheffield	28/05/2013	9	6	1.21	0.5 (ref <2.6)	0.17 (ref <0.5)	Not detected	Born at 39 weeks

Table 6 – GA1 True Positive cases

GA1 True Positives									
	Centre	Date of birth (DOB)	DOB – Date of referral (days)	Diagnostic test date – Date of referral (days)	Screen C5DC µmol/L (COV = 0.70)	Diagnostic C5DC µmol/L	3OH glutarate µmol /mmol creatinine	DNA confirmed	Comments
1	Sheffield	01/05/2013	8	14	1.98	4.43 (ref <0.06)	Significantly increased	Yes	
2	GOSH	08/03/2013	10	2	6.35	1.73 (ref <0.1)	Increased (to 188)	Yes	Homozygous
3	GOSH	21/09/2012	7	7	0.93	0.28 (ref <0.1)	Mild increase	Yes	Compound heterozygote
4	GSTS	29/12/2012	13	4	0.96	1.0 (ref <0.3)	Significantly increased	Yes	Compound heterozygote

Table 7 – LCHADD/MTP True and False Positive Cases

LCHADD/MTP True Positives								
	Centre	Date of birth (DOB)	DOB – Date of referral (days)	Diagnostic test date – Date of referral (days)	Screen C16OH µmol/L (COV 0.20)	Diagnostic C16OH µmol/L (ref <0.1)	G1528C Mutation analysis	Comments
1	GOSH	08/07/2013	10	0	0.35	1.89	Not detected	Baby was on treatment prior to screening sample
LCHADD/MTP False Positives								
1	GOSH	17/05/2013	11	2	0.17	0.03	Not detected	
2	GOSH	24/06/2013	14	4	0.15	Normal	Not detected	

Table 8 – HCU True and False Positive Cases

	Centre	Date of birth (DOB)	DOB – Date of referral (days)	Diagnostic test date – Date of referral (days)	Screen Met (COV = 50) µmol/L	Screen Thcys (Ref <15) µmol/L	Diagnostic Met µmol/L	Diagnostic Thcys µmol/L (Ref <18)	Comments
HCU True Positives									
1	Birmingham	01/07/2013	17	4	128	36.0	468	214	
2	Leeds	08/02/2013	13	5	234	40	646	156	
HCU False Positives									
1	Birmingham	25/05/2013	10	3	56	21	72	46	Ongoing follow up

Table 9 – MSUD True and False Positive Cases

	Centre	Date of birth (DOB)	DOB – Date of referral (days)	Diagnostic test date – Date of referral (days)	Screen xLeu (COV = 600) µmol/	Diagnostic Leu/Ile/Allo µmol/L	Comments
MSUD True Positives							
1	Birmingham	04/09/2012	9	0	2507	1758/615/264	
MSUD False Positives							
1	Birmingham	10/09/2012	10	4	1484	64/26/<5	Baby on total parenteral nutrition

The average age at referral (excluding the outlier, 381) = 11 days (range 7 – 24) and the time mean time taken to report diagnostic findings from referral was 4 days (range 0 – 14). Taken together the 12 positive cases exceeded the cut-off value by a mean of 4.1x (range 1.02 – 11.9).

c. Overview of cases identified within and outside the screened areas during the period 16 July 2012 – 19 July 2013

Table 10 – Cases identified within and outside the screened area during the pilot programme

Condition	True positive	False positive	Clinical diagnoses within the screening area	Diagnoses outside the screening area
IVA	4	14	1 sibling, Leeds 1 sibling, Birmingham	1 sibling, Cardiff
LCHADD	1	2	1 post mortem diagnosis, Leeds 1 post mortem diagnosis, Manchester 1 sibling, Sheffield 1 below initial cut-off, Birmingham	1 sibling, Newcastle 1 Southampton
MSUD	1	1	0	1 Oxford 2 Southampton
GA1	4	0	0	1 Worthing 1 Oxford
Hcys	2	1	0	
Total	**12**	**18**	**6**	**8**

As shown in Table 10 and 11, within the screening area three cases were identified by prospective sibling screening in families with a known risk. Two cases were identified at autopsy in the first week of life before screening took place. One child was identified clinically with LCHADD because the screening C16-OH result was below the cut off value at that time. As a consequence the cut off value was evaluated and lowered.

Outside the project sites there were two sibling diagnoses, and five clinical diagnoses. The remaining case, a child with MSUD, was diagnosed through an alternative route: the screening lab noticed a high leucine level, and referred the child on to a clinical team, even though the screening lab wasn't within the ENBS study area. The child was already unwell, and the parents were planning on taking the child to hospital.

Table 11 - Clinical and sibling diagnoses within the screening area, and diagnoses outside the ENBS project area

Disorder	Site	Date of birth	Date of diagnosis	Date of diagnosis – date of birth	Notes
IVA	Leeds	21/09/2012	27/09/2012	6	Sibling
	Birmingham	12/10/2012	17/10/2012	5	Sibling
	Cardiff	28/05/2013	29/05/2013	1	Sibling*
LCHADD /MTP	Leeds	12/08/2012	31/08/2012	19	Died on day 2
	Manchester	09/01/2013	05/02/2013	27	Died on day 5
	Sheffield	21/09/2012	25/09/2012	4	Sibling
	Birmingham	07/10/2012	07/11/2012	31	Missed by screening; cut of value lowered
	Newcastle	09/08/2012	10/08/2012	1	Sibling*
	Southampton	29/01/2013	02/02/2013	4	Clinical diagnosis*
MSUD	Oxford	17/02/2013	02/03/2013	13	Alternate route to diagnosis*
	Southampton	31/03/2013	16/04/2013	16	Clinical diagnosis*
	Southampton	09/07/2013	23/07/2013	14	Clinical diagnosis*
GA1	Worthing	23/09/2012	19/06/2013	234	Clinical diagnosis*
	Oxford	19/09/2012	18/10/2012	29	Clinical diagnosis*

*cases outside the project area

d. Declines

The study was separately consented and few declines were experienced (0.05% overall). There were more at the beginning of the study and this settled down throughout the year, perhaps due to increasing familiarity with the screening on the part of midwives, although that is speculation. The numbers are shown below in Table 12:

Table 12 - The number of families declining screening from each site during each quarter

Site	Declines				Total declines	Total number of births
	Quarter 1	Quarter 2	Quarter 3	Quarter 4		
Birmingham Children's	23	9	1	4	37	72,228
Great Ormond Street	36	12	8	6	62	129,231
Guy's and St Thomas'	4	3	0	1	8	58,472
Leeds	3	2	0	0	5	47,105
Manchester	18	13	6	4	41	55,944
Sheffield	38	12	10	5	65	74,207
Total declines	122	51	25	20	218	437,187

e. Operation of the website

Table 13 - Website statistics

Month	**Total visits**	**Unique visitors**	**% visitors staying for longer than 30 seconds**	**% visitors from UK**
May 2012	170	63	57.14	65.76
June 2012	543	211	56.45	86.86
July 2012	886	280	62.34	90.26
August 2012	599	319	35.40	86.01
September 2012	558	380	33.21	71.31
October 2012	668	364	33.80	69.56
November 2012	751	369	37.98	79.2
December 2012	537	375	27.68	63.5
January 2013	718	398	30.00	67.7
February 2013	906	521	29.45	68.7
March 2013	1338	826	39.56	64.72
April 2013	1033	648	37.12	69.26
May 2013	652	453	33.23	47.42
June 2013	690	487	32.06	49.40
July 2013	644	420	36.71	63.91
August 2013	632	433	26.00	50.69
September 2013	591	358	41.45	82.94
Totals	**11916**	**6905**		

The website attracted 6905 unique visitors (calculated on a month by month basis) from when the site was launched until 30 September 2013.

Downloads of information leaflets from the website

To minimise the likelihood of parents of screened children not being able to understand the expanded screening pilot we developed an English language information leaflet, which was translated into 16 of the most commonly spoken languages in England. All 17 versions were available through our website, along with selected audio translations. The total number of download of the leaflet in each language is shown in Table 14.

Table 14 - Total downloads of English and translated parent and professional information leaflet

Language	Total downloads
English	580
Arabic	72
Bengali	61
Chinese simplified	32
Chinese traditional	21
French	36
Gujarati	22
Hindi	45
Kurdish	21
Lithuanian	14
Polish	200
Portuguese	33
Romanian	40
Russian	46
Somali	37
Spanish	38
Turkish	127
Urdu	59
Overall total	1484

Between 1 July 2012 and 30 September 2013 the parent and professional information leaflets for suspected and confirmed conditions were downloaded 4774 times (see Table 15).

Table 15 - Total downloads of conditions suspected and confirmed parent and professional information leaflets 01 July 2013 – 30 September 2013

Leaflet	**Total downloads**
GA1 is suspected	415
GA1 is confirmed	1926*
MSUD is suspected	384
MSUD is confirmed	457
LCHADD is suspected	139
LCHADD is confirmed	246
IVA is suspected	123
IVA (mild) is confirmed	153
IVA is confirmed	308
HCU is suspected	152
HCU is confirmed	471
Overall total	4774*

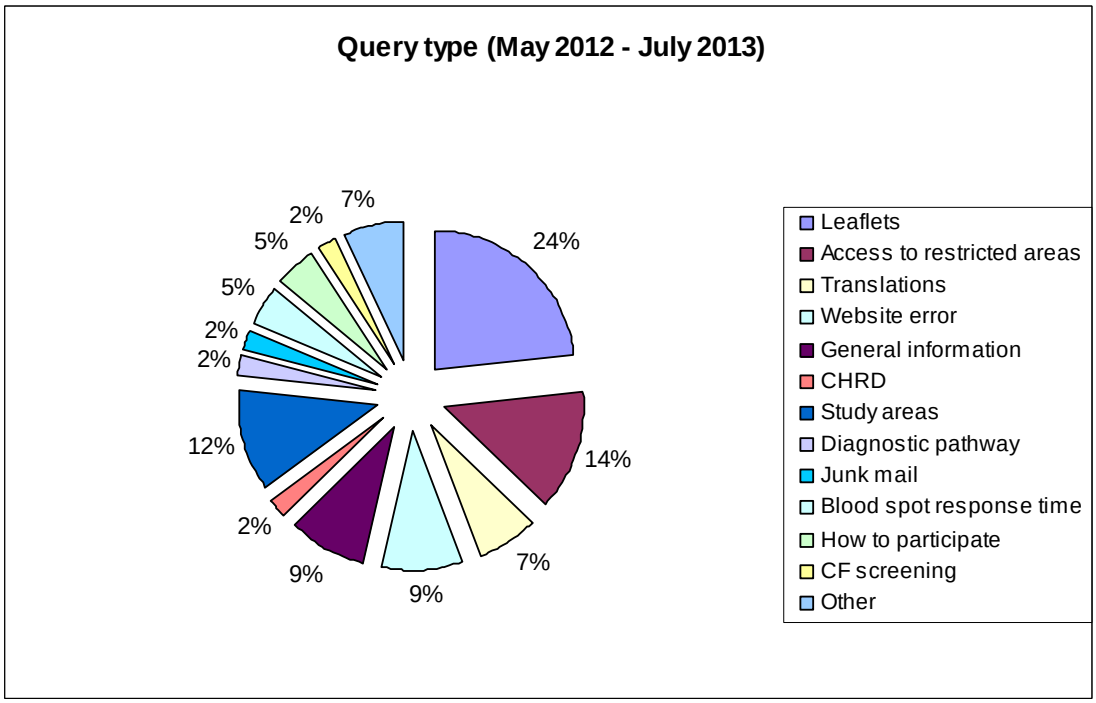
*This figure includes an uncharacteristically high, and inexplicable, monthly download total of 1106 in February 2013.

Queries to the website

At the outset of the study we agreed that any queries received would receive a response time within 3 days. During the study 43 queries were received and this target was achieved in 42/43 instances.

12/43 queries received were from members of the public and the remainder were from health professionals. The types of queries received are shown below (Figure 11):

Figure 11 – Types of query received through the website



f. Feedback on the project from clinicians, midwives and laboratory staff

Those involved in the pilot project were offered the opportunity to comment on how the aspects of the project that they had been involved in had progressed, and on whether they think the operational and logistic aspects of the expanded screening would benefit from further development if expanded screening is implemented nationally.

31 individuals responded. Their responses are summarised below.

- 81% agreed that the Expanded Newborn Screening Project proceeded without significant issue
- 19% would alter some aspects of the project if it started again.
- 100% thought the clinical management guidelines, dietetic protocols, screening protocols, information leaflet, condition suspected, condition confirmed, suspected letter for GPs, and the condition confirmed letter for GPs were clear.
- No midwife responders reported difficulties in obtaining consent for newborn screening.

- 67% of responders had used the www.expandedscreening.org website to access information, and 46% had advised parents to access it.
- None of those responding suggested changes to the website.
- 40% of midwives didn't know about the audio translations of the information leaflet, and 33% didn't know about the written translations.
- 39% of responders had watched the short films about the conditions.

This suggests that if the conditions were implemented at a national level documents should be reviewed and if audio or written translations of pre-screening information were used this should be better publicised.

5. Observations

i. The conduct of the screening pilot was well received by both the public and health professionals and there were few declines (0.05% overall). Queries that arose were mainly minor administrative issues and the responses were timely and appropriate.

ii. The study did not appear to cause an undue burden within the maternity services, laboratory, clinical or dietetic services.

iii. The number of babies tested was in line with the original predictions (437,187 vs. 430,000).

iv. The assays were well controlled during the study period and none of the six centres reported serious analytical problems during the study period.

v. The number of screen positive results was a little lower than predicted (30 vs. 41), as was the number of true positive (12 vs. 16) and false positive cases (18 vs. 25). Given the rarity of the conditions this may be subject to year on year fluctuation.

vi. The observed positive predicted value was very close to that predicted (40% vs. 39%)

vii. The age at referral was in line with expectations (mean = 11 days).

viii. The time from referral to the availability of diagnostic results was generally within that anticipated (4 days (mean) vs. 7 days anticipated, range 0 - 14). There were 3/30 instances where this 7 day turn around standard was not achieved.

ix. Three patients were identified as siblings of known patients and not included within the screen positive cases.

x. Two patients were identified at autopsy following death in the first week of life prior to screening.

xi. There were no recorded false negative cases in the screening areas.

xii. The laboratories did not report logistic difficulties when conducting the diagnostic testing.

xiii. There was one instance of a condition other than the target being identified: Short/branched chain acyl-CoA dehydrogenase (SBCAD) deficiency was identified following an increased C5 (the marker used to identify IVA).

xiv. One case died despite early recognition (isovaleric acidaemia)

xv. One case had been recognised before the screening result was available (LCHADD/MTP)

xvi. Three of the four true positive IVA cases were biochemically mild and received only advice in relation to the use of an emergency regimen.

6. Conclusions

i. The public and professional reaction to screening, so far as it can be judged, did not cause concern.
ii. Few of the potential dysbenefits of "expanded newborn screening" i.e. a very low PPV% and "over identification of patients" were evident during the study.
iii. No false negative cases were reported within the screened areas.
iv. The workload for laboratory and clinical staff was not undue and managed within the resources provided as part of the study, as outlined on p44.
v. The screening and diagnostic protocols performed well and the testing seemed robust and timely.
vi. It is likely that the GA1, MSUD and homocystinuria patients identified during the study gained very significant benefit. The LCHADD/MTP patient had been recognised clinically and the positive screening result did not offer additional benefit.
vii. The patients diagnosed with isovaleric acidaemia were mostly (3 of 4) mild from a biochemical perspective and the remaining patient sadly died. The intervention (emergency regimen only in mild cases) may not have offered significant dysbenefit to the families but the medicalisation of a healthy child is not trivial.

In comparison, the Heidelberg centre, using the same cut-off of 1.0 µmol/L:

- Identified 19 true positive cases over a 14 year period from 1,550,000 tested.
- 14/19 were classified as mild/uncertain phenotype on the basis of organic acid analysis and C5 acylcarnitine, never >10 µmol/L. These "mild" cases were not treated with diet although some were given carnitine.
- 5/19 were described as having "classical IVA" with IVG >1000µmol/mmol cr and C5 > 10µmol/L, all responded well to treatment.

The total number of true and false positive cases identified in our study and in Heidelberg are consistent as is the proportion of "mild"/"severe" cases. There have been no recorded illnesses in the mild cases in the German experience.

It is possible to maintain the view that:

- The number of IVA cases identified during the study was in line with expectations and international experience.

- The inclusion of IVA within the screening panel is common international practice and has not been abandoned in any of the countries in which it is included where it is believed to offer benefit to patients.
- The identification of classical IVA by screening offers significant benefit in most cases.
- The identification of an elevated C5 acylcarnitine in the newborn period in "mild" cases is commonly regarded as an important risk factor in unfavourable clinical circumstances.
- The intervention, "emergency regimen", is minimally invasive for the family and is likely to be effective in cases of potential decompensation.
- The developmental delay that accompanies later presenting clinical cases could potentially be avoided by the early recognition offered by screening.

Conversely, it is possible to maintain the view that:

- "Classical" IVA is very rare (approx 1: 400,000).
- The PPV% for IVA was lower than the other conditions included at 18% using the exiting cut-off value.
- That biochemically "mild" IVA is common and any long-term risk to the child is unproven.
- That medicalising these families is a significant dysbenefit and should be avoided.

7. The overall case and the case for individual conditions

The five candidate disorders selected for study feature commonly in newborn screening programmes around the world where these operate and enjoyed the support of clinicians within the UK when asked about which conditions should be included. Overall detection of these disorders by newborn screening does not appear to have resulted in a large number of false positive results nor in a marked over diagnosis when compared with the unscreened population, although data is limited.

Diagnostic follow-up testing has been achieved in a timely way and the clinical services have coped with the resulting referral activity. Few parents declined screening during the study and these low numbers fell progressively as the study proceeded. The website has proven a valuable asset to provide information to parents and professionals and a useful means of responding to queries as they arose.

Overall there is little evidence of dysbenefit and the cost effectiveness study suggests that screening all five of the disorders is likely to be cost saving.

On an individual disorder basis, while numbers are limited, our understanding of the disorders and the results suggest:

- **Homocystinuria** – that the two patients identified will have benefited significantly as a result. There was one false positive result.

- **Glutaric aciduria type 1** – that the four patients identified will have benefited significantly. There were no false positive results.

- **Maple syrup urine disease** – The single case identified during the study benefited significantly in the view of her clinician and without screening severe encephalopathy may not have been avoided. There was one false positive result.

- **Long chain hydroxyl acyl CoA dehydrogenase/MTP deficiency**. The single case identified during the study did not benefit from screening. Two false positive cases were identified. During the study period seven cases were identified in total:

- Two died before screening
- One was diagnosed as a sibling of a known case
- One was identified in the unscreened area as a result of a previous sibling
- One was diagnosed clinically in the unscreened area
- One was missed on screening and the protocol was modified as a result
- One was detected as part of screening

These results provide pause for thought in relation to LCHADD/MTP as a candidate for screening. In reality the numbers are small and likely to be variable year on year and should be viewed in the context of international experience. Experience in Germany[6] supports the view that a significant number of patients (3/6 in Germany) presented acutely or died before screening. This compares with 3/7 in our study. It is argued that the remaining cases identified asymptomatically receive significant benefit from the early detection offered by screening. In practice this disorder displays marked clinical heterogeneity. Taking these findings together it is likely that two or three patients per year identified by screening would benefit while two or three may present acutely or die before screening or shortly afterwards. There appears to be little direct dysbenefit from screening.

- **Isovaleric acidaemia.** Four cases were identified. Three of these were classified as "mild" and offered emergency regimen as needed without dietary intervention. The remaining case was severely ill and died. There were 14 false positive results.

  The number identified, 4/437,000 is similar to the Heidelberg experience, 19/1,550,000, and the ratio of "mild" cases not requiring dietary intervention: "classic" cases requiring dietary intervention is also similar, 3:1 (UK) vs. 14:5 (Germany). Few international studies include good outcome data to guide us. Perhaps the most useful is a recent German study that investigated 21 patients with IVA and compared their outcome with 155 in the world literature. They conclude that *"Within the group of "classical" organic acidurias, IVA appears to be exceptional considering its milder neuropathologic implications. The potential to avoid neonatal mortality and to improve neurologic and cognitive outcome under early treatments reinforces IVA to be qualified for newborn screening".*[7]

From the results of our study screening for IVA results in a significant number of false positive cases and some “mild” cases that require little intervention but may medicalise the family. Increasing the cut off value from 1.0 µmol/L to 2.0 µmol/L would reduce the number of false positives from 14 to 1 and remove two of the three “mild” cases. In the German experience dried blood spot C5 acylcarnitine was >10µ/L in “classic” cases and was 11.9 µmol/L in our single case. It would be reasonable to increase the current cut off to 2 µmol/L to avoid false positive results.

References

1. Burton H and Moorthie S. Expanded Newborn Screening: a review of the evidence. PHG Foundation (2010). ISBN 978-1-907198-03-8.
2. Cooksey D. A review of UK health funding research. HM Treasury (2006). ISBN-10: 0-11-840488-1, ISBN-13: 978-0-11-840488-4
3. Moody, L. and Choudhry, K. (2013), Parental views on informed consent for expanded newborn screening. Health Expectations, 16: 239–250.
4. Moorthie S, Cameron L, Sagoo G and Burton H. Birth prevalence of five inherited metabolic disorders. PHG Foundation (2013). ISBN to be confirmed.
5. Dixon S, Shackley P, Bonham J, and Ibbotson R. Putting a value on the avoidance of false positive results when screening for inherited metabolic disease in the newborn. J Inherit Metab Dis. 2012 Jan;35(1):169-76.
6. Sperk A, Mueller M, and Spiekerkoetter U, 2010. Outcome in six patients with mitochondrial trifunctional protein disorders identified by newborn screening. Mol Genet Metab. 2010 Oct-Nov;101(2-3):205-7.
7. Grunert S, Wendelz U, Lindner M, Leichsenring M, Schwab K, Vockley J, Lehnert W and Ensenauer R, 2012. Clinical and neurocognitive outcome in symptomatic isovaleric acidemia. Orphanet Journal of Rare Diseases *2012, **7**:9*

This report presents independent research by the National Institute for Health Research Collaboration for Leadership in Applied Health Research and Care for South Yorkshire (NIHR CLAHRC SY). The views and opinions expressed are those of the authors, and not necessarily those of the NHS, the NIHR or the Department of Health.

This project was kindly supported by The Children's Hospital Charity (Registered Charity No. 505002) via NIHR CLAHRC for South Yorkshire.

Appendix A – agreed Expanded Newborn Screening general information leaflet

What does the treatment involve?
The most important aspect of treatment for these disorders is dietary and special diets have been designed for each condition. During periods of common childhood illness additional advice is given to parents and on rare occasions, admission to hospital may be needed.

Could existing children be affected?
As "genetic" conditions these disorders could affect existing or future children with the same parents. In the event of a positive result, existing children would be checked and advice about future pregnancies offered.

Does the test involve any risk?
No additional blood sample is required and the only risk is the anxiety caused by a false positive result. Like the conditions themselves false positive results are very rare so we anticipate around 16 true positive and 24 false positive cases from the 430,000 screened. The chances of a false positive result is around 1 in 20,000.

Can cases be missed?
Yes, while most babies with these conditions will be detected by the programme it is possible that a baby with one of the conditions may not give a positive result.

Is this "Research"?
No, similar screening programmes are already undertaken in many countries throughout Europe and the USA. This evaluation will not answer new research questions but will ensure that the way that the tests are conducted is carefully evaluated and optimised for use in the UK. For the small number of cases which turn out to be positive, parents will be asked to participate in a research study to help improve treatment and evaluate the screening.

What will happen at the end of the pilot?
Newborn screening for these five disorders will stop in July 2013. A report will be produced and a cost effectiveness study based upon the data will be completed. The National Screening Committee (UKNSC) will then decide whether to recommend to the Department of Health that newborn screening for these conditions should be formally adopted or not.

How will parents hear that everything is OK if the test is normal?
Parents receive their baby's newborn screening results for PKU, CF, CHT, SCD and MCADD either by a "not suspected" letter which is sent to parents in some areas or given via the health visitor. If results for conditions being screened as part of the pilot are also normal they will receive the same letter, but the extra five normal results will not be named individually.

Can parents decline this testing for their baby?
Yes, parents are free to decline this testing if they wish and it will not affect participation in screening for the other conditions which form part of the UK Newborn Screening Programme

Can parents and health professionals get further information?
Further information on the pilot and the conditions to be included can be found at: www.expandedscreening.org

Expanded Newborn Screening

A one year pilot

Advice for parents and health professionals

NIHR CLAHRC for South Yorkshire

NHS

Expanded Newborn Screening
www.expandedscreening.org

NHS
National Institute for Health Research

In 2004 a study began which led to the successful introduction of screening for medium-chain acyl-CoA dehydrogenase deficiency (MCADD) in England. Since then around 500 cases of MCADD have been identified and treated. It was known at the time that much rarer conditions would also benefit greatly from early detection, and could be identified using the same blood spot sample.

After careful consideration and discussion with the National Screening Committee (NSC) and the Health Technology Assessment programme, it was agreed to undertake an evaluation of screening for an additional five very rare conditions. These specific conditions already form part of newborn screening programmes in both the USA and Europe.

The additional conditions being screened are:
Maple syrup urine disease (MSUD)
Homocystinuria, pyridoxine unresponsive, (Hcys)
Glutaric aciduria type 1 (GA1)
Isovaleric acidaemia (IVA)
Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)

While clearly beneficial for the children and families detected, taken together these conditions are expected to identify only 25 cases each year in the UK, approximately 1 in 30,000 of those tested, or around two or three in a typical screening laboratory region.
Studies undertaken in focus groups and published in Health Expect. 2011 Aug 12. 10.1111/j.1369 – 7625; indicated that women want to know that the pilot evaluation is taking place and be given the right to accept or decline if they wished.

They did not want detailed information in relation to each individual rare condition. This is the approach that has been adopted and the information within this leaflet is supplemented by on-line information available at www.expandedscreening.org for those parents who wish to access it. This approach has worked well in other screening programmes.

The pilot will take place from 16th July 2012 until 19th July 2013 in six screening laboratories and the areas they serve: these are Leeds, Manchester, Sheffield, Birmingham, London (Guys) and London (GOSH) Newborn Screening Laboratories. Approximately 430,000 babies will be screened for the five extra conditions, if mothers/parents consent, during this time. This leaflet is designed to help answer questions that may arise at the time of blood sampling. Please see the following questions and answers below which may be helpful:

What kind of additional disorders are being screened for?
Five very rare disorders will be screened for (they are listed by name earlier in the leaflet). They typically occur in between 1 in 100,000 to 1 in 200,000 births, they all benefit from the early detection offered by screening.

What causes these conditions?
They are "genetic" disorders in which each parent, although well themselves, unavoidably passes the disorder to their child.

What would happen if the baby's test was positive?
Positive results are extremely rare for these conditions. Parents will be contacted by telephone by the health professional concerned to check that the baby is well. An appointment will be made for the baby to be seen at a hospital for an assessment and some tests. The results of these tests, usually available within 7 days, will either confirm the condition or indicate that the result was a false positive result. In the meantime parents will be given advice about how to look after their baby and be given particular advice about feeding. In rare circumstances the baby may have a brief stay in hospital.

How effective is treatment and what would happen if the conditions were not detected?
All these disorders are serious if untreated and while the baby seems to be well at birth can become ill. This can be very early in some cases or can take up to one or two years.

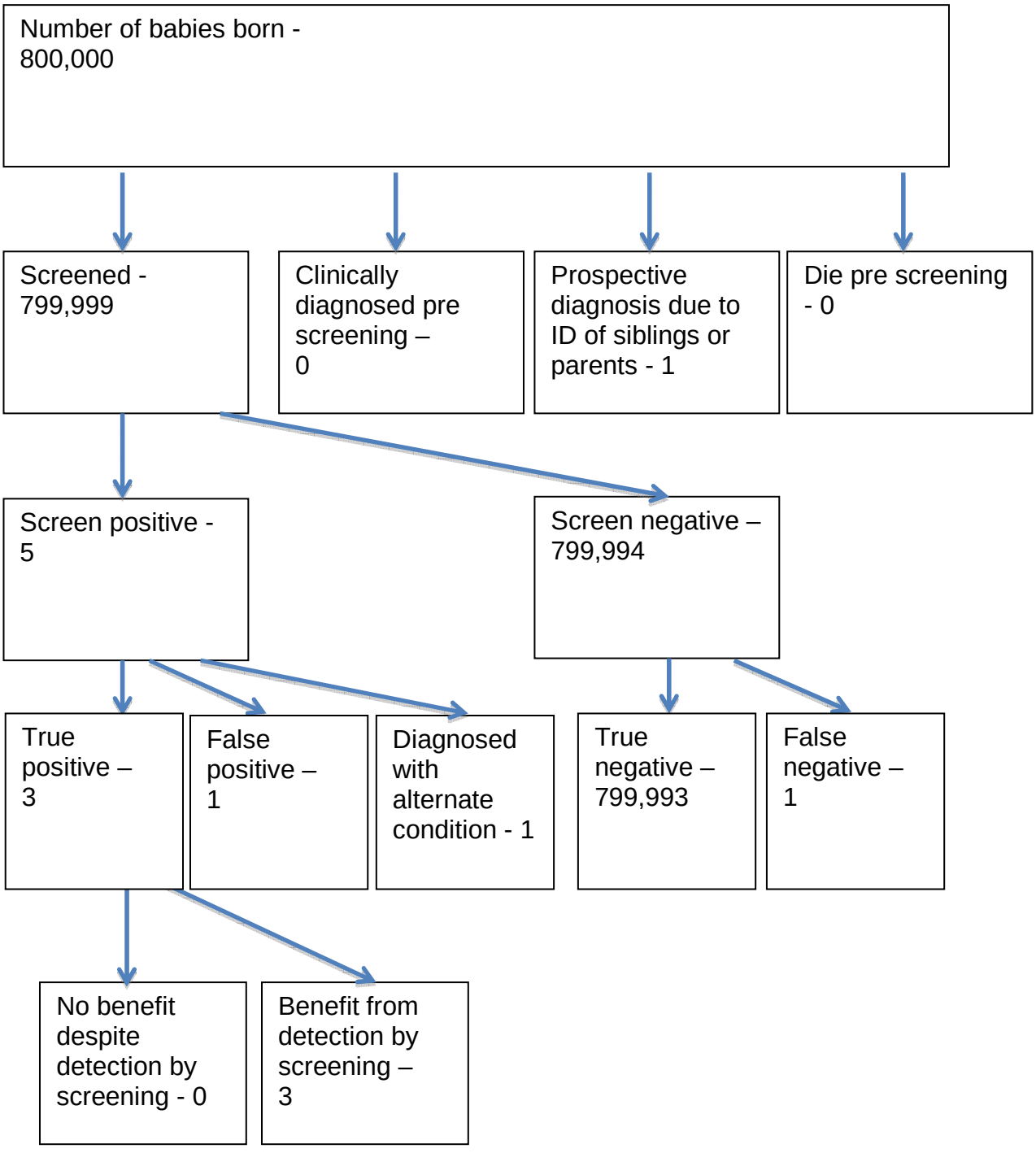
Treatment started early is much more effective than delayed treatment. In general when detected by screening the outlook for the affected baby is much improved although in rare cases even this can be ineffective. Serious effects, including severe mental retardation or death may result if untreated.

NHS
National Institute for
Health Research

Appendix B

An appraisal of the condition homocystinuria (HCU) against the National Screening Committee Criteria

Figure 1 - Approximate instances of each eventuality HCU



The Condition – HCU

1. The condition should be an important health problem

HCU is currently screened for in the USA and in parts of Europe. HCU was estimated to occur in the UK at a rate of 1:144,000[1]. The recent systematic review by the PHG Foundation revised this estimation to 1:204,081[2]. The former estimation was used throughout the ENBS project. These may be underestimates, as many countries have shown higher incidences where mutation analysis was used to determine actual incidence.

Although this individual disease is rare, the consequences for the child and family are severe, and a high proportion of the children will require expensive care from health services, education and social care services. This is particularly the case as treatments improve survival; survival may be with disability, and the burden of care extends for life.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.

The HCU prevalence in the UK is estimated to be 1:204,081.

Whilst lacking the strength of epidemiological evidence using large numbers, the aetiology of HCU has been studied in detail in individuals, so there is a precise understanding of the underlying abnormal biochemistry (abnormal levels of metabolites, enzyme activity and underlying genetic defect)[3].

HCU is an autosomal recessive condition, usually affecting the gene *CBS* that encodes the enzyme cystathionine beta-synthase. Defects in this enzyme disrupt metabolism of the amino acids methionine and homocysteine, leading to a build up of methionine and homocysteine in the blood and excess homocysteine excretion in the urine. The proposed screening analysis involves assaying the levels of methionine and total homocysteine in the patient's blood spot sample, with diagnosis based

around blood levels of these analytes. HCU can also occasionally be caused by mutations in other genes; this still results in a build up of homocysteine but not methionine.

There are two phenotypic forms of homocystinuria. The pyridoxine non- responsive form is detectable by screening, whereas the pyridoxine responsive form may not be. In both phenotypes and especially in the more severe pyridoxine non-responsive, a normal outcome including IQ and other abnormalities is only achievable in those treated from childhood.

Early symptoms of HCU are vague, becoming apparent between 6 months and adulthood. Diagnosis is not usually made until the first 2-3 years of life. Untreated HCU affects four major systems: the eye (myopia and lens dislocation), the central nervous system (mental retardation, seizures, psychosis), the skeleton (premature osteoporosis, dolichosternomelia, archnodactyly) and the vasculature (premature thromboembolic events). Vascular events are the major cause of morbidity and mortality. Without treatment, 25% of patients will die before the age of 30, usually as a result of thromboembolic events.

3. All the cost-effective primary prevention interventions should have been implemented as far as practicable.

HCU is a rare recessive genetic condition and is not amendable to primary preventative measures. Screening can detect the condition and lead to suitable treatment following diagnosis. Prenatal and pre-implantation diagnosis may be appropriate for individual families identified as being at high risk, but pre-natal or pre-implantation diagnosis is not suitable at the population level.

4. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

As HCU is autosomal recessive, both parents of an affected child are presumed to be carriers. Parents and siblings can choose to have their carrier status confirmed, and genetic counselling and support given for future reproductive choices.

5. There should be a simple, safe, precise and validated screening test.

Elevated levels of particular analytes are screened by tandem mass spectrometry. This is performed in laboratories all over the world and the methodology has been shown to be simple and safe. The infrastructure already exists, including sample collection. External quality assurance and CE marked reagents are available.

As part of the ENBS project the laboratories across the six sites have worked closely together to optimise and standardise screening and calibrations. The positive predictive value (PPV) during the ENBS project was 50%.

6. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.

Information from existing screening programmes can give some indication of test values in Caucasian populations. However, this data cannot be directly extrapolated to the UK as differences in age at screening and method of assay calibration will have an impact on the values.

A cut off level has been defined and agreed across the six screening laboratories as part of the ENBS project. The positive predictive value (PPV) during the ENBS project was 50%.

7. The test should be acceptable to the population.

No additional procedure or additional blood is required.

During the Expanded Newborn Screening programme the number of parents opting out of the additional screening was recorded, and was found to be 0.05% across the programme by the final quarter, suggesting the test is acceptable to the large majority of the public.

A contingent value study conducted among 160 families in South Yorkshire indicated that 93% would welcome a newborn screening programme including HCU[4].

8. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.

Further diagnostic investigations on individuals with a positive screening test include plasma amino acid analysis. Clearly defined diagnostic protocols have been developed within the ENBS project through consultation with multidisciplinary project team members across all sites. The confirmatory assays are externally quality assured and well know to diagnostic laboratories. The protocols developed by the ENBS project are similar to those existing for disorders that are already screened for.

9. If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out.

Not applicable. Mutation analysis is not involved in the proposed screening or diagnostic tests.

The Treatment

10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.

There is evidence that early detection and compliance with treatment leads to better outcomes, including IQ and fewer complications[5,6,7,8].

Agreed treatment guidelines were developed and formally adopted for the ENBS pilot study.

Treatment for non-pyridoxine responsive HCU is a protein restricted diet with supplemental amino acid mixture, devoid of methionine and homocysteine, and co-factor supplementation. Other adjunctive treatments may be required to minimise the impact of HCU symptoms.

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

There is expert consensus and evidence from high quality observational studies that individuals who have HCU are at increased risk of death, mental retardation and other symptoms, and that they should be offered a special diet.

All individuals diagnosed with HCU will be offered appropriate treatment.

12. Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.

There are specialised services in most regions in England. A screening programme may simplify the diagnostic process for some of the infants and ensure that positive screening results are immediately flagged up by the specialised services.

The Screening Programme

13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.

There are no randomised trials of HCU screening.

There is evidence that early and appropriate treatment can help individuals avoid long-term mental and physical symptoms, and improve survival[5,6,7,8].

HCU screening is not being proposed for the purpose of informing reproductive choices.

Information provided to parents of screen positives has been developed. Pre-screen information, and leaflets for parents and healthcare professionals for screen positive results have been developed. Multiple translations and audio translations of patient information materials are available, as is a website providing information. Parents/carers are supported by clinicians and allied health professionals in understanding and developing appropriate care routines as required.

14. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.

HCU screening is similar to screening for other disorders currently offered for which public acceptance has been demonstrated.

In a study conducted prior to the start of the ENBS evaluation it was shown that of 160 parents, 93% supported expansion of screening including HCU[3].
Parent groups contacted through CLIMB (Children Living with Inherited Metabolic Diseases, registered charity 1089588) took part in the study.

The screening test involves no additional procedure or effort on behalf of the performing health professional, and as the existing newborn screening tests are already deemed socially and ethically acceptable there should be no issue in expanding the range of disorders being tested for from a social and ethical perspective. There is some additional laboratory time and resource required for the screening test.

Diagnostic procedures involve collection of blood and urine. These are routinely seen as clinically, ethically and socially acceptable.

Parents may experience significant distress in learning that their child may have an illness. This is difficult to avoid but qualitative research carried out during the

MCADD screening proposal in 2006 suggests that the vast majority of parents perceived the benefits of screening to outweigh any short-term psychosocial outcomes they had experienced. As part of the ENBS project we are undertaking a qualitative study to discuss with parents the timing and methods of communication such that the timing and methods can be optimised.

Treatment of HCU is dietary so is unlikely to cause ethical or clinical concerns. However, as the diet must be adhered to for the patient's lifespan it may have social or psychological implications. We believe the risk of social implications is outweighed by the benefits of the restricted diet.

15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).

Pre-emptive detection of affected children before they exhibit symptoms improves survival, improves outcomes including IQ, and reduces complications[5,6,8].

The screening test requires no additional procedure other than routine newborn screening (the heel prick test) that is already collected/carried out. The diagnostic procedures require urine and blood samples, so cause minimal harm if correctly collected.

Treatment for the disorder is dietary, with an emergency regimen that may include hospital admission in addition to dietary treatment.

In a willingness to pay study conducted prior to the ENBS project it was shown that 93% of parents would accept screening, and were willing to pay a mean of £178[4], suggesting that parents believe the benefits of screening outweigh physical and psychological harm. During the ENBS project only 0.05% of parents declined screening, thereby further supporting the suggestion that parents believe the benefits of screening outweigh the harms.

The psychological implications of the test and diagnostic procedures are parental anxiety and helplessness between the confirmation of a positive screen result and the diagnostic outcome. The ENBS pilot is conducting a qualitative communication research study through consultation with parents of true and false positives, and

clinicians, to optimise the communication of results and information in order to minimise anxiety. The outcome of this study will inform the timing and nature of information flow should the National Screening Committee decide to roll out screening for this condition nationally.

16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money). Assessment against this criterion should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.

The health economic evaluation of the expanded newborn screening programme indicates that national screening for HCU would be cost saving.

17. All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.

Suitable treatment options already exist.

Improved education to facilitate rapid diagnosis upon clinical presentation would be difficult to achieve given the rarity of the disorder, and is not likely to be economically viable or result in greatly improved outcomes. Age at clinical presentation varies widely. Screening may enable pre-emptive treatment before affected individuals display symptoms so may avoid death or the development of lifelong disabilities with associated treatment costs.

18. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.

An External Quality Assurance (EQA) process is already in place for other conditions that are screened and diagnosed in similar ways to those proposed for HCU. Throughout the ENBS project we have developed QA measures for HCU that could

be rolled out nationally. We are currently working to minimise variation between screening labs and to standardise calibrations. If HCU screening is implemented nationally, these QA measures and the standardisation activities that are underway will inform the implementation.

19. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.

Our assessment shows that the additional screening burden associated with screening for HCU can be absorbed into existing facilities and hardware.

As all diagnostically confirmed cases of HCU would be expected to engage with emergency services during the first metabolic crisis if they hadn't already been identified by screening, identifying patients by screening could potentially reduce the staff/facilities implications by reducing the likelihood of metabolic crises and emergency service involvement.

If HCU screening is implemented across England the implementation itself will require short-term funding for management, and staff included in the screening and diagnostic pathways would require a small amount of information and training. The information and training could be adapted from the materials developed as part of the ENBS evaluation.

20. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.

A parent/professional information leaflet has been developed for all parents who agree to their child being screened. Feedback on these leaflets has been requested from various sources both during and since their development. Written and oral translations are available in 17 languages.

Disease-specific leaflets have been developed for parents and professionals to be given in the case of a screen positive result (the 'condition suspected' leaflet).

Disease-specific leaflets have been developed for parents and professionals to be given upon the confirmation of a true positive result (the 'condition confirmed' leaflet).

As part of the ENBS pilot a qualitative communication study is underway to confirm the timing and usefulness of information provision from the perspectives of screen positive parents, and healthcare professionals.

21. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.

We propose that all babies born in England be offered HCU screening so eligibility for newborn screening cannot be extended further.

The interval between birth and screening may be subject to scrutiny for this and other conditions already included within the programme.

As part of the ENBS pilot we are collating data on screen false negatives. Data collection is ongoing although no false negative HCU cases have yet presented.

The ENBS project suggests that the cut off levels are appropriate, with the caveat that the low frequency of screen positives means that ongoing monitoring is required to ensure rigorous statistical support for the cut off level.

22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members.

Screening is not for a mutation. HCU is an autosomal recessive disorder, so by implication it is assumed that both parents of an affected child are carriers of a mutation in one of the genes that encodes the proteins that constitute the dysfunctional complex. Siblings may also carry mutations. Parents will be given

genetic counselling and support should they choose to have more children, and all siblings can be tested.

References for Appendix B

1. Burton H and Moorthie S, 2010. Expanded Newborn Screening: a review of the evidence. PHG Foundation. ISBN 978-1-907198-03-8.

2. Moorthie S, Cameron L, Sagoo G and Burton H, 2013. Birth prevalence of five inherited metabolic disorders. PHG Foundation. ISBN to be confirmed.

3. Mudd H, Skovby F, Levy H, Pettigrew K, Wilcken B, Pyeritz R, Andria G, Boers G, Bromberg I, Cerone R, Fowler B, Grobe H, Schmidt H, and Schweitzer L, 1985. The natural history of homocystinuria due to cystathionine β-synthase deficiency. Am J Hum Genet. 1985 January; 37(1): 1–31.

4. Dixon S, Shackley P, Bonham J and Ibbotson R, 2012. Putting a value on the avoidance of false positive results when screening for inherited metabolic disease in the newborn. J Inherit Metab Dis. 35(1):169-76.

5. Yap S, Rushe H, Howard PM, and Naughten ER, 2001. The intellectual abilities of early-treated individuals with pyridoxine-nonresponsive homocystinuria due to cystathionine beta-synthase deficiency. J Inherit Metab Dis. 2001 Aug;24(4):437-47.

6. Yap S and Naughten E, 1998.
Homocystinuria due to cystathionine beta-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. J Inherit Metab Dis. 1998 Oct;21(7):738-47.

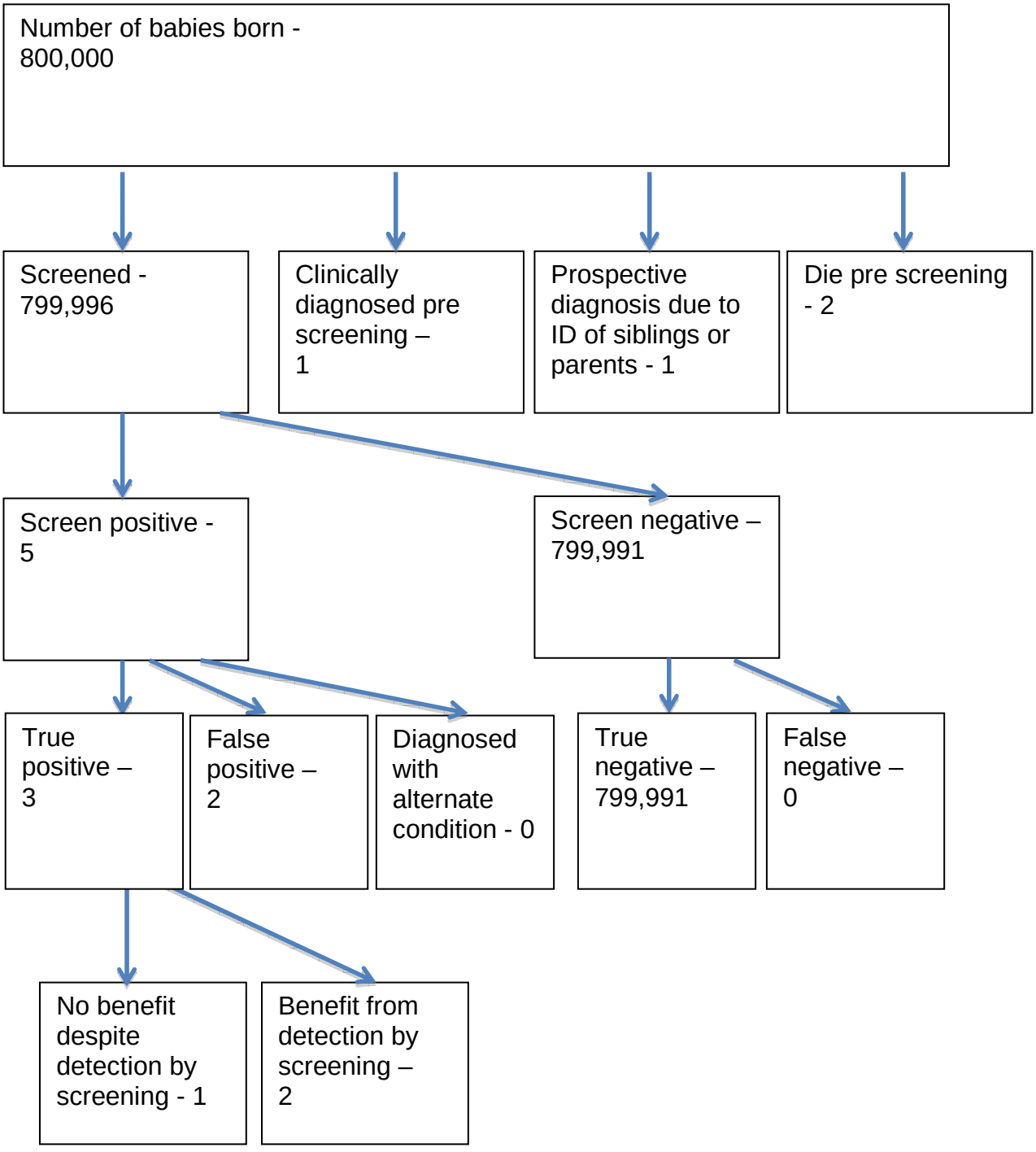
7. Yap S, Boers G, Wilcken B, Wilcken D, Brenton D, Lee P, Walter J, Howard P, and Naughten E, 2001. Vascular outcome in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. Arterioscler Thromb Vasc Biol. 2001 Dec;21(12):2080-5.

8. Yap S, 2012. Classical homocystinuria: newborn screening with early treatment effectively prevents complications. Hamdan Medical Journal 2012; 5:351-362.

Appendix C

An appraisal of the conditions Long Chain 3-Hydroxy-acyl-CoA Dehydrogenase Deficiency (LCHADD) and Mitochondrial Trifunctional Protein (MTP) Deficiency against the National Screening Committee Criteria

Figure 1 - Approximate instances of each eventuality - LCHADD/MTP deficiency



The Condition – LCHADD/MTP deficiency

1. The condition should be an important health problem

LCHADD/MTP deficiency are currently screened for in the USA and widely across Europe; prevalence figures are variable. LCHADD was estimated to occur in the UK at a rate of 1:218,564[1]. The recent systematic review by the PHG Foundation revised this estimation to 1:149,254[2]. The former estimation was used throughout the ENBS project.

Although this individual disease is rare, the consequences for the child and family are severe, and a high proportion of the children will require expensive care from health services, education and social care services. This is particularly the case as treatments improve survival; survival may be with disability, and the burden of care extends for life.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.

The LCHADD prevalence in the UK is estimated to be 1:149,254[2].

Whilst lacking the strength of epidemiological evidence using large numbers, the aetiology of LCHADD/MTP deficiency has been studied in detail in individuals, so there is a precise understanding of the underlying abnormal biochemistry (abnormal levels of metabolites, enzyme activity and underlying genetic defect).

Mitochondrial trifunctional protein (MTP) is an enzyme complex that is required for long chain fatty acid oxidation. The complex is encoded for by two genes; *HADHA* and *HADHB*. *HADHA* encodes for the enzyme long chain 3-hydroxyacyl Co-A dehydrogenase (LCHAD) and the enzyme enoyl-CoA hydratase, and *HADHB* encodes for long chain thiolase. The common G1528C mutation in the *HADHA* gene leads to LCHAD deficiency (LCHADD). A combination of any other two mutations in

either the *HADHA* or *HADHB* genes leads to deficiency of all three enzymes i.e. mitochondrial trifunctional protein (MTP) deficiency.

LCHADD and MTP deficiency are autosomal recessive conditions that prevent the body from converting long chain fatty acids into energy. Long chain fatty acids are found in foods such as milk. The fatty acids are stored in the body's fat tissues, and are a major source of energy for the heart and muscles. During periods of fasting, fatty acids undergo fatty acid oxidation in the liver to produce ketone bodies that are exported to other tissues as a major energy source. Both LCHADD and MTP deficiency result in disruption/failure of long chain fatty acid oxidation.

Signs and symptoms of LCHADD typically appear during infancy or early childhood and can include feeding difficulties, lethargy, hypoglycaemia, weak muscle tone, liver problems, and abnormalities in the retina. Later in childhood, people with this condition may experience muscle pain, breakdown of muscle tissue, and a loss of sensation in their arms and legs (peripheral neuropathy). Individuals with LCHADD are also at risk of serious heart problems, breathing difficulties, coma, and sudden death. Problems related to LCHADD can be triggered by periods of fasting or by intercurrent illnesses such as viral infections.

The severe form of MTP deficiency is early onset, with liver, heart, skeletal and neural abnormalities, and is usually fatal. There is a less severe form that has many of the features of isolated LCHADD and patients generally respond favourably to appropriate treatment. There is a milder form that is usually triggered by intercurrent illness, exercise, fasting, or exposure to heat or cold. Symptoms include muscle defects, neuropathy, and occasionally retinal problems.
The proposed screening analysis involves assaying the levels of acyl carnitines (which are products of dysregulation of fatty acid oxidation) in the patient's blood spot sample, with diagnosis based around mutation analysis, and blood and urine levels of analytes. Lymphocyte and fibroblast assays may also be used during diagnosis.

3. **All the cost-effective primary prevention interventions should have been implemented as far as practicable.**

LCHADD and MTP deficiency are rare recessive genetic conditions and are not amendable to primary preventative measures. Screening can detect the condition

and lead to suitable treatment following diagnosis. Prenatal and pre-implantation diagnosis may be appropriate for individual families identified as being at high risk, but pre-natal or pre-implantation diagnosis is not suitable at the population level.

4. **If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.**

   As LCHADD and MTP deficiency are autosomal recessive, both parents of an affected child are presumed to be carriers. Parents and siblings can choose to have their carrier status confirmed, and genetic counselling and support given for future reproductive choices.

The Test

5. **There should be a simple, safe, precise and validated screening test.**

   Elevated levels of particular analytes (acyl carnitines) are screened by tandem mass spectrometry. This is performed in laboratories all over the world and the methodology has been shown to be simple and safe. The infrastructure already exists, including sample collection. External quality assurance and CE marked reagents are available.

   As part of the ENBS project the laboratories across the six sites have worked closely together to optimise and standardise screening and calibrations. The positive predictive value (PPV) during the ENBS project was 33%.

6. **The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.**

   Information from existing screening programmes can give some indication of test values in Caucasian populations. However, this data cannot be directly extrapolated

to the UK as differences in age at screening and method of assay calibration will have an impact on the values.

A cut off level has been defined and agreed across the six screening laboratories as part of the ENBS project. The positive predictive value (PPV) during the ENBS project was 33%.

During the ENBS project an individual with LCHADD/MTP deficiency was identified clinically before screening who would not have been identified by screening. As a consequence the cut off value was lowered. No further false negatives have been detected since.

7. **The test should be acceptable to the population.**

No additional procedure or additional blood is required.

During the Expanded Newborn Screening programme the number of parents opting out of the additional screening was recorded, and was found to be 0.05% across the programme by the final quarter, suggesting the test is acceptable to the large majority of the public.

A contingent value study conducted among 160 families in South Yorkshire indicated that 93% would welcome a newborn screening programme including LCHADD[3].

8. **There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.**

Further diagnostic investigations on individuals with a positive screening test include mutation analysis, blood acyl carnitine analysis, and urinary organic acid analysis. In some cases lymphocyte and occasionally fibroblast assays may also be used. Clearly defined diagnostic protocols have been developed within the ENBS project through consultation with multidisciplinary project team members across all sites. The confirmatory assays are externally quality assured and well know to diagnostic laboratories. The protocols developed by the ENBS project are similar to those existing for disorders that are already screened for.

9. **If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out.**

   The screening test is not for a mutation. Screen positive cases are investigated further, with mutation analysis forming part of the diagnostic process. A specific mutation tested for during LCHADD diagnosis is G1528C as 87% of mutant alleles carry this mutation[4]. Extended mutation analysis is not currently routinely available in England.

10. **There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.**

    Agreed treatment guidelines were developed and formally adopted for the ENBS pilot study.

    Treatment is aimed at controlling dietary intake of long chain fatty acids, thereby avoiding the build up of toxic metabolic byproducts. Medium chain triglycerides are also used as an alternative energy source to long chain fatty acids. Many patients who receive treatment develop normally although vigilance is required to detect the onset of a metabolic crisis, which can be induced by stressors such as infection and fasting.

    Treatment for those identified through screening and on presentation of acute metabolic crises involves strict dietary modifications, substitutions and supplements. An emergency regimen is available for LCHADD/MTP deficiency patients undergoing metabolic crisis. Metabolic crises may require hospital admission even when the emergency regimen has been employed, depending on the specific clinical presentation.

11. **There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.**

There is expert consensus and evidence from high quality observational studies that individuals who have LCHADD/MTP deficiency are at increased risk of death or severe metabolic decompensation and that they should be offered dietary advice and management with the emergency regimen during acute illnesses[5].

All individuals diagnosed with LCHADD/MTP deficiency will be offered appropriate treatment. Some patients have some residual enzyme activity, so may have a milder clinical phenotype.

12. **Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.**

    There are specialised services in most regions in England. A screening programme may simplify the diagnostic process for some of the infants and ensure that positive screening results are immediately flagged up by the specialised services.

The Screening Programme

13. **There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.**

    There are no randomised trials of LCHADD/MTP deficiency screening. There is increasing evidence that mortality is lower in screened cases than in those presenting clinically, though the prognosis is uncertain[5,6].

    LCHADD/MTP deficiency screening is not being proposed for the purpose of informing reproductive choices.

    Information provided to parents of screen positives has been developed. Pre-screen information, and leaflets for parents and healthcare professionals for screen positive

results have been developed. Multiple translations and audio translations of patient information materials are available, as is a website providing information. Parents/carers are supported by clinicians and allied health professionals in understanding and developing appropriate care routines as required.

14. **There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.**

LCHADD/MTP deficiency screening is similar to screening for other disorders currently offered for which public acceptance has been demonstrated.

In a study conducted prior to the start of the ENBS evaluation it was shown that of 160 parents, 93% supported expansion of screening including LCHADD[3]. Parent groups contacted through CLIMB (Children Living with Inherited Metabolic Diseases, registered charity 1089588) took part in the study.

The screening test involves no additional procedure or effort on behalf of the performing health professional, and as the existing newborn screening tests are already deemed socially and ethically acceptable there should be no issue in expanding the range of disorders being tested for from a social and ethical perspective. There is some additional laboratory time and resource required for the screening test.

Diagnostic procedures involve collection of blood, urine, and DNA for mutation analysis. These are routinely seen as clinically, ethically and socially acceptable. In some cases lymphocyte and occasionally fibroblast assays may also be used. Fibroblast assays require a skin biopsy.

Parents may experience significant distress in learning that their child may have an illness. This is difficult to avoid but qualitative research carried out during the MCADD screening proposal in 2006 suggests that the vast majority of parents perceived the benefits of screening to outweigh any short-term psychosocial outcomes they had experienced. As part of the ENBS project we are undertaking a qualitative study to discuss with parents the timing and methods of communication such that the timing and methods can be optimised.

15. **The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).**

There is increasing evidence that mortality is lower in screened cases of LCHADD[5]/MTP[6] deficiency than in those presenting clinically, though the prognosis is not clear.

The screening test requires no additional procedure other than routine newborn screening (the heel prick test) that is already tested for. The diagnostic procedures require urine, DNA and blood samples, so cause minimal harm if correctly collected.

Treatment for the disorder is dietary, with an emergency regimen that may include hospital admission in addition to dietary treatment.

In a willingness to pay study conducted prior to the ENBS project it was shown that 93% of parents supported screening[3], suggesting that parents believe the benefits of screening outweigh physical and psychological harm. During the ENBS project only 0.05% of parents declined screening, thereby further supporting the suggestion that parents believe the benefits of screening outweigh the harms

The psychological implications of the test and diagnostic procedures are parental anxiety and helplessness between the confirmation of a positive screen result and the diagnostic outcome. The ENBS pilot is conducting a qualitative communication research study through consultation with parents of true and false positives, and clinicians, to optimise the communication of results and information in order to minimise anxiety. The outcome of this study will inform the timing and nature of information flow should the National Screening Committee decide to roll out screening for this condition nationally.

16. **The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.**

The health economic evaluation of the expanded newborn screening programme indicates that national screening for LCHADD/MTP would be cost saving.

17. **All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.**

    Suitable treatment options already exist.

    Improved education to facilitate rapid diagnosis upon clinical presentation would be difficult to achieve given the rarity of the disorder, and is not likely to be economically viable or result in greatly improved outcomes. Clinical presentation is usually during the first episode of metabolic decompensation. Screening may enable pre-emptive treatment before affected individuals have episodes of metabolic decompensation so may avoid death or the development of lifelong disabilities with associated treatment costs.

18. **There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.**

    An External Quality Assurance (EQA) process is already in place for other conditions that are screened and diagnosed in similar ways to those proposed for LCHADD/MTP deficiency. Throughout the ENBS project we have developed QA measures for LCHADD/MTP deficiency that could be rolled out nationally. We are currently working to minimise variation between screening labs and to standardise calibrations. If LCHADD/MTP deficiency screening is implemented nationally, these QA measures and the standardisation activities that are underway will inform the implementation.

19. **Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.**

Our assessment shows that the additional screening burden associated with screening for LCHADD/MTP deficiency can be absorbed into existing facilities and hardware.

As all diagnostically confirmed cases of LCHADD/MTP deficiency would be expected to eventually engage with emergency or non emergency services during the first metabolic crisis if they hadn't already been identified by screening, identifying patients by screening could potentially reduce the staff/facilities implications by reducing the likelihood of metabolic crises and emergency service involvement.

If LCHADD/MTP deficiency screening is implemented across England the implementation itself will require short-term funding for management, and staff included in the screening and diagnostic pathways would require a small amount of information and training. The information and training could be adapted from the materials developed as part of the ENBS evaluation.

20. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.

A parent/professional information leaflet has been developed for all parents who agree to their child being screened. Feedback on these leaflets has been requested from various sources both during and since their development. Written and oral translations are available in 17 languages.

Disease-specific leaflets have been developed for parents and professionals to be given in the case of a screen positive result (the 'condition suspected' leaflet).

Disease-specific leaflets have been developed for parents and professionals to be given upon the confirmation of a true positive result (the 'condition confirmed' leaflet).

As part of the ENBS pilot a qualitative communication study is underway to confirm the timing and usefulness of information provision from the perspectives of screen positive parents, and healthcare professionals.

21. **Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.**

We propose that all babies born in England be offered LCHADD/MTP deficiency screening so eligibility for newborn screening cannot be extended further.

The interval between birth and screening may be subject to scrutiny for this and other conditions already included within the programme.

During the ENBS study a baby with LCHADD/MTP deficiency was identified clinically before screening, and whom screening would not have identified. As a result the screening cut off value was lowered. Data collection on false negatives is ongoing although no further false negative LCHADD/MTP deficiency cases have yet presented. Due to the rarity of the condition the cut off value should be re-assessed in the instance of implementation of national screening.

22. **If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members.**

Screening is not for a mutation, though mutation analysis is performed as part of the diagnostic process. LCHADD is an autosomal recessive disorder, so by implication it is assumed that both parents of an affected child are carriers of a mutation in one of the genes that encodes the proteins that constitute the dysfunctional complex. Siblings may also carry mutations. Parents will be given genetic counselling and support should they choose to have more children, and all siblings can be tested.

It is assumed that all LCHADD sufferers in the UK will present clinically and eventually be diagnosed, at which point the parents' carrier status would be known anyway; screening may hasten the inevitable diagnosis.

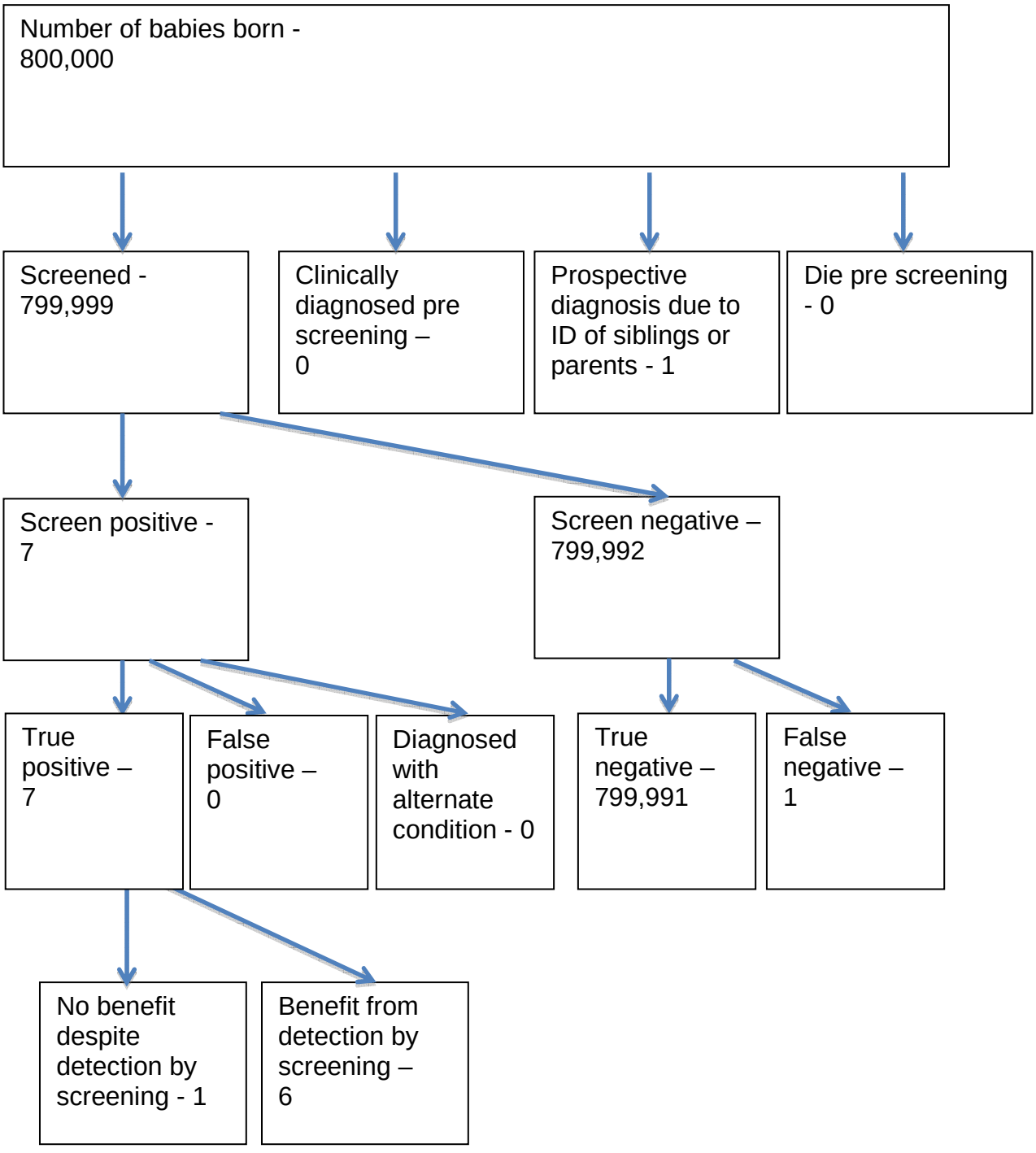
References for Appendix C

1. Burton H and Moorthie S, 2010. Expanded Newborn Screening: a review of the evidence. PHG Foundation. ISBN 978-1-907198-03-8.
2. Moorthie S, Cameron L, Sagoo G and Burton H, 2013. Birth prevalence of five inherited metabolic disorders. PHG Foundation. ISBN to be confirmed.
3. Dixon S, Shackley P, Bonham J and Ibbotson R, 2012. Putting a value on the avoidance of false positive results when screening for inherited metabolic disease in the newborn. J Inherit Metab Dis. 35(1):169-76.
4. Ijist L, Ruiter J, Hoovers J, Jakobs M and Wanders R, 1996. - Common Missense Mutation G1528C in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency. Characterization and Expression of the Mutant Protein, Mutation Analysis on Genomic DNA and Chromosomal Localization of the Mitochondrial Trifunctional Protein alpha Subunit Gene. J Clin Invest. 1996;98(4):1028–1033.
5. Sykut-Cegielska J, Gradowska W, Piekutowska-Abramczuk D, Andresen B, Olsen R, Oltarzewski M, Pronicki M, Pajdowska M, Bogdanska A, Jablonska E, Radomyska B, Kusmierska K, Krajewska-Walasek M, Gregersen N and Pronicka E, 2011. Urgent metabolic service improves survival in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency detected by symptomatic identification and pilot newborn screening. J Inherit Metab Dis. 2011 Feb;34(1):185-95.
6. Sperk A, Mueller M, and Spiekerkoetter U, 2010. Outcome in six patients with mitochondrial trifunctional protein disorders identified by newborn screening. Mol Genet Metab. 2010 Oct-Nov;101(2-3):205-7.

Appendix D

An appraisal of the condition Glutaric Aciduria type 1 (GA1) against the National Screening Committee Criteria

Figure 1 - Approximate instances of each eventuality GA1



The Condition – GA1

1. The condition should be an important health problem

GA1 is currently screened for in the USA and widely across Europe. GA1 was estimated to occur in the UK at a rate of 1:109,191[1]. The recent systematic review by the PHG foundation revised this estimation to 1:96,154[2]. The former estimation was used throughout the ENBS project.

Although this individual disease is rare, the consequences for the child and family are severe, and a high proportion of the children will require expensive care from health services, education and social care services. This is particularly the case as treatments improve survival; survival may be with disability, and the burden of care extends for life.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.

The GA1 prevalence in the UK is estimated to be 1:96,154[2].

Whilst lacking the strength of epidemiological evidence using large numbers, the aetiology of GA1 has been studied in detail in individuals, so there is a precise understanding of the underlying abnormal biochemistry (abnormal levels of metabolites, enzyme activity and underlying genetic defect).

GA1 is an autosomal recessive genetic condition caused by mutations in the glutaryl CoA dehydrogenase (*GCHD*) gene, which encodes an enzyme. GA1 patients are therefore unable to correctly metabolise the amino acids lysine, hydroxylysine and tryptophan. Breakdown products accumulate and cause damage to the brain and other organs.

GA1 varies in severity. There is little relation between metabolite levels, enzyme activity and severity or outcome of disease; genotype appears to be the best predictor. Some GA1 patients have a milder phenotype, or remain asymptomatic into

adulthood, or possibly for their whole life. It is known that some symptomatic patients have remained undiagnosed with GA1 in the past[3].

GA1 patients can be classified according to whether they've suffered an encephalopathic crisis or not. About 70% of patients have an encephalopathic crisis, most commonly at around 9 months, with 95% by 2 years of age. Encephalopathic crisis is usually triggered by an infection and usually leads to permanent movement and muscle tone abnormalities, and occasionally mental retardation. A proportion of patients develop these abnormalities without suffering a reported encephalopathic crisis. About 50% of clinically diagnosed patients die before the age of 25 years. The risk of encephalopathic crisis decreases after early childhood.

The proposed screening analysis involves assaying the levels of glutaryl carnitine (C5-DC) in the patient's blood spot sample, with diagnosis based around blood and urine levels of analytes and DNA analysis.

3. **All the cost-effective primary prevention interventions should have been implemented as far as practicable.**

   GA1 is a rare recessive genetic condition and is not amendable to primary preventative measures. Screening can detect the condition and lead to suitable treatment following diagnosis. Prenatal and pre-implantation diagnosis may be appropriate for individual families identified as being at high risk, but pre-natal or pre-implantation diagnosis is not suitable at the population level.

4. **If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.**

   As GA1 is autosomal recessive, both parents of an affected child are presumed to be carriers. Parents and siblings can choose to have their carrier status confirmed, and genetic counselling and support given for future reproductive choices.

The Test

5. **There should be a simple, safe, precise and validated screening test.**

Elevated levels of particular analytes are screened by tandem mass spectrometry. This is performed in laboratories all over the world and the methodology has been shown to be simple and safe. The infrastructure already exists, including sample collection. External quality assurance and CE marked reagents are available.

As part of the ENBS project the laboratories across the six sites have worked closely together to optimise and standardise screening and calibrations. The positive predictive value (PPV) during the ENBS project was 100%.

A proportion of GA1 patients (up to 10%) do not have abnormal metabolites and may be missed by newborn screening[4,5].

6. **The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.**

Information from existing screening programmes can give some indication of test values in Caucasian populations. However, this data cannot be directly extrapolated to the UK as differences in age at screening and method of assay calibration will have an impact on the values.

A suitable cut off level has been defined and agreed across the six screening laboratories as part of the ENBS project. The PPV during the ENBS project was 100%.

A proportion of GA1 patients (up to 10%) do not have abnormal metabolites and may be missed by newborn screening[4,5].

7. **The test should be acceptable to the population.**

No additional procedure or additional blood is required.

During the Expanded Newborn Screening programme the number of parents opting out of the additional screening was recorded, and was found to be 0.05% across the programme by the final quarter, suggesting the test is acceptable to the large majority of the public

A contingent value study conducted among 160 families in South Yorkshire indicated that 93% would welcome a newborn screening programme including GA1[6].

8. **There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.**

   Further diagnostic investigations on individuals with a positive screening test include plasma acylcarnitine analysis and urinary organic acids, as well as mutation analysis. Clearly defined diagnostic protocols have been developed within the ENBS project through consultation with multidisciplinary project team members across all sites. The confirmatory assays are externally quality assured and well know to diagnostic laboratories. The protocols developed by the ENBS project are similar to those existing for disorders that are already screened for.

9. **If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out.**

   The screening test is not for a mutation. Screen positive cases are investigated further, with mutation analysis forming part of the diagnostic process.

The Treatment

10. **There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.**

If diagnosed in a timely manner and treated with a special diet and emergency regimen, the development of acute encephalopathic crises can be prevented in 65–95% of children[7,8,9,10]. Neurological outcomes are best when treatment has been adhered to[11]. Treatment has little effect once neuropathic symptoms have occurred[7,12]. The risk of encephalopathic crisis decreases greatly after early childhood.

There is little relation between metabolite levels, enzyme activity and severity or outcome of disease. Genotype appears to be the best predictor. A proportion of GA1 patients (up to 10%) do not have abnormal metabolites and may be missed by newborn screening[4,5].

Agreed treatment guidelines were developed and formally adopted for the ENBS pilot study.

Treatment for GA1 is through a low protein, lysine-restricted diet, with supplementation, and carnitine to assist detoxification. An emergency regimen of oral or intravenous glucose is available for times of intercurrent illness in order to decrease the likelihood of metabolic crisis. Hospital admission may be required even when the emergency regimen has been employed, depending on the specific clinical presentation. Although the risk of encephalopathic crisis diminishes greatly after early childhood many adults continue to follow a restricted diet.

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

There is expert consensus and evidence from high quality observational studies that individuals who have GA1 are at increased risk of death or severe metabolic crisis and that they should be offered dietary advice and management with the emergency regimen during acute illnesses.

All individuals diagnosed with GA1 will be offered appropriate treatment, which is dietary, carnitine, and emergency regimen. Some GA1 patients may have a milder clinical phenotype. The dietary treatment can be modified for each patient to obtain biochemical control.

12. **Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.**

    There are specialised services in most regions in England. A screening programme may simplify the diagnostic process for some of the infants and ensure that positive screening results are immediately flagged up by the specialised services.

The Screening Programme

13. **There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.**

    There are no randomised trials of GA1 screening.
    Early treatment, and use of the emergency regimen can improve outcomes. The risk of metabolic crisis leading to permanent damage can be reduced in 65-95% of children by appropriate treatment[7,8,9,10]. Neurological outcomes are best when treatment has been adhered to[11]. A proportion of GA1 patients (up to 10%) do not have abnormal metabolites and may be missed by newborn screening[4,5].

    GA1 screening is not being proposed for the purpose of informing reproductive choices.

    Information provided to parents of screen positives has been developed. Pre-screen information, and leaflets for parents and healthcare professionals for screen positive results have been developed. Multiple translations and audio translations of patient information materials are available, as is a website providing information.
    Parents/carers are supported by clinicians and Allied Health Professionals in understanding and developing appropriate care routines as required.

14. **There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.**

GA1 screening is similar to screening for other disorders currently offered for which public acceptance has been demonstrated.

In a study conducted prior to the start of the ENBS evaluation it was shown that of 160 parents, 93% supported expansion of screening including GA1[6]. Parent groups contacted through CLIMB (Children Living with Inherited Metabolic Diseases, registered charity 1089588) took part in the study.

The screening test involves no additional procedure or effort on behalf of the performing health professional, and as the existing newborn screening tests are already deemed socially and ethically acceptable there should be no issue in expanding the range of disorders being tested for from a social and ethical perspective. There is some additional laboratory time and resource required for the screening test.

Diagnostic procedures involve collection of blood, urine, and DNA for mutation analysis. These are routinely seen as clinically, ethically and socially acceptable.

Parents may experience significant distress in learning that their child may have an illness. This is difficult to avoid but qualitative research carried out during the MCADD screening proposal in 2006 suggests that the vast majority of parents perceived the benefits of screening to outweigh any short-term psychosocial outcomes they had experienced. As part of the ENBS project we are undertaking a qualitative study to discuss with parents the timing and methods of communication such that the timing and methods can be optimised.

15. **The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).**

If diagnosed in a timely manner and treated with a special diet and emergency regimen, the development of acute encephalopathic crises can be prevented in 65–

95% of children[7,8,9,10]. Treatment has little effect once neuropathic symptoms have occurred[7]. The risk of encephalopathic crisis decreases greatly after early childhood. A proportion of GA1 patients (up to 10%) do not have abnormal metabolites and may be missed by newborn screening[4,5].

The screening test requires no additional procedure other than the routine heel prick sample that is already taken and tested for other approved newborn screening conditions. The diagnostic procedures require urine, DNA and blood samples, so cause minimal harm if correctly collected.

Treatment for the disorder is dietary, with an emergency regimen that may include hospital admission for initial stabilisation and during acute decompensation.

In a willingness to pay study conducted prior to the ENBS project it was shown that 93% of parents would accept screening, and were willing to pay a mean of £178[6], suggesting that parents believe the benefits of screening outweigh physical and psychological harm. During the ENBS project only 0.05% of parents declined screening, thereby further supporting the suggestion that parents believe the benefits of screening outweigh the harms.

The psychological implications of the test and diagnostic procedures are parental anxiety and helplessness between the confirmation of a positive screen result and the diagnostic outcome. The ENBS pilot is conducting a qualitative communication research study through consultation with parents of true and false positives, and clinicians, to optimise the communication of results and information in order to minimise anxiety. The outcome of this study will inform the timing and nature of information flow should the National Screening Committee decide to roll out screening for this condition nationally.

16. **The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e.. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.**

The health economic evaluation of the expanded newborn screening programme indicates that national screening for GA1 would be cost saving.

17. **All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.**

    Suitable treatment options already exist.

    Improved education to facilitate rapid diagnosis upon clinical presentation would be difficult to achieve given the rarity of the disorder, and is not likely to be economically viable or result in greatly improved outcomes. Clinical presentation is usually during the first episode of encephalopathic crisis. Screening may enable pre-emptive treatment before affected individuals have episodes of metabolic decompensation so may avoid the development of lifelong disabilities with high treatment costs.

18. **There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.**

    An External Quality Assurance (EQA) process is already in place for other conditions that are screened and diagnosed in similar ways to those proposed for GA1. Throughout the ENBS project we have developed QA measures for GA1 that could be rolled out nationally. We are currently working to minimise variation between screening labs and to standardise calibrations. If GA1 screening is implemented nationally, these QA measures and the standardisation activities that are underway will inform the implementation.

19. **Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.**

Our assessment shows that the additional screening burden associated with screening for GA1 can be absorbed into existing facilities and hardware.

As all diagnostically confirmed cases of GA1 would be expected to engage with emergency services during the first metabolic crisis if they hadn't already been identified by screening, identifying patients by screening could potentially reduce the staff/facilities implications by reducing the likelihood of metabolic crises and emergency service involvement.

If GA1 screening is implemented across England the implementation itself will require short-term funding for management, and staff included in the screening and diagnostic pathways would require a small amount of information and training. The information and training could be adapted from the materials developed as part of the ENBS evaluation.

20. **Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.**

A parent/professional information leaflet has been developed for all parents who agree to their child being screened. Feedback on these leaflets has been requested from various sources both during and since their development. Written and oral translations are available in 17 languages.

Disease-specific leaflets have been developed for parents and professionals to be given in the case of a screen positive result (the 'condition suspected' leaflet).

Disease-specific leaflets have been developed for parents and professionals to be given upon the confirmation of a true positive result (the 'condition confirmed' leaflet).

As part of the ENBS pilot a qualitative communication study is underway to confirm the timing and usefulness of information provision from the perspectives of screen positive parents, and healthcare professionals.

21. **Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.**

We propose that all babies born in England be offered GA1 screening so eligibility for newborn screening cannot be extended further.

The interval between birth and screening may be subject to scrutiny for this and other conditions already included within the programme.

As part of the ENBS pilot we are collating data on screen false negatives. Data collection is ongoing although no false negative GA1 cases have yet presented.

The ENBS project suggests that the cut off levels are appropriate, with the caveat that the low frequency of screen positives means that ongoing monitoring to ensure rigorous statistical support for the cut off level is required.

22. **If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members.**

Screening is not for a mutation. GA1 is an autosomal recessive disorder, so by implication it is assumed that both parents of an affected child are carriers of a mutation in one of the genes that encodes the proteins that constitute the dysfunctional complex. Siblings may also carry mutations. Parents will be given genetic counselling and support should they choose to have more children, and all siblings can be tested.

It is assumed that all GA1 sufferers in the UK will present clinically and eventually be diagnosed, at which point the parents' carrier status would be known anyway; screening may hasten the inevitable diagnosis.

References for Appendix D

1. Burton H and Moorthie S, 2010. Expanded Newborn Screening: a review of the evidence. PHG Foundation. ISBN 978-1-907198-03-8.

2. Moorthie S, Cameron L, Sagoo G and Burton H, 2013. Birth prevalence of five inherited metabolic disorders. PHG Foundation. ISBN to be confirmed.

3. Kölker S, Garbade S, Boy N, Maier E, Meissner T, Mühlhausen C, Hennermann J, Lücke T, Häberle J, Baumkötter J, Haller W, Muller E, Zschocke J, Burgard P, and Hoffmann G, 2007. Decline of acute encephalopathic crises in children with glutaryl-CoA dehydrogenase deficiency identified by newborn screening in Germany. Pediatr Res. 62:357-63.

4. Gallagher R, Cowan T, Goodman S, and Enns G,2005. Glutaryl CoA dehydrogenase deficiency and newborn screening: Retrospective analysis of a low excretor provides further evidence that some cases may be missed. Mol Genet Metabol 86: 417-420

5. Treacy E, Lee-Chong A, Roche G, Lynch G, Ryan S and Goodman S, 2003. Profound neurological presentation resulting from homozygosity for a mild glutaryl-CoA dehydrogenase mutation with a minimal biochemical phenotype. J Inherit Metab Dis. 26**:** 72-74

6. Dixon S, Shackley P, Bonham J and Ibbotson R, 2012. Putting a value on the avoidance of false positive results when screening for inherited metabolic disease in the newborn. J Inherit Metab Dis. 35(1):169-76.

7. Kölker S, Garbade SF, Greenberg CR, Leonard JV, Saudubray JM, Ribes A, Kalkanoglu HS, Lund AM, Merinero B, Wajner M, Troncoso M, Williams M, Walter JH, Campistol J, Martí-Herrero M, Caswill M, Burlina AB, Lagler F, Maier EM, Schwahn B, Tokatli A, Dursun A, Coskun T, Chalmers RA, Koeller DM, Zschocke J, Christensen E, Burgard P, and Hoffmann GF, 2006. Natural History, Outcome, and Treatment Efficacy in Children and Adults with Glutaryl-CoA Dehydrogenase Deficiency. Pediatric Research (2006) 59, 840–847.

8. Strauss K, Puffenberger E, Robinson D, and Morton D, 2003. Type I glutaric aciduria, part 1: natural history of 77 patients. Am J Med Gen Semin Med Genet 121:38–52

9. Hoffmann G, Athanassopoulos S, Burlina A, Duran M, de Klerk J, Lehnert W, Leonard J, Monavari A, Müller E, Muntau A, Naughten E, Plecko-Starting B, Superti-

Furga A, Zschocke J, and Christensen E, 1996. Clinical course, early diagnosis, treatment, and prevention of disease in glutaryl-CoA dehydrogenase deficiency. Neuropediatrics 27:115–123

10. Naughten E, Mayne P, Monavari A, Goodman S, Sulaiman G, and Croke D, 2004. Glutaric aciduria type I, outcome in the Republic of Ireland. J Inherit Metab Dis 27:917–920

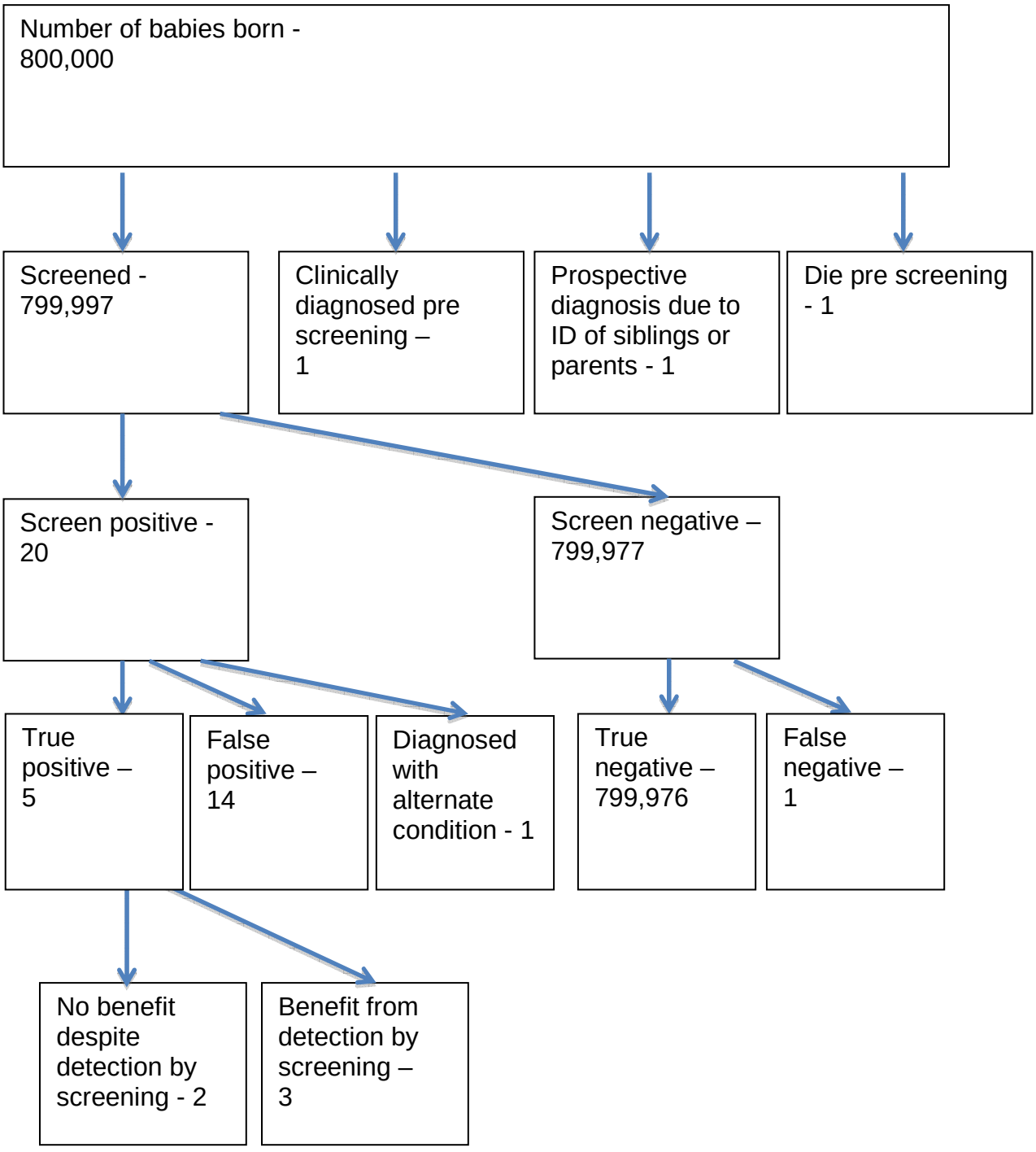
11. Heringer J, Boy SP, Ensenauer R, Assmann B, Zschocke J, Harting I, Lücke T, Maier EM, Mühlhausen C, Haege G, Hoffmann GF, Burgard P, Kölker S, 2010. Use of guidelines improves the neurological outcome in glutaric aciduria type I. Ann Neurol. 2010 Nov;68(5):743-52.

12. Kyllerman M, Skjeldal O, Christensen E, Hagberg G, Holme E, Lonnquist T, Skov L, Rotwelt T and Dobleln U, 2004. Long-term follow up, neurological outcome and survival rate in 28 Nordic patients with glutaric aciduria type 1. Eur J Paediatr Neurol. 2004;8(3):121-9.

Appendix E

An appraisal of the condition isovaleric acidemia (IVA) against the National Screening Committee Criteria

Figure 1 - Approximate instances of each eventuality IVA



The Condition – IVA

1. The condition should be an important health problem

IVA is currently screened for in the USA and widely across Europe. IVA was estimated to occur in the UK at a rate of 1:155,396[1]. The recent systematic review by the PHG foundation revised this estimation to 1:123,457[2]. The former estimation was used throughout the ENBS project.

Although this individual disease is rare, the consequences for the child and family can be severe, and depending on the severity of the condition, children will require expensive care from health services, education and social care services. This is particularly the case as treatments improve survival; survival may be with disability, and the burden of care extends for life.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.

The IVA prevalence in the UK is 1:123,457[2].

Whilst lacking the strength of epidemiological evidence using large numbers, the aetiology of IVA has been studied in detail in individuals, so there is a precise understanding of the underlying abnormal biochemistry (abnormal levels of metabolites, enzyme activity and underlying genetic defect).

IVA is an autosomal recessive genetic condition caused by mutations in the isovaleryl-CoA dehydrogenase (*IVD*) gene. Due to the mutation IVA patients are deficient in an enzyme that is involved in the catabolism of the amino acid leucine, thereby allowing toxic compounds, including isovaleric acid, to build up in the body. In severe cases this presents in the first two weeks of life, leading to vomiting and lethargy, and progressing to seizures and/or coma, and possibly death. In the long term, IVA can be associated with cognitive defects and mild motor dysfunction. Less severely affected patients may present at a later age, or with chronic symptoms such as failure to thrive or developmental delay, usually within the first year. Clinical

diagnoses are usually between 2 weeks of age and 5 years. Many affected patients are prone to acute decompensation following minor illnesses, which may prove fatal.

The proposed screening analysis involves assaying the levels of acylcarnitine (C5) in the patient's blood spot sample, with diagnosis based around blood and urine levels of analytes and DNA analysis.

3. **All the cost-effective primary prevention interventions should have been implemented as far as practicable.**

   IVA is a rare recessive genetic condition and is not amendable to primary preventative measures. Screening can detect the condition and lead to suitable treatment following diagnosis. Prenatal and pre-implantation diagnosis may be appropriate for individual families identified as being at high risk, but pre-natal or pre-implantation diagnosis is not suitable at the population level.

4. **If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.**

   As IVA is autosomal recessive, both parents of an affected child are presumed to be carriers. Parents and siblings can choose to have their carrier status confirmed, and genetic counselling and support given for future reproductive choices.

The Test

5. **There should be a simple, safe, precise and validated screening test.**

   Elevated levels of particular analytes are screened by tandem mass spectrometry. This is performed in laboratories all over the world and the methodology has been shown to be simple and safe. The infrastructure already exists, including sample collection. External quality assurance and CE marked reagents are available.

As part of the ENBS project the laboratories across the six sites have worked closely together to optimise and standardise screening and calibrations. The positive predictive value (PPV) during the ENBS project was 22%.

6. **The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.**

Information from existing screening programmes can give some indication of test values in Caucasian populations. However, this data cannot be directly extrapolated to the UK as differences in age at screening and method of assay calibration will have an impact on the values.

A suitable cut off level has been defined and agreed across the six screening laboratories as part of the ENBS project. The positive predictive value for IVA during the ENBS evaluation was 22%.

7. **The test should be acceptable to the population.**

No additional procedure or additional blood is required.

During the Expanded Newborn Screening programme the number of parents opting out of the additional screening was recorded, and was found to be 0.05% across the programme by the final quarter, suggesting the test is acceptable to the large majority of the public

A contingent value study conducted among 160 families in South Yorkshire indicated that 93% would welcome a newborn screening programme including IVA[3].

8. **There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.**

Further diagnostic investigations on individuals with a positive screening test include urine isovalerylglycine analysis and urinary organic acids, plasma acylcarnitine

analysis as well as mutation analysis. Clearly defined diagnostic protocols have been developed within the ENBS project through consultation with multidisciplinary project team members across all sites. The confirmatory assays are externally quality assured and well know to diagnostic laboratories. The protocols developed by the ENBS project are similar to those existing for disorders that are already screened for.

9. **If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out.**

   The screening test is not for a mutation. Screen positive cases are investigated further, with DNA testing forming part of the diagnostic process. DNA testing includes testing for a benign mutation[4], which if detected, would indicate that the patient would remain asymptomatic.

The Treatment

10. **There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.**

    Early detection and treatment could help avoid episodes of metabolic decompensation, which may result in death. Due to the range in severity and presentation of IVA, it is possible that detection through newborn screening may identify and medicalise patients who would otherwise have remained asymptomatic.

    Agreed treatment guidelines were developed and formally adopted for the ENBS pilot study.

    Treatment for IVA is through a low protein diet, with supplementation and carnitine and/or glycine. An emergency regimen is available for times of intercurrent illness in order to decrease the likelihood of metabolic crisis. Hospital admission may be required even when the emergency regimen has been employed, depending on the

specific clinical presentation. Mild cases may be treated without dietary changes but with advice in relation to the use of an emergency regimen.

11. **There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.**

    There is expert consensus and evidence from high quality observational studies that individuals with IVA who have metabolic crises are at increased risk of death or long term effects. However, it is difficult to determine which IVA patients are at risk of metabolic crises. Those who are known to be heterozygous for a benign mutation are thought to remain asymptomatic, so should be observed rather than treated.

12. **Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.**

    There are specialised services in most regions in England. A screening programme may simplify the diagnostic process for some of the infants and ensure that positive screening results are immediately flagged up by the specialised services.

The Screening Programme

13. **There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (egg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.**

    There are no randomised trials of IVA screening.

Early treatment could avert metabolic crisis, thereby reducing the likelihood of long-term effects, short-term illness, or condition-related death.

IVA screening is not being proposed for the purpose of informing reproductive choices.

Information provided to parents of screen positives has been developed. Pre-screen information, and leaflets for parents and healthcare professionals for screen positive results have been developed. Multiple translations and audio translations of patient information materials are available, as is a website providing information. Parents/carers are supported by clinicians and allied health professionals in understanding and developing appropriate care routines as required.

14. **There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.**

IVA screening is similar to screening for other disorders currently offered for which public acceptance has been demonstrated.

In a study conducted prior to the start of the ENBS evaluation it was shown that of 160 parents, 93% supported expansion of screening[3].
Parent groups contacted through CLIMB (Children Living with Inherited Metabolic Diseases, registered charity 1089588) took part in the study.

The screening test involves no additional procedure or effort on behalf of the performing health professional, and as the existing newborn screening tests are already deemed socially and ethically acceptable there should be no issue in expanding the range of disorders being tested for from a social and ethical perspective. There is some additional laboratory time and resource required for the screening test.

Diagnostic procedures involve collection of blood, urine, and DNA for mutation analysis. These are routinely seen as clinically, ethically and socially acceptable.

Parents may experience significant distress in learning that their child may have an illness. This is difficult to avoid but qualitative research carried out during the MCADD screening proposal in 2006 suggests that the vast majority of parents perceived the benefits of screening to outweigh any short-term psychosocial outcomes they had experienced. As part of the ENBS project we are undertaking a qualitative study to discuss with parents the timing and methods of communication such that the timing and methods can be optimised.

15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).

If severe IVA is diagnosed in a timely manner and treated with a special diet and emergency regimen, metabolic crisis and the associated long-term effects may be avoided. Diagnosis and long-term treatment in patients with a lower risk may be managed by observation, education (for the caregivers, and later the individual), and provision of the emergency regimen.

The screening test requires no additional procedure other than the routine heel prick sample that is already taken and tested for other approved newborn screening conditions. The diagnostic procedures require urine, DNA and blood samples, so cause minimal harm if correctly collected.

Treatment for the disorder is dietary, with an emergency regimen that may include hospital admission for initial stabilisation and during acute decompensation.

In a willingness to pay study conducted prior to the ENBS project it was shown that 93% of parents would accept screening, and were willing to pay a mean of £178[4], suggesting that parents believe the benefits of screening outweigh physical and psychological harm. During the ENBS project only 0.05% of parents declined screening, thereby further supporting the suggestion that parents believe the benefits of screening outweigh the harms.

The psychological implications of the test and diagnostic procedures are parental anxiety and helplessness between the confirmation of a positive screen result and the diagnostic outcome. The ENBS pilot is conducting a qualitative communication research study through consultation with parents of true and false positives, and

clinicians, to optimise the communication of results and information in order to minimise anxiety. The outcome of this study will inform the timing and nature of information flow should the National Screening Committee decide to roll out screening for this condition nationally.

16. **The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.**

    The health economic evaluation of the expanded newborn screening programme indicates that national screening for IVA would be cost saving.

17. **All other options for managing the condition should have been considered (egg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.**

    Suitable treatment options already exist.

    Improved education to facilitate rapid diagnosis upon clinical presentation would be difficult to achieve given the rarity of the disorder, and is not likely to be economically viable or result in greatly improved outcomes. Clinical presentation is usually during the first episode of metabolic crisis. Screening may enable pre-emptive treatment before affected individuals have episodes of metabolic decompensation so may avoid the development of lifelong disabilities with high treatment costs.

18. **There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.**

An External Quality Assurance (EQA) process is already in place for other conditions that are screened and diagnosed in similar ways to those proposed for IVA. Throughout the ENBS project we have developed QA measures for IVA that could be rolled out nationally. We are currently working to minimise variation between screening labs and to standardise calibrations. If IVA screening is implemented nationally, these QA measures and the standardisation activities that are underway will inform the implementation.

19. **Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.**

    Our assessment shows that the additional screening burden associated with screening for IVA can be absorbed into existing facilities and hardware.

    An unknown percentage of diagnostically confirmed cases of IVA would be expected to engage with emergency services during the first metabolic crisis. Identifying patients by screening could potentially reduce the staff/facilities implications by reducing the likelihood of metabolic crises and emergency service involvement.

    If IVA screening is implemented across England the implementation itself will require short-term funding for management, and staff included in the screening and diagnostic pathways would require a small amount of information and training. The information and training could be adapted from the materials developed as part of the ENBS evaluation.

20. **Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.**

    A parent/professional information leaflet has been developed for all parents who agree to their child being screened. Feedback on these leaflets has been requested from various sources both during and since their development. Written and oral translations are available in 17 languages.

Disease-specific leaflets have been developed for parents and professionals to be given in the case of a screen positive result (the 'condition suspected' leaflet).

Disease-specific leaflets have been developed for parents and professionals to be given upon the confirmation of a true positive result (the 'condition confirmed' leaflet).

As part of the ENBS pilot a qualitative communication study is underway to confirm the timing and usefulness of information provision from the perspectives of screen positive parents, and healthcare professionals.

21. **Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.**

We propose that all babies born in England be offered IVA screening so eligibility for newborn screening cannot be extended further.

The interval between birth and screening may be subject to scrutiny for this and other conditions already included within the programme.

As part of the ENBS pilot we are collating data on screen false negatives. Data collection is ongoing although no false negative IVA cases have yet presented.

The ENBS project suggests that the cut off values require scrutiny to reduce the number of false positive results.

22. **If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members.**

Screening is not for a mutation. IVA is an autosomal recessive disorder, so by implication it is assumed that both parents of an affected child are carriers of a

mutation in one of the genes that encodes the proteins that constitute the dysfunctional complex. Siblings may also carry mutations. Parents will be given genetic counselling and support should they choose to have more children, and all siblings can be tested.

An unknown percentage of IVA sufferers in the UK will present clinically and eventually be diagnosed, at which point the parents' carrier status would be known anyway; screening may hasten the inevitable diagnosis in families where the patient is symptomatic.

References for Appendix E

1. Burton H and Moorthie S, 2010. Expanded Newborn Screening: a review of the evidence. PHG Foundation. ISBN 978-1-907198-03-8.

2. Moorthie S, Cameron L, Sagoo G and Burton H, 2013. Birth prevalence of five inherited metabolic disorders. PHG Foundation. ISBN to be confirmed.

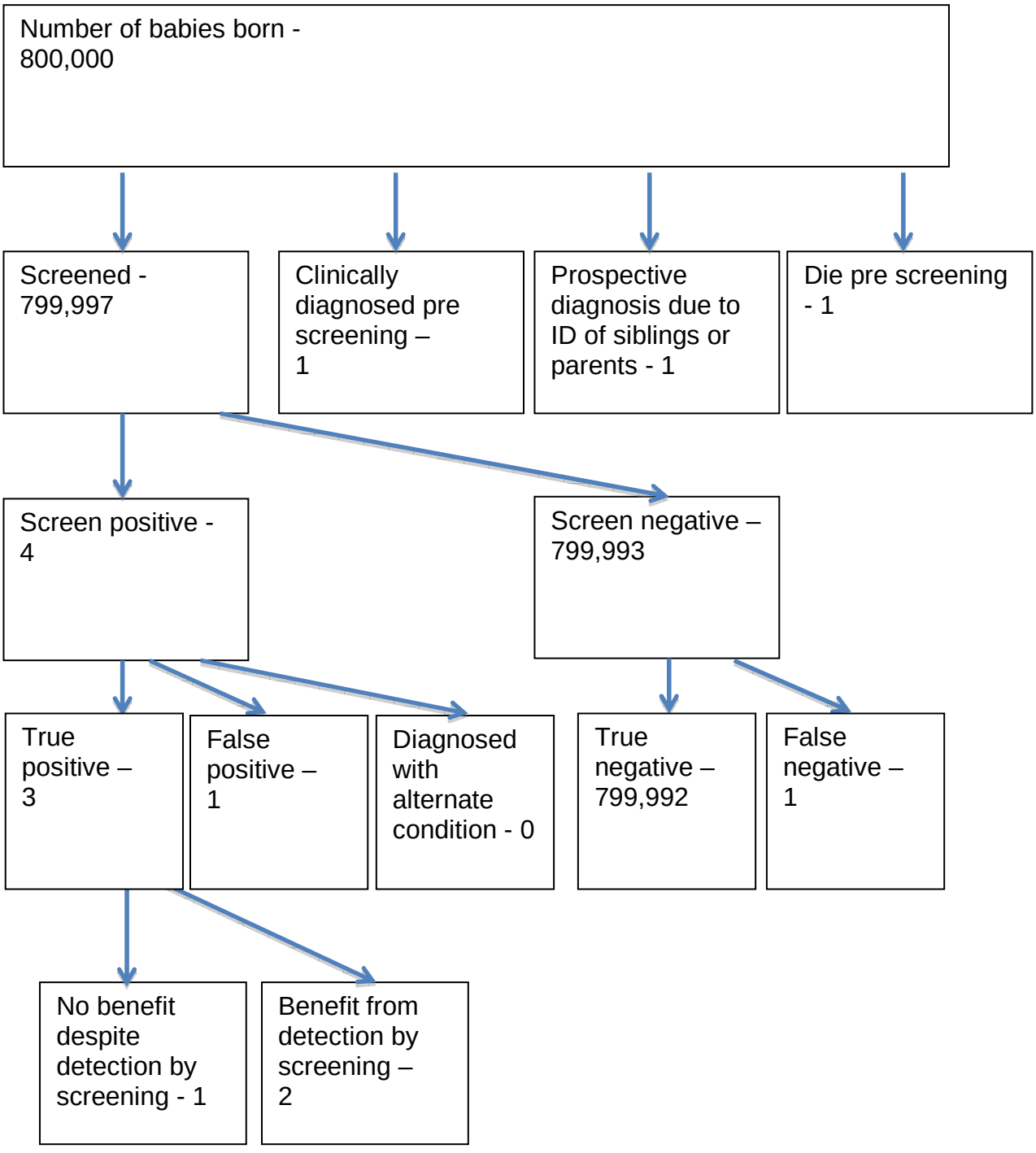
3. Dixon S, Shackley P, Bonham J and Ibbotson R, 2012. Putting a value on the avoidance of false positive results when screening for inherited metabolic disease in the newborn. J Inherit Metab Dis. 35(1):169-76.

4. Ensenauer R, Vockley J, Willard JM, Huey JC, Sass JO, Edland SD, Burton BK, Berry SA, Santer R, Grunert S, Koch HG, Marquardt I, Rinaldo P, Hahn S and Matern D, 2004. A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. Am J Hum Genet. 75(6):1136-42.

Appendix F

An appraisal of the condition Maple Syrup Urine Disease (MSUD) against the National Screening Committee Criteria

Figure 1 - Approximate instances of each eventuality - MSUD



The Condition – MSUD

1. The condition should be an important health problem

Maple Syrup Urine Disease (MSUD) is currently screened for in the USA and widely across Europe. MSUD was estimated to occur in the UK at a rate of 1:116,000[1]. The recent systematic review by the PHG foundation revised this estimation to 1:140,845[2]. The former estimation was used throughout the ENBS project.

Although this individual disease is rare, the consequences for the child and family are severe, and a high proportion of the children will require expensive care from health services, education and social care services. This is particularly the case as treatments improve survival; survival may be with disability, and the burden of care extends for life.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.

The estimated MSUD prevalence in the UK is 1:140,845[2]. Worldwide it has an estimated incidence of 1:185,000[3].

Whilst lacking the strength of epidemiological evidence using large numbers, the aetiology of MSUD has been studied in detail in individuals, so there is a precise understanding of the underlying abnormal biochemistry (abnormal levels of metabolites, enzyme activity and underlying genetic defect).

MSUD is an autosomal recessive condition caused by mutations in any of the three genes that make up the branched chain 2-keto acid dehydrogenase (BCKAD) enzyme complex. This prevents the BCKAD complex from correctly metabolising the amino acids leucine, isoleucine and valine. These branched chain amino acids and their byproducts therefore build up and reach toxic levels in the patient's blood and urine. The proposed screening analysis involves assaying the levels of leucines in

the patient's blood spot sample, with diagnosis based around blood and urine levels of analytes.

The majority of MSUD patients have a severe classic form, with little or no BCKAD complex activity. There is a milder intermediate form of MSUD, an intermittent form, and a thiamine responsive form.

MSUD patients may die during acute episodes of decompensation. Symptoms of the classic form of MSUD include neonatal encephalopathy and cerebral oedema, poor feeding, increased/decreased tone, ketoacidosis and seizures. Learning difficulties, spasticity and cortical visual impairment are also associated with classic MSUD. Onset is usually around days 2-4 of life in bottle fed babies, and can be up to 2 weeks in breastfed babies. Acute episodes of metabolic decompensation can be triggered by childhood illnesses e.g. infection. The intermediate form is typically characterised by a failure to thrive, developmental delay, and often with no ketoacidosis. Patients with the intermittent form typically present clinically in response to an infection or increase in protein intake, which may not be until 12-24 months of age. Thiamine responsive MSUD patients may present after infancy, and still require some dietary controls.

3. **All the cost-effective primary prevention interventions should have been implemented as far as practicable.**

   MSUD is a rare recessive genetic condition and is not amendable to primary preventative measures. Screening can detect the condition and lead to suitable treatment following diagnosis. Prenatal and pre-implantation diagnosis may be appropriate for individual families identified as being at high risk, but pre-natal or pre-implantation diagnosis is not suitable at the population level.

4. **If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.**

As MSUD is autosomal recessive, both parents of an affected child are presumed to be carriers. Parents and siblings can choose to have their carrier status confirmed, and genetic counselling and support given for future reproductive choices.

The Test

5. **There should be a simple, safe, precise and validated screening test.**

Elevated levels of particular analytes are screened by tandem mass spectrometry. This is performed in laboratories all over the world and the methodology has been shown to be simple and safe. The infrastructure already exists, including sample collection. External quality assurance and CE marked reagents are available.

As part of the ENBS project the laboratories across the six sites have worked closely together to optimise and standardise screening and calibrations. The positive predictive value (PPV) during the ENBS project was 50%.

6. **The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.**

Information from existing screening programmes can give some indication of test values in Caucasian populations. However, this data cannot be directly extrapolated to the UK as differences in age at screening and method of assay calibration will have an impact on the values.

A suitable cut off level has been defined and agreed across the six screening laboratories as part of the ENBS project. The PPV for MSUD during the ENBS project was 50%.

7. **The test should be acceptable to the population.**

No additional procedure or additional blood is required.

During the Expanded Newborn Screening programme the number of parents opting out of the additional screening was recorded, and was found to be 0.05% across the programme by the final quarter, suggesting the test is acceptable to the large majority of the public

A contingent value study conducted among 160 families in South Yorkshire indicated that 93% would welcome a newborn screening programme including MSUD[4].

8. **There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.**

   Further diagnostic investigations on individuals with a positive screening test include plasma amino acid analysis and urinary organic acids. Clearly defined diagnostic protocols have been developed within the ENBS project through consultation with multidisciplinary project team members across all sites. The confirmatory assays are externally quality assured and well know to diagnostic laboratories. The protocols developed by the ENBS project are similar to those existing for disorders that are already screened for.

9. **If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out.**

   Not applicable. Mutation analysis is not involved in the proposed screening or diagnostic tests.

The Treatment

10. **There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.**

Pre-emptive detection of affected newborns, before they exhibit neurologic signs of MSUD, significantly reduces lifetime risk of mental illness and global functional impairment[5,6]. Early treatment, and use of the emergency regimen can avoid long-term mental disabilities and improves survival[7].

Agreed treatment guidelines were developed and formally adopted for the ENBS pilot study.

Treatment is aimed at controlling branched chain amino acid levels (leucine, isoleucine and allo-isoleucine). Many patients who receive treatment develop normally although vigilance is required to detect the onset of a metabolic crisis, which can be induced by stressors such as infection and fasting.

Treatment for those identified through screening and on presentation of acute metabolic crises involves strict dietary modifications, substitutions and supplements. An emergency regimen in available for identified patients undergoing metabolic crisis. Metabolic crises may require hospital admission even when the emergency regimen has been employed, depending on the specific clinical presentation. Early treatment, and use of the emergency regimen can avoid long-term mental disabilities[5,6], and improves survival[7].

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

There is expert consensus and evidence from high quality observational studies that individuals who have the classic MSUD phenotype are at increased risk of death or severe metabolic decompensation and that they should be offered dietary advice and management with the emergency regimen during acute illnesses.

All individuals diagnosed with MSUD will be offered appropriate treatment, which is dietary for whichever form of MSUD is diagnosed (severe, intermediate, intermittent, or thiamine responsive), plus emergency regimen. Some MSUD patients have some residual enzyme activity, so may have a milder clinical phenotype. The dietary treatment can be modified for each patient to obtain biochemical control.

12. **Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.**

    There are specialised services in most regions in England. A screening programme may simplify the diagnostic process for some of the infants and ensure that positive screening results are immediately flagged up by the specialised services.

The Screening Programme

13. **There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.**

    There are no randomised trials of MSUD screening.

    There is increasing evidence that early treatment, and use of the emergency regimen can avoid long-term mental disabilities[5,6], and improves survival[7].

    MSUD screening is not being proposed for the purpose of informed choice.

    Information provided to parents of screen positives has been developed. Pre-screen information, and leaflets for parents and healthcare professionals for screen positive results have been developed. Multiple translations and audio translations of patient information materials are available, as is a website providing information. Parents/carers are supported by clinicians and allied health professionals in understanding and developing appropriate care routines as required.

14. **There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and**

ethically acceptable to health professionals and the public.

MSUD screening is similar to screening for other disorders currently offered for which public acceptance has been demonstrated.

In a study conducted prior to the start of the ENBS evaluation it was shown that of 160 parents, 93% supported expansion of screening including MSUD[4]. Parent groups contacted through CLIMB (Children Living with Inherited Metabolic Diseases, registered charity 1089588) took part in the study.

The screening test involves no additional procedure or effort on behalf of the performing health professional, and as the existing newborn screening tests are already deemed socially and ethically acceptable there should be no issue in expanding the range of disorders being tested for from a social and ethical perspective. There is some additional laboratory time and resource required for the screening test.

Diagnostic procedures involve collection of blood and urine. These are routinely seen as clinically, ethically and socially acceptable.

Parents may experience significant distress in learning that their child may have an illness. This is difficult to avoid but qualitative research carried out during the MCADD screening proposal in 2006 suggests that the vast majority of parents perceived the benefits of screening to outweigh any short-term psychosocial outcomes they had experienced. As part of the ENBS project we are undertaking a qualitative study to discuss with parents the timing and methods of communication such that the timing and methods can be optimised.

15. **The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).**

Pre-emptive detection of newborns, before they exhibit neurologic signs of MSUD, significantly reduces lifetime risk of mental illness and global functional impairment[5,6], and improves survival[7].

The screening test requires no additional procedure other than routine newborn screening (the heel prick test) that is already collected/carried out. The diagnostic

procedures require urine and blood samples, so cause minimal harm if correctly collected.

Treatment for the disorder is dietary, with an emergency regimen that may include hospital admission for initial stabilisation and during acute decompensation in addition to dietary treatment.

In a willingness to pay study conducted prior to the ENBS project it was shown that 93% of parents would accept screening, and were willing to pay a mean of £178[4], suggesting that parents believe the benefits of screening outweigh physical and psychological harm. During the ENBS project only 0.05% of parents declined screening, thereby further supporting the suggestion that parents believe the benefits of screening outweigh the harms.

The psychological implications of the test and diagnostic procedures are parental anxiety and helplessness between the confirmation of a positive screen result and the diagnostic outcome. The ENBS pilot is conducting a qualitative communication research study through consultation with parents of true and false positives, and clinicians, to optimise the communication of results and information in order to minimise anxiety. The outcome of this study will inform the timing and nature of information flow should the National Screening Committee decide to roll out screening for this condition nationally.

16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money). Assessment against this criterion should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.

The health economic evaluation of the expanded newborn screening programme indicates that national screening for MSUD would be cost saving.

17. **All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.**

    Suitable treatment options already exist.

    Improved education to facilitate rapid diagnosis upon clinical presentation would be difficult to achieve given the rarity of the disorder, and is not likely to be economically viable or result in greatly improved outcomes. Clinical presentation is usually during the first episode of metabolic decompensation. Screening may enable pre-emptive treatment before affected individuals have episodes of metabolic decompensation so may avoid the development of lifelong disabilities with high treatment costs.

18. **There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.**

    An External Quality Assurance (EQA) process is already in place for other conditions that are screened and diagnosed in similar ways to those proposed for MSUD. Throughout the ENBS project we have developed QA measures for MSUD that could be rolled out nationally. We are currently working to minimise variation between screening labs and to standardise calibrations. If MSUD screening is implemented nationally, these QA measures and the standardisation activities that are underway will inform the implementation.

19. **Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.**

    Our assessment shows that the additional screening burden associated with screening for MSUD can be absorbed into existing facilities and hardware.

    As all diagnostically confirmed cases of MSUD would be expected to engage with emergency services during the first metabolic crisis if they hadn't already been identified by screening, identifying patients by screening could potentially reduce the

staff/facilities implications by reducing the likelihood of metabolic crises and emergency service involvement.

If MSUD screening is implemented across England the implementation itself will require short-term funding for management, and staff included in the screening and diagnostic pathways would require a small amount of information and training. The information and training could be adapted from the materials developed as part of the ENBS evaluation.

20. **Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.**

    A parent/professional information leaflet has been developed for all parents who agree to their child being screened. Feedback on these leaflets has been requested from various sources both during and since their development. Written and oral translations are available in 17 languages.

    Disease-specific leaflets have been developed for parents and professionals to be given in the case of a screen positive result (the 'condition suspected' leaflet).

    Disease-specific leaflets have been developed for parents and professionals to be given upon the confirmation of a true positive result (the 'condition confirmed' leaflet).

    As part of the ENBS pilot a qualitative communication study is underway to confirm the timing and usefulness of information provision from the perspectives of screen positive parents, and healthcare professionals.

21. **Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.**

We propose that all babies born in England be offered MSUD screening so eligibility for newborn screening cannot be extended further.

The interval between birth and screening may be subject to scrutiny for this and other conditions already included within the programme.

As part of the ENBS pilot we are collating data on screen false negatives. Data collection is ongoing although no false negative MSUD cases have yet presented.

The ENBS project suggests that the cut off levels are appropriate, with the caveat that the low frequency of screen positives means that ongoing monitoring to ensure rigorous statistical support for the cut off level is required.

22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members.

Screening is not for a mutation. MSUD is an autosomal recessive disorder, so by implication it is assumed that both parents of an affected child are carriers of a mutation in one of the genes that encodes the proteins that constitute the dysfunctional complex. Siblings may also carry mutations. Parents will be given Genetic Counselling and support should they choose to have more children, and all siblings can be tested.

It is assumed that all MSUD sufferers in the UK will present clinically and eventually be diagnosed, at which point the parents' carrier status would be known anyway; screening may hasten the inevitable diagnosis.

References for Appendix F

1. Burton H and Moorthie S, 2010. Expanded Newborn Screening: a review of the evidence. PHG Foundation. ISBN 978-1-907198-03-8.
2. Moorthie S, Cameron L, Sagoo G and Burton H, 2013. Birth prevalence of five inherited metabolic disorders. PHG Foundation. ISBN to be confirmed.
3. Chuang T, and Shih V, 2001. Maple syrup urine disease (branched-chain ketoaciduria). In: Scriver, C. R.; Beaudet, A. L.; Sly, W. S.; Valle, D. (eds.) : The Metabolic and Molecular Bases of Inherited Disease. Vol. II. New York: McGraw-Hill (8th ed.): 2001. Pp. 1971-2005.
4. Dixon S, Shackley P, Bonham J and Ibbotson R, 2012. Putting a value on the avoidance of false positive results when screening for inherited metabolic disease in the newborn. J Inherit Metab Dis. 35(1):169-76.
5. Muelly E, Moore G, Bunce S, Mack J, Bigler D, Morton D, and Strauss K, 2013. Biochemical correlates of neuropsychiatric illness in maple syrup urine disease. J Clin Invest. 2013 Apr 1;123(4):1809-20.
6. Strauss K, Puffenberger E, and Morton D, 2012. One community's effort to control genetic disease. Am J Public Health. 2012;102:1300–6.
7. Nellis M, Kasinski A, Carlson M, Allen R, Schaefer A, Schwartz E, and Danner D, 2003. Relationship of causative genetic mutations in maple syrup urine disease with their clinical expression. Molec. Genet. Metab. 80: 189-195, 2003.