A Laboratory Guide to Newborn Screening in the UK for

**MCADD**

**MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY**
A Laboratory Guide to Newborn Screening in the UK for MCADD

Handbook for laboratories incorporating:

• Background of MCADD newborn screening programme
• Screening laboratory organisation
• Screening protocol
• Pre-analytical aspects
• Analysis of octanoylcarnitine
• Acylcarnitine full scan analysis
• Quality and performance monitoring
• Clinical referral
• Reporting to child health records departments
• Laboratory standards
• Associated protocols and documents

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UK Newborn Screening Programme Centre
www.newbornbloodspot.screening.nhs.uk
This is the 2nd edition of the guide


October 2010
Review date October 2012

This document has been published by the UK Newborn Screening Programme Centre, which is funded by the Department of Health on behalf of all four UK countries.

A UK National Screening Committee publication

ISBN number 978-0-9562374-1-5

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1. Introduction

This handbook is provided for newborn screening laboratories as a guide for the implementation of newborn screening for MCADD in the UK. The handbook is available as a pdf file on the UK Newborn Screening Programme Centre (UKNSPC) website www.newbornbloodspot.screening.nhs.uk.

At the time of going to print, every attempt has been made to provide the correct up to date information.

If there are any errata or comments, please send them to the UK Newborn Screening Programme Centre, Level 5, Frontage Building, Great Ormond Street Hospital, Great Ormond Street, London, WC1N 3JH (uknewbornscreen@gosh.nhs.uk) for incorporation into the next edition.

1.1 Background

In March 2004, the Department of Health and the National Screening Committee (NSC) commissioned a pilot newborn screening study, ‘The UK Collaborative Study of Newborn Screening for Medium-chain acyl-CoA Dehydrogenase Deficiency (UKCSNS-MCADD)’, from six laboratories in England (see Appendix 1 for further details). This study provided data / information on the clinical and cost effectiveness of screening for MCADD and the feasibility of implementation. Subsequently, in May / June 2006 sufficient evidence and analysis was made available for the NSC to make the recommendation that newborn screening of all babies would be clinically and cost effective in the UK.

In February 2007, the Minister for Public Health (England) announced that all parents would be offered screening for MCADD for their baby. MCADD screening was officially announced in the Chief Executive’s Bulletin (Issue 355, 2-8 February 2007) and has Gateway reference number 7801. The UK Newborn Screening Programme Centre was asked to lead implementation across England. Screening for MCADD became universal in England from April 2009. The original screening protocol was updated in February 2010 to include the C8:C10 ratio which was endorsed by the MCADD Screening Programme Board. The C8:C10 ratio was introduced on the 1st April 2010.

1.2 Definition of MCADD and clinical presentation

MCADD is an autosomal recessively inherited defect of fatty acid oxidation due to deficiency of the enzyme medium-chain acyl-CoA dehydrogenase. This enzyme is required for the metabolism of medium-chain fatty acids and is necessary to enable the body to use its own fat reserves to produce energy in periods of fasting or stress. Deficiency of medium-chain acyl-CoA dehydrogenase causes a block in the medium-chain length step of fat oxidation (carbon chain lengths C6-C12). This leads to a build up of medium-chain fatty acids, in particular octanoylcarnitine (C8) and its metabolites and results in inefficient breakdown of fat.

Medium chain fatty acid metabolism in fat oxidation defects

<table>
<thead>
<tr>
<th>Fatty acids with chain lengths as below</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12</td>
</tr>
<tr>
<td>C10</td>
</tr>
<tr>
<td>C8</td>
</tr>
<tr>
<td>C6</td>
</tr>
<tr>
<td>C4</td>
</tr>
<tr>
<td>Octanoylcarnitine 7-hydroxyoctanoate</td>
</tr>
<tr>
<td>Hexanoylglycine 5-hydroxyhexanoate</td>
</tr>
<tr>
<td>sebacic acid</td>
</tr>
<tr>
<td>suberic acid</td>
</tr>
<tr>
<td>adipic acid</td>
</tr>
</tbody>
</table>
Appendix H

Complications typically arise during periods of stress caused by an illness / situation associated with fasting and / or vomiting, when the infant needs to break down fat quickly. Hypoglycaemia and a decompensated state develops which can result in serious life threatening symptoms including seizures, brain damage and even death. Symptoms are not apparent at birth and about one-third of cases of MCADD remain asymptomatic throughout life, however, symptoms can develop very quickly in affected infants who are not feeding well. Episodes of metabolic decompensation can be prevented through avoidance of fasting by close monitoring of the infant to determine ‘safe’ time periods between meals and following a strict feeding schedule. MCADD mainly presents before the age of two years with a mean age of thirteen months, although neonatal presentations have also been reported (Wilcken et al. 1993).

The diagnostic hallmark of MCADD is hypoketotic hypoglycaemia. An acidosis, a raised blood ammonia and abnormal liver function may also be present. The profile of blood acylcarnitines is unique and specific for MCADD and is usually diagnostic in both sick and well children, although false negatives can occur. Urine organic acids usually show characteristic acylglycine elevations (hexanoyl- and suberyl-glycine), although concentrations in non-crisis periods may be only minimally elevated. Fatty acid oxidation studies in cultured skin fibroblasts usually reveal abnormal profiles in MCADD patients.

The gene for MCADD is located on chromosome 1p31. About 88% of clinically diagnosed MCADD cases are homozygous for the common c.985A>G mutation (Pollitt and Leonard 1998), however this percentage is lower (45-55%) in cases picked up through newborn screening in the UK. MCADD affects between one in 10,000 and one in 20,000 babies born in the UK and it has been reported that the UK population has a carrier frequency of between 1 in 52 and 1 in 83 (Seddon 1995). Published data suggest that MCADD due to the c.985A>G mutation is a disease of white ethnic origin and proposes a Northern European founder effect for this mutation (Gregersen et al. 1993). Results from the UKCSNS-MCADD support this, as the c.985A>G homozygous MCADD has not been found in black and Asian ethnic populations that have migrated to England (UKCSNS-MCADD, currently unpublished data).

With early detection and monitoring, and avoidance of fasts, children diagnosed with MCADD can lead normal lives particularly as ‘safe’ time between meals expands as they grow older.

Further information can be obtained from a presentation prepared by the UKNSPC – www.newbornbloodspot.screening.nhs.uk/mcadd.
MCADD screening is fully integrated within the existing blood spot screening programme and no additional blood sample is required. The screening test, assay of octanoylcarnitine (C8), is undertaken at the same time as the tests for other disorders, (phenylketonuria, cystic fibrosis, hypothyroidism and sickle cell disease), using blood from the same heel-prick blood sample, collected on the standard newborn screening collection card. C8 is measured by electrospray tandem mass spectrometry (MS/MS). No alternative method should be used. A back-up to the main instrument must be in place, i.e. a second MS/MS or arrangement with a neighbouring laboratory for measurement of the samples by MS/MS for short term instrument failures. All screening laboratories should have a formalised contingency plan for testing in the event of a major disaster.
3.0 Screening protocol

3.1 The national screening protocol

The newborn screening protocol has been devised using the results from the UKCSNS-MCADD pilot study and been agreed and endorsed by the MCADD Screening Programme Board. It is expected that all centres will comply with the protocol.

The protocol is intended to:

Maximise early detection of MCADD so that appropriate early pre-symptomatic treatment can be initiated and the risk of acute, life-threatening episodes can be reduced and death avoided.

Minimise detection of unaffected heterozygotes and other potential false positive cases. The standard protocol is summarised diagrammatically in Appendix 2. No alternative is to be offered. The first step is the C8 and C10 assay on underivatised blood spot samples using multiple reaction monitoring (MRM) mode acquisitions. If the initial C8 is ≥0.40 μmol/L, the original blood spot card should be re-tested in duplicate on the same day. If the mean of the three results is <0.50 μmol/L, the result is negative i.e. MCADD not suspected and there is no further action. If the mean of the three results is ≥0.50 μmol/L, calculate the C8:C10 ratio for each spot, then calculate the mean C8:C10 ratio from the triplicate results. If the mean C8 ≥0.50 μmol/L AND the mean C8:C10 ratio ≥1.0, this is a presumptive positive (MCADD suspected) and the baby must be referred for clinical / diagnostic follow-up. A full acylcarnitine scan analysis should be carried out as soon as possible, ideally the same day, and if available, the result provided at the time of the referral. Referral is on the basis of the raised octanoylcarnitine and C8:C10 ratio result and independent of scan results. Full scan analysis should not delay referral to clinician. The scan can be undertaken on the original eluate (underivatised), or a further blood spot from the card can be punched for derivatised analysis.

3.2 Sibling testing

Next baby

A new baby has a 1:4 risk of having MCADD and it is important to test at the earliest opportunity. For guidance on testing and management whilst awaiting results – refer to MCADD genetic / biochemical sibling testing protocol (Appendix 3, Section A).

Older siblings

Older siblings may be at risk of MCADD even though they may have been asymptomatic to date. It is therefore very important that they are tested as soon as the diagnosis on the proband has been confirmed. Please refer to MCADD genetic / biochemical sibling testing protocol (Appendix 3, Section B).

3.3 Late testing

Babies who have not been screened during the newborn period should be screened (dried blood spot octanoylcarnitine) up to 12 months of age in line with the UKNSPC standards. It should be noted that older infants with MCADD may have C8 levels below the screening cut-off and there is a potential for false negative screening results (see 4.3). If MCADD is suspected, the infant should be referred to a clinician for diagnostic testing.
4. Pre-analytical aspects

4.1 Specimen requirements
MCADD screening requires the quantitative assay of octanoylcarnitine (C8) and decanoylcarnitine (C10). As for all screening tests, good quality blood spots are essential. Specimens that are overlaid by multiple applications are likely to give erroneous/misleading results and may result in false negatives or babies being recalled and investigated unnecessarily.

Specimens should be transported to the laboratory in the usual way and be kept in a dry environment at room temperature or 4 °C before analysis; storage after analysis should follow the guidelines provided by the UKNSPC ‘Policies and standards for newborn blood spot screening in the UK’. Further information is available at www.newbornbloodspot.screening.nhs.uk.

4.2 Factors affecting the screening result
There is some evidence that C8 values are higher in the immediate neonatal period (1-2 days of life) compared to the rest of the neonatal period. Results from the UKCSNS-MCADD showed that C8 concentrations show little variation with age in normal babies during the screening time window, i.e. 5-8 days of age, and remain relatively constant during the first few weeks of life (Phillips et al. 2005, Khalid et al. 2010). Results also showed that C8 concentrations decrease slightly with increasing birth weight and in general, males have slightly higher C8 concentrations than females; these observations however are not significant for screening purposes.

There are a number of factors which could theoretically reduce or increase C8 concentration in babies and could therefore pose a risk of false negative or false positive screening results. These are discussed below.

4.3 Potential for false negatives
- The effects of blood transfusion are unclear. Transfusions could result in a false negative result, as for other screening tests, and a repeat sample should therefore be taken after a reasonable time has elapsed. At least 72h is recommended, as for the other screening tests, to allow pre-transfusion levels to be reached.

- Dextrose administration in a sick neonate with MCADD prior to blood collection may reduce octanoylcarnitine level.

- Carnitine depletion has resulted in C8 levels below the screening cut-off in older children with MCADD who have presented clinically. Carnitine stores in newborns generally reflect maternal levels and low carnitine is sufficiently rare for this to be an exceedingly low risk of false negative screening results. No cases were found during the pilot study. This theoretical risk should be borne in mind if testing is delayed beyond the normal postnatal time-frame.

- Short delays in transit of the specimen have not been associated with altered C8 levels, blood spot cards can be accepted up to 14 days post specimen date, as for other tests. Hydrolysis of C8 can take place on blood spot cards which have not been stored dry and, theoretically, could result in false negative screening values. This has been observed on control/calibrator specimens stored over long periods. Blood spot cards which have been exposed to moisture should not be accepted.

- It is known that C8 falls in older infants (after approx 1 month of age) and infants with MCADD may have C8 levels below the screening cut-off (see Section 3.3 on late testing).
4.4 Potential for false positives

- Physiological stress in newborns can be associated with elevations of C8 above normal levels, particularly in heterozygote carriers of MCADD. False positives when screening at 5-8 days however is very rare. For further information on the interpretation of raised C8 in other conditions / situations, please refer to Section 6.2.

- Early sampling in the immediate postnatal period may give higher results as discussed above.

- Card contamination of unknown cause has resulted in 3 false positive results during the pilot study; a protocol for dealing with this is outlined in Section 5.5 Action on discrepant replicates and Appendix 4.
5. Analysis of octanoylcarnitine and decanoylcarnitine

Detailed laboratory methods are not provided.

5.1 Methodology

Both octanoylcarnitine (C8) and decanoylcarnitine (C10) are measured in underivatised solvent extracts from dried blood spots using a tandem mass spectrometer with liquid chromatography sample introduction (LC/MS/MS). Multiple reaction monitoring (MRM) acquisition mode must be used and analysis restricted to the specified analyte.

There are no additional sample requirements as C8 and C10 analysis is incorporated into the same MS/MS method as the MRM analysis of phenylalanine (Phe) for phenylketonuria (PKU) screening. Table 1 shows MRM ion transitions used.

Internal standards (IS) of deuterium labelled C8 and phenylalanine (e.g. $^2$H$_3$-C8 and $^2$H$_5$-Phe) prepared in a suitable solvent (e.g. 80% methanol or ethanol) are used to elute C8 and Phe from a punched dried blood spot disc in multiwell plates.

Table 1. MRM transitions for C8, C10 and Phe analysis

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Transition</th>
<th>Example IS</th>
<th>Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8</td>
<td>288 → 85</td>
<td>$^2$H$_3$ - C8</td>
<td>291 → 85</td>
</tr>
<tr>
<td>C10</td>
<td>316 → 85</td>
<td>$^2$H$_3$ - C8</td>
<td>291 → 85</td>
</tr>
<tr>
<td>Phe</td>
<td>166 → 120</td>
<td>$^2$H$_5$ – Phe</td>
<td>171 → 125</td>
</tr>
</tbody>
</table>

MS/MS sampling can be direct from the original plate (with blood spots *in situ*) or the eluants may be transferred to a fresh plate before sampling. Preference for transfer will depend on systems in use and is a balance between transfer possibly reducing blockage rates and the fact that it has its own risks e.g. sample mix up / contamination (see Section 5.5 and Appendix 4 for guidance on discrepant replicates).

Calibration options include simple ratio to a calibrated IS or use of a dried blood spot calibration curve on each batch. In-house calibrators or commercial kits may be used.

Validation of analysis shall include automatic flagging of inadequate internal standard abundances by the analytical software and since sensitivity requirements for C8 are 100 fold that of Phe. Inspection of individual flow profiles and ion abundances is also recommended.

5.2 Calculation of C8:C10 ratio

C10 should be measured in triplicate (i.e. singlicate analysis at the same time as the initial C8 measurement and further duplicate analyses when carrying out C8 repeat analyses where initial C8 ≥0.40 μmol/L). If the triplicate C8 result is ≥0.50 μmol/L then calculate the mean C8:C10 ratio.

For consistency between laboratories the ratio should be calculated using C10 measured using the $^{13}$C8 internal standard (it is recognised that some laboratories have $^{13}$C10 in their internal standard solution). It would be of value if those laboratories could collect comparative data for C10 and C8:C10 ratios obtained from using the $^{13}$C10 internal standard – but to use the C10 value calculated using $^{13}$C8 for reporting.
5.3 Internal quality control (IQC)
It is recommended that relevant levels of dried blood spot IQC are included at the beginning and end of each plate. Three levels of C8 and C10 are recommended:

i) Normal
ii) Medium, level around initial cut-off (i.e. 0.40 – 0.50 μmol/L)
iii) High (e.g. 1 – 2 μmol/L)

Suitable levels are not always available commercially so there may be a need for in-house preparations. The Centers for Disease Control and Prevention (CDC, USA) QC sets are a very useful supplement to any internal QC for monitoring long term stability of the assay (see Section 7 Quality and performance monitoring).

5.4 Performance criteria
Based on the performance during the pilot study, it is expected that laboratories should achieve the following:

Precision:
- 4% at C8 = 1.0 μmol/L and 10% at C8 = 0.05 μmol/L
- 7% at C10 = 1.0 μmol/L and 10% at C10 = 0.05 μmol/L

Sensitivity / linearity:
- C8 and C10 assay range 0.01 to 5.0 μmol/L
5.5 Action on discrepant replicates

If a sample has an initially raised C8 (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial C8 is suspected and the protocol outlined in Appendix 4 should be followed. The same should apply to C10 values.
6. Acylcarnitine full scan analysis

Full scan analysis should only be undertaken by laboratories with appropriate experience (see standards in Section 10.4). Screening laboratories may need to make arrangements for the scan to be done by a diagnostic laboratory; noting the urgency for the result.

The choice of sample preparation (i.e. derivatised vs. underivatised) for acylcarnitine full scan analysis is dependent on local preference and experience. It is tempting to analyse acylcarnitines underivatised because of safety, cost and ease of sample preparation. However, derivatisation increases both the sensitivity and specificity of acylcarnitine analysis by MS/MS. Tandem mass spectrometric analysis of the crude blood extract for acylcarnitines by precursor ion scanning of m/z 85 is remarkably specific for these compounds. However interferences are known to occur when samples are analysed underivatised where a compound(s) with a molecular ion of m/z 288 gives rise to a fragment ion of m/z 85 mimicking octanoylcarnitine. Derivatisation by means of butylation adds 56 amu to the molecular weight of the native acylcarnitine. Hence, if after butylation a signal at m/z 288 shifts by 56 amu and its ion intensity ratio to octanoylcarnitine internal standard remains unchanged there is a very good chance that the signal represents only octanoylcarnitine otherwise it is due to interference or contamination.

6.1 Procedure for the extraction and analysis of acylcarnitines from dried blood spots

It is advised that screening laboratories request details of the method from a laboratory in their ‘buddy’ group which is experienced in the analysis of diagnostic acylcarnitines.

6.2 Interpretation of acylcarnitine scan

All cases with a triplicate average of C8 ≥0.50 μmol/L and C8:C10 ratio ≥1.0 are referred immediately and regardless of the scan pattern. The additional information from the scan is a useful guide in the consultation and should be provided to the clinician as soon as possible.

MCADD metabolites – MCADD suspected

MCADD patients have increased blood octanoylcarnitine (C8) levels. In most patients the acylcarnitine scan profile also shows elevated C6, C10 and C10:1 signals with raised C8:C10 and C8:C6 ratios. There is often a low acetylcarnitine (C2) level with raised C8:C2 ratio. There is no accumulation of acylcarnitine species >C10 or <C6. In older patients carnitine insufficiency may develop and these patients tend to have a lower concentration of C8 together with low C2 and free carnitine (C0).

Other conditions and multiple acyl-CoA dehydrogenase deficiency (MADD) metabolites

Increased C8 concentration may also be associated with conditions other than MCADD most notably prematurity and state of metabolic stress and induced lipolysis. In addition multiple acyl-CoA dehydrogenase deficiency is characterised by elevated C8 together with accumulation of other short-, medium- and long-chain acylcarnitines. Some of the rare causes of elevated blood C8 levels are listed in Table 2 on page 17. By using a second marker e.g. C8:C10, C8:C6 or C8:C2 ratios the specificity of C8 measurement for the diagnosis of MCADD may be improved (Chace et al. 1997, Clayton et al. 1998, Pourfarzam et al. 2001). Typical examples of blood full acylcarnitine profiles from MCADD and some non-MCADD cases, analysed as butyl ester derivatives, are shown in Figure 1 under Section 6.3 Stability of acylcarnitines in DBS.
Table 2: Rare causes of elevated blood octanoylcarnitine (C8) other than MCADD

<table>
<thead>
<tr>
<th>Condition</th>
<th>Acylcarnitine affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prematurity / very low birth weight</td>
<td>C6 and C8</td>
</tr>
<tr>
<td>Hypoxia / circulatory failure</td>
<td>C4, C6, C8, C10 and C12</td>
</tr>
<tr>
<td>State of metabolic stress and induced lipolysis</td>
<td>C8, C10, C10:1, C12, C12:1, C14 and C14:1</td>
</tr>
<tr>
<td>MCT supplementation</td>
<td>C8 and C10</td>
</tr>
<tr>
<td>Valproate therapy</td>
<td>C8 and C10</td>
</tr>
<tr>
<td>Neonate born to riboflavin deficient mother</td>
<td>C6, C8, C10, C12 and C14</td>
</tr>
<tr>
<td>Riboflavin deficiency</td>
<td>C6, C8, C10, C12 and C14</td>
</tr>
<tr>
<td>Mitochondrial myopathy</td>
<td>C8 and C10</td>
</tr>
<tr>
<td>Multiple acyl-CoA dehydrogenase deficiency</td>
<td>C4, C5, C6, C8, C10, C12, C14 and C14:1</td>
</tr>
</tbody>
</table>

A report should be issued for all acylcarnitine scans to the referring clinician as a defined pattern / category, see Table 3.

Table 3: Reporting of acylcarnitine full scan analysis
(Refer to Section 6.2 Interpretation of acylcarnitine scan).

<table>
<thead>
<tr>
<th>Acylcarnitine findings</th>
<th>Report to paediatrician</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8 with C6, C10, C10:1</td>
<td>C8 and acylcarnitine profile consistent with MCADD but requires confirmation</td>
</tr>
<tr>
<td>C8 with C4, C5, C6, C10, C12, C14:1 and C14</td>
<td>C8 and acylcarnitine profile consistent with MADD but requires confirmation</td>
</tr>
<tr>
<td>C8 with findings not typical of MCADD or MADD e.g. C4-hydroxy</td>
<td>C8 with acylcarnitine profile not typical of MCADD or MADD, but requires further investigation</td>
</tr>
</tbody>
</table>

MCADD – medium-chain acyl-CoA dehydrogenase deficiency, MADD – multiple acyl-CoA dehydrogenase deficiency

In the unusual event that the acylcarnitine scan does not suggest MCADD, further investigations may be required at the first consultation, dependent on the findings from the scan and the clinical picture.

6.3 Stability of acylcarnitines in DBS

At room temperature acylcarnitines in blood spotted on filter paper are slowly hydrolysed to free carnitine and their corresponding fatty acids. The rate of degradation depends on the carbon chain-length of the acyl group and the presence of functional groups. For saturated straight-chain acylcarnitines the stability of the carnitine esters in stored blood spots in general increases with the increasing chain-length of the acyl group. Thus at room temperature acetyl carnitine is degraded at the rate of up to about 20% per year and medium-chain acylcarnitines (i.e. C6, C8 & C10) degrade at the rate of up to 10% per year. The stability of acylcarnitines is significantly improved if dried blood spots are stored in sealed bags at low temperature. At 4 °C the decrease in the concentrations of C6 and C8 is about 4% per year. In specimens stored at -20 °C the decrease in the concentration of acetylcarnitine is less than 10% per year and those of C6, C8 and C10 are less than 3%.
Figure 1: Acylcarnitine profiles from dried blood spots analysed as butyl ester derivatives

A) a healthy neonate at the age of 5 days, B) and C) a neonate with MCADD at the age of 2 days and 8 days respectively, D) a neonate with multiple acyl-CoA dehydrogenase deficiency (MADD) at the age of 5 days and E) a neonate in catabolic state and severe ketosis at the age of 6 days (NB. Please note different scale on y-axis in E).
7. Quality and performance monitoring

Laboratories must establish appropriate quality and performance monitoring procedures as set out below.

7.1 External quality control assurance
A scheme for monitoring the performance of the C8 assay set up at Birmingham Children’s Hospital was in operation from March 2004 to April 2010. From 1st April 2010, UKNEQAS included C8 and C10 in the newborn screening blood spot scheme. The purpose of the scheme is to provide a quality assessment of precision and accuracy for the analysis of C8 and C10 for laboratories that screen for MCADD by MS/MS. Four specimens are circulated on a monthly basis. All laboratories in the UK which screen for MCADD by MS/MS are expected to take part in this scheme. For more details please contact Finlay MacKenzie, UKNEQAS (Finlay.MacKenzie@uhb.nhs.uk).

Laboratories undertaking acylcarnitine full scan analysis must participate and demonstrate good performance in an appropriate EQA scheme, e.g. ERNDIM (www.erndima.nl) or CDC (www.cdc.gov/labstandards).

7.2 Normal population data (octanoylcarnitine)
A programme for monitoring the performance of the octanoylcarnitine (C8) assay using whole population newborn screening data coordinated by the UCL Institute of Child Health (ICH), London, compared data from the six Phase 1 laboratories. This demonstrated good concordance in the population distribution of C8 values both within and between laboratories, and proved valuable as a quality assurance measure (see Khalid et al. 2010).

7.3 Reporting of positive cases to UKNSPC
The ongoing monitoring of all cases which are presumptive positive at screening is being undertaken by the UKNSPC. This requires that ethnic group, screening results and diagnostic test results are reported using the Laboratory Notification Form (dated 14/06/2010). All screening laboratories are expected to contribute to this reporting.

The laboratory notification form of presumptive positive screen is available at: www.newbornbloodspot.screening.nhs.uk

Completed forms should be sent by email to: christine.cavanagh@nhs.net

Or by post to:
Christine Cavanagh
Programme Manager
UK Newborn Screening Programme Centre
Level 5, Frontage Building
Great Ormond Street Hospital for Children NHS Trust
Great Ormond Street
London WC1N 3JH
Tel: 020 7829 7884
8. Clinical referral

8.1 Responsibility for communications / clinical liaison

It is essential that each screening laboratory, in collaboration with commissioners and local implementation groups, make detailed local arrangements for the follow-up of presumptive positive cases for all districts covered by their laboratory and clarifies responsibility for undertaking the MCADD Clinical Liaison Service (CLS) role as part of the referral process – see protocol: Appendix 10 – MCADD initial clinical referral guidelines and standards.

The CLS role may be undertaken by person(s) based in the screening laboratory (i.e. a screening clinical nurse specialist or duty biochemist), in the designated clinical team or in the community, depending on local arrangements.

8.2 Follow-up for presumptive positive cases consistent with MCADD

(See Section 3.1 and Appendix 2)

These are babies with mean of triplicate C8 ≥0.50 μmol/L and C8:C10 ratio ≥1.0

- All presumptive positives should be referred to the designated (or specialist) MCADD team via the CLS (as per local arrangements) on the same day that the final screening result has become available. This must be reported both verbally as well as in writing – a template is provided (Appendix 5).

- The first clinic appointment should take place within 24hrs of the final result becoming available.

- A confirmatory acylcarnitine scan should have been undertaken and reported to the MCADD designated team (see Section 6.2) – if possible in time for the clinic appointment.

The CLS must contact the GP and:

- Coordinate local support

- Obtain a telephone number for the family

- Ensure that the family is seen by a health professional that day (e.g. GP / midwife / health visitor / clinical nurse specialist – as per local protocol)

- Fax information as follows:
  - MCADD GP letter (Appendix 6)
  - MCADD is suspected leaflet (includes UK Newborn Screening Programme Centre website address and links to parent support group)
  - MCADD A&E letter (Appendix 7)
  - Contact numbers for the MCADD designated (or specialist) team
  - Details of the time and location of an appointment with the MCADD designated (or specialist) team.

The recommended MCADD dietary (and other) guidelines can be accessed via the UKNSPC website (www.newbornbloodspot.screening.nhs.uk).
8.3 Diagnostic specimen requirements

The diagnostic protocol is summarised diagrammatically in Appendix 8.

Diagnostic tests provided externally will need to be charged for. In approximately 50% of cases the diagnosis will be confirmed following repeat C8, urine organic acids and DNA analysis for the common mutation (c.985A>G). For C8, qualitative urine organic acids and DNA (c.985A>G), results should be available within 5 working days after specimen collection.

For the other cases, further investigations will be required, these are:-

**Extended mutation screen (EMS)**

Until end of June 2010 samples for EMS were sent to:

Dr. Brage Andresen  
Department of Biochemistry and Molecular Biology  
University of South Denmark  
Campusvej 55  
5230 Odense M.  
Denmark

Phone: +45-65502413  
Fax: +45-65502467  
Email: bragea@bmb.sdu.dk

As of 1st July 2010, samples for EMS studies should be sent to one of two designated UK EMS laboratories as outlined below. It is the responsibility of the metabolic diagnostic laboratory to communicate with their EMS laboratory and to ensure samples are sent in a timely manner.

EMS referral forms can be found at: [http://newbornbloodspot.screening.nhs.uk/mcadd](http://newbornbloodspot.screening.nhs.uk/mcadd)

West Midlands, Sheffield, Leeds, Liverpool, Manchester, Newcastle and Oxford are to send samples for EMS to:-

**Sheffield Laboratory**  
Service Lead: Richard Kirk or Dr. Ann Dalton  
Sheffield Diagnostic Genetics Service  
Sheffield Children's NHS Foundation Trust  
Western Bank  
Sheffield S10 2TH

Tel: 0114 271 7014  
Fax: 0114 275 0629  
Email: Richard.Kirk@sch.nhs.uk or Ann.Dalton@sch.nhs.uk
Appendix H

Bristol, Cambridge, GOSH, Portsmouth, SE Thames (Guy's) and SW Thames are to send samples for EMS to:-

Guy's Laboratory

Service lead: Kirsty Stewart or Dr. Stephen Abbs
DNA Laboratory
GSTS Pathology
Floor 5 Tower Wing
Guy's Hospital
London SE1 9RT

Tel: 0207 188 2582
Fax: 0207 188 7273
Email: Kirsty.Stewart@gsts.com or Stephen.Abbs@gsts.com

The Metabolic Diagnostic laboratory working with the Inherited Metabolic Disorder team is responsible for sending samples for c.985A>G testing.

Samples should be collected on 3 blood spot cards (ideally 4 good quality spots on each) at the first appointment by the Inherited Metabolic Disorder team. All 3 cards should be sent to the Metabolic Diagnostic laboratory.

- Card 1 is used for confirmatory biochemistry.
- Card 2 is sent for c.985A>G testing at partner molecular biology laboratory.
- Card 3 is held by the Metabolic Diagnostic laboratory until c.985A>G results have been obtained and is then sent to EMS laboratory (Guy's or Sheffield laboratory) if EMS is required.
- Samples for EMS screening should be sent with the appropriate EMS referral form (see: www.newbornbloodspot.screening.nhs.uk/mcadd) by the Metabolic Diagnostic laboratory via first class post.
- The Metabolic Diagnostic laboratory should alert the EMS laboratory that the sample has been dispatched for testing.
- The EMS laboratory should acknowledge receipt of card.
- EMS laboratory are to send reported results, via fax or email to an NHS.net account to the Metabolic Diagnostic laboratory that requested the EMS.

Quantitative organic acids (Hexanoylglycine)

Quantitation requires a sensitive stable isotope dilution method, e.g. gas chromatography mass spectrometry, employing a standard curve or equivalent. UK Laboratories providing Quantitative Hexanoylglycine can be found on the MetBioNet website (www.metbio.net).

Specimen requirements

2 mL fresh random urine (no preservative), aliquoted from the sample used for qualitative organic acid analysis. Frozen -20 °C. Send on dry ice.

Note: Skin fibroblast fat oxidation studies are no longer included as part of the formal diagnostic protocol. If undertaken as part of any further follow-up this would be at local discretion and charged individually.
8.4 Coordination of screening follow-up results / requesting of extended mutation analysis

All samples should be sent to the local metabolic diagnostic laboratory * for the following:

- DNA analysis for c.985A>G – send sample
- Biochemistry
- Extended Mutation Screening (EMS) – On learning the c.985A>G result and the biochemistry result the Metabolic Diagnostic laboratory will send a sample for EMS if required.

Note: It is the responsibility of the Metabolic Diagnostic laboratory* to review results of c.985A>G analysis and biochemistry and, together with input from the clinician and decide whether EMS is required.

EMS is required in the following circumstances

- 1 copy c.985A>G (regardless of biochemistry)
- No c.985A>G but with abnormal biochemistry
- Clinical reasons

* If local arrangements differ from this for logistical reasons the responsibility for deciding whether EMS is required must be clear.
9. Reporting to child health records departments

Results should be reported using the latest version of the screening status codes - see below status codes in Section 9.1. It is recommended that the screening report for MCADD should be either ‘MCADD not suspected’ (code 04) or ‘MCADD suspected’ (code 08). It is a requirement that screening results (MCADD not suspected or suspected) are fed back to the child health records departments. It is recommended that child health records departments notify normal results to parents by letter.

9.1 Status codes

The UKNSPC agreed status codes version 2.0 are detailed in Table 4 with specific comments with reference to MCADD.
### Table 4. Status codes for MCADD

<table>
<thead>
<tr>
<th>Screening Status Code</th>
<th>Suggested term displayed in child health system</th>
<th>Comment with reference to MCADD</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Specimen received in laboratory</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>MCADD declined</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>MCADD repeat / further sample required</td>
<td>“Reason for repeat test” will include the following: Too young for reliable screening Too soon after transfusion (&lt;72 hours) Unsuitable sample Insufficient sample Unsatisfactory analysis</td>
</tr>
<tr>
<td>04</td>
<td>MCADD not suspected</td>
<td>C8 &gt; 0.4 μmol/L</td>
</tr>
<tr>
<td>05</td>
<td>Not applicable to MCADD</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>Not applicable to MCADD</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>Not applicable to MCADD</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>MCADD suspected</td>
<td>According to the following criteria: C8 ≥ 0.4 μmol/L (singlicate analysis) C8 ≥ 0.5 μmol/L (re-test in duplicate, mean of 3 results) AND C8:C10 ratio ≥ 1.0 (mean from triplicate results)</td>
</tr>
<tr>
<td>09</td>
<td>MCADD not screened / screening incomplete*</td>
<td>“Reason for no result” will include the following: Died Unreliable result Moved out of area Not contactable, reasonable efforts made Too old for screening (&gt;1 year)</td>
</tr>
<tr>
<td>10</td>
<td>Not applicable to MCADD</td>
<td></td>
</tr>
</tbody>
</table>
10. Laboratory standards for newborn screening for MCADD

10.1 Generic
See the UKNSPC Policies and Standards at [www.newbornbloodspot.screening.nhs.uk](http://www.newbornbloodspot.screening.nhs.uk) for process standards relating to timely sample collection, despatch and programme coverage.

10.2 Organisation

- Newborn screening for MCADD should be provided within the organisational structure of the newborn blood spot screening programme. It should be undertaken by specialist newborn screening laboratories already providing screening programmes for phenylketonuria, congenital hypothyroidism, cystic fibrosis and sickle cell disorders.

- Laboratories screening for MCADD must be accredited by Clinical Pathology Accreditation (UK). There must be a member of staff at consultant level responsible for MCADD screening with defined lines of accountability for all aspects of the service.

- There should be local policies and standard operating procedures describing the whole screening process including pre-analytical, analytical and post-analytical processes; these include reporting normal and abnormal results, referral and follow-up arrangements for presumptive positive cases. Processes must be provided in line with relevant national standards and guidance and should be reviewed periodically taking into account audit data, accumulating results, technical developments and local changes in healthcare provision.

10.3 Analytical processes

- Newborn screening for MCADD should be provided using the nationally agreed screening protocol with the screening tests, octanoylcarnitine (C8) and decanoylcarnitine (C10), performed using an underivatised MRM tandem mass spectrometric technique.

- The laboratory must undertake appropriate internal quality control procedures for the screening test and demonstrate satisfactory performance in an approved External Quality Assurance Scheme as part of UKNSPC Quality Management arrangements.

- Laboratories are expected to provide a completed notification form on each presumptive positive to the UKNSPC.

- Acylcarnitine full scan analysis must be undertaken in a timely manner by a laboratory with appropriate analytical and interpretive expertise. Laboratories must participate and demonstrate acceptable performance in an approved EQA scheme.

- Laboratories should provide data on screening performance to the UKNSPC as well as regional and local audit / quality management groups as required.

- The results and performance of the MCADD screening programme should be included within an annual report produced by the screening laboratory for circulation to local Directors of Public Health (and others as required).

- There must be a documented risk management policy for the laboratory aspects of the MCADD screening programme as part of an overall newborn screening risk management policy.
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10.4 Performance standards, including diagnostic tests

- The laboratory analytical service should be configured to enable the MCADD newborn screening protocol (a raised octanoylcarnitine confirmed in triplicate, a raised C8:C10 ratio in triplicate and acylcarnitine full scan) to be completed within 4 working days from receipt of an adequate sample as core standard.

- Presumptive positive results (average of triplicate C8 and C8:C10 ratio) should be referred by the laboratory to the appropriate clinical team on the day they become available. This includes referral on Fridays prior to a weekend and a bank holiday.

- Follow-up diagnostic tests (including full acylcarnitine scan, urine organic acids and c.985A>G mutation analysis) must be undertaken in line with the diagnostic protocol by accredited laboratories who must participate and demonstrate acceptable performance in the relevant / accredited EQA schemes. Provision should be made to ensure results of these investigations are available within 5 working days from sample collection. The responsibility for this must be defined locally.

- Results of further follow-up diagnostic tests (EMS and quantitative hexanoylglycine) should be available within 20 working days of sample collection.
11. References and further reading


Appendix H


Appendix 1 UKCSNS-MCADD study*

Overview and early findings can be found at:
www.newbornbloodspot.screening.nhs.uk/mcadd

* The UK Collaborative Study of Newborn Screening – Medium-chain acyl-CoA dehydrogenase deficiency
Appendix 2 MCADD newborn screening protocol

Routine newborn screening dried blood spot samples:
Underivatised MRM
Octanoylcarnitine (C8)

Yes

C8 ≥0.40 µmol/L
[A]

No

Re-test C8 in duplicate [B, C] ¹
Test for C10 ²

C8 ≥0.50 µmol/L
Mean [A,B,C]

No

MCADD not suspected
No further action

Yes

Obtain C10 results ²
Calculate C8:C10 ratio

Ratio ²
C8:C10 ≥1.0

No

Yes

MCADD suspected
Referral to designated clinician³

See MCADD diagnostic protocol

¹ If insufficient blood to re-test, but raised initial C8 (≥0.40) treat as MCADD suspected.

² Refer to methodology for calculating C8:C10 ratio.

³ Recommended to provide clinician with full acylcarnitine scan (on eluate and/or derivatised) as soon as possible. See MCADD Lab Handbook Section 6.2 for further guidance.
A. Protocol for management of at risk delivery – neonatal testing for siblings born after proband diagnosis

When to test and samples taken

24 - 48 hours:  
- C8, qualitative urinary organic acids and genotyping
- Write on blood spot card ‘Family history of MCADD’

Day 5 - 8:  
- Routine newborn screen
- Write on blood spot card ‘Family history of MCADD’

Management

Prior to results
It is essential to ensure that the baby maintains a good milk intake. A term baby should be fed every 4 hours and a preterm baby every 3 hours. Exclusively breast fed babies are particularly at risk in the first 72 hours when the supply of breast milk is poor; top up feeds of expressed breast or formula milk may be necessary in the first 48 - 72 hours until a good milk supply is established. If oral feeds are not tolerated or if the baby is unwell in any way, urgent referral should be made to a paediatrician for review and consideration of nasogastric tube feeds or commencing intravenous glucose*.

If MCADD confirmed: Follow the standard MCADD clinical and dietary management guidelines*

B. Testing siblings born before proband diagnosis

When to test
Offer to test if sibling has not been previously screened for MCADD and if:

- Proband has abnormal biochemistry at follow-up visit
- 2 recognised disease causing mutations on genotyping

Sibling samples

1. C8 and qualitative urinary organic acids

2. DNA – send for genotyping once definite MCADD diagnosis secured in proband (2 disease causing mutations identified)

Management

Before the results are available and thereafter if MCADD confirmed: Follow the standard MCADD clinical and dietary management guidelines*

C. Genetic counselling

In most instances questions about genetic inheritance and risk will be dealt with by the metabolic team, but if there are any outstanding issues, or discussion about prenatal diagnosis, further genetic counselling is available through the local Genetics Service.

*For more information please refer to MCADD management and dietary guidelines at www.newbornbloodspot.screening.nhs.uk
Further information / protocols can also be accessed at www.bimd.org.uk/mcadd.asp
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Appendix 4 Guidance on discrepant replicates

If a sample has an initially raised C8 (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial C8 is suspected. If there is no obvious contamination from the plates then the following procedure is recommended.

Check persistence of C8 (mass 288) by performing an underivatised acylcarnitine full scan on the well that gave the raised result plus 2 normal samples and High QC from the same plate for comparison (if necessary reconstitute the well).

<table>
<thead>
<tr>
<th>If 288 persists:</th>
<th>If no 288:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivatise the transfer plate well that gave the raised result plus the same 2 normal and high QC samples from the plate</td>
<td>Contamination not of sample origin, no further testing of samples in batch necessary but other non-sample sources of contamination may be sought.</td>
</tr>
<tr>
<td>Perform a derivatised acylcarnitine full scan (or product ion scan).</td>
<td></td>
</tr>
</tbody>
</table>

If C8 confirmed i.e. 344 detected *

*When method involves eluate transfer from elution to transfer plate, can first attempt to identify any sample mix up at transfer stage by performing an underivatised acylcarnitine scan of the whole elution plate (reconstituted with 75 μL methanol). If any elution well shows increased C8 – retest that sample. However, re-analysis of the elution plate is often not successful due to insufficient residual sample.

<table>
<thead>
<tr>
<th>If C8 confirmed i.e. no 344</th>
<th>If C8 not confirmed i.e. no 344</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-test the plate / whole batch to eliminate the possibility of sample mix up.</td>
<td>Contamination is not due to genuine C8, no further testing of samples in batch necessary but should investigate other non-sample sources of 288 contamination.</td>
</tr>
</tbody>
</table>
Appendix 5 Template for notification of presumptive positive for designated (or specialist) MCADD team (by Screening Laboratory to clinician)

For Patient’s Notes

Baby’s Name _____________________________________________________

Gender ________________________________________________________

D.O.B _________________________________________________________

NHS Number ___________________________________________________

Address _______________________________________________________

GP ___________________________________________________________

To the Medical Consultant, _______________________________________

Screening specimen date: _________________________________________

The above baby was found to have a positive (abnormal) newborn screening test result for medium-chain acyl-CoA dehydrogenase deficiency (MCADD). The blood spot octanoylcarnitine (C8) was ____________ μmol/L whole blood (mean of triplicate results) with the C8:C10 ratio as _____________. (The screening tests for phenylketonuria, congenital hypothyroidism and sickle cell disease are ____________, and cystic fibrosis is _______________).

The acylcarnitine full scan analysis shows:-

___________________________________________________________________________________________

___________________________________________________________________________________________

___________________________________________________________________________________________

Recommended Action as per MCADD Screening Programme clinical management protocol and diagnostic protocol:-

• First review appointment to take place within 24 hrs

• Venous blood from baby for acylcarnitines (C8 and full scan), two (2) blood spot cards for DNA (c.985A>G and extended mutation analysis)

• Urine for qualitative / quantitative organic acid analysis

Signed: ___________________________________ Date ________________________________________

Screening laboratory contact details: ______________________________________________________
Appendix 6 Template for GP letter

[Date]

Dear Doctor

Re: [insert full name and date of birth of child]

[Name of child] has been detected on newborn screening to have a positive (abnormal) test for medium-chain acyl-CoA dehydrogenase deficiency (MCADD). This is a rare inherited enzyme deficiency which reduces the metabolism of fat into energy. A child with this condition is at risk from hypoglycaemia, coma and death with fasting and particularly during intercurrent illnesses when the demand for energy increases and calorie intake is often reduced. He/she may appear drowsy or lethargic, vomit, have seizures or have a deteriorating conscious level.

Hypoglycaemia is a late sign; treatment must be initiated if [name of child] is unwell even if the blood sugar is normal.

A patient with MCADD requires no special treatment when well apart from avoiding prolonged fasts. Breastfeeding is not contraindicated but it is important to ensure that the infant is feeding adequately. Formula feeds rich in medium-chain triglycerides (MCT) should be avoided. The family will be taught to use an Emergency Regimen (ER) during intercurrent illness, details of which will be sent to you. The aim is to supply readily available calories to avoid mobilising the fat stores and therefore avoid decompensation.

Oral Rehydration Therapy (ORT) solutions do not contain sufficient calories to avoid decompensation, and if used require fortifying with glucose polymer. The family will have a recipe for this. If the ER is not tolerated, or the child's condition deteriorates, then urgent admission to the local hospital should be arranged for an intravenous 10% dextrose infusion with appropriate electrolyte additives. A copy of the A&E letter the parents will be given is attached. When the child is well, they should return to their usual feeds.

The positive test so far is a screening test, and therefore it is essential to meet with the family to further explain the condition and to confirm the diagnosis. This will entail blood and urine tests. As discussed on the phone, the parents are to attend [appointment location] at [appointment time] to be seen by the metabolic team. If the parents would like to discuss any matters prior to this review, [name of clinician] may be contacted on [contact number].

The long-term prognosis for MCADD is very good once diagnosed providing that the emergency regimen is followed as directed. 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. Once the diagnosis has been confirmed, screening of any siblings will be offered.

If you have any further questions, please do not hesitate to contact [name and contact details].

With kind regards

Yours sincerely

Enc: A&E letter
To Whom It May Concern:

This child has (or is currently being investigated for a positive newborn screening test for) medium-chain acyl-CoA dehydrogenase deficiency (MCADD). Children with MCADD have a reduced ability to metabolise fat to provide energy. Infections, fasting, vomiting or diarrhoea result in the accumulation of medium-chain fats, which form toxic metabolites. This can lead to serious illness with encephalopathy and even death. Hypoglycaemia may only occur at a relatively late stage; treatment must not be delayed just because the blood glucose is normal.

Treatment aims to inhibit mobilisation of fat by providing ample glucose. During intercurrent infections, parents will use an Emergency Regimen (ER) of frequent glucose polymer drinks but if this is not tolerated or there is clinical deterioration they have been instructed to attend the hospital urgently for further management.

### Brief parent-held guide to MCADD hospital management*

**Assess**
- Responsiveness / conscious level – record Glasgow coma score
- U&E, Blood Gases, Glucose (stick test + laboratory measurement), other tests as indicated

**Vomiting or Diarrhoea or Not tolerating feed/ER or Altered consciousness or Blood Glucose <3.0 mmol/L**

1. IV 10% glucose bolus 2 mL/kg (200 mg/kg)
2. If poor circulation / shock, follow with 20 mL/kg 0.9% sodium chloride
3. Whilst the maintenance fluid is being made up, continue 10% glucose at 5mL/kg/hr
4. Maintenance fluid given as 10% glucose with 0.45% sodium chloride.

   This solution can be made up as follows:
   - Remove and discard 50 mL from a 500 mL bag of 0.45% sodium chloride & 5% glucose solution, then add 50 mL of 50% glucose to the fluid remaining in the bag

5. Correct any fluid / electrolyte deficits; add potassium once U&E status is known
6. Admit & notify metabolic team (contact details below)
7. Monitor blood glucose 4 hourly during acute phase
8. Adjust IV infusion rate to maintain blood glucose 4-8 mmol/L
9. Continue infusion until blood glucose stable and tolerating usual oral feeds

**Asymptomatic and glucose <3.0 mmol/L**

- Oral or nasogastric ER until glucose within normal range and clinically recovered and stable

Note: Oral rehydration solutions do not contain sufficient glucose to avoid decompensation and therefore, if prescribed, must be fortified with glucose polymer - see MCADD Dietary Information Sheets for ER recipe, available at [www.newbornbloodspot.screening.nhs.uk/mcadd](http://www.newbornbloodspot.screening.nhs.uk/mcadd)
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Please notify the metabolic team below if this child is admitted.

[INSERT CONTACT DETAILS FOR METABOLIC TEAM]

If you have any questions, please do not hesitate to contact the metabolic team as above.

*MCADD dietary management guidelines are available at www.newbornbloodspot.screening.nhs.uk

*Further detailed information / protocols can also be accessed at www.bimdg.org.uk/mcadd.asp
All babies presumptive positive for MCADD at newborn screening

FOLLOW-UP ANALYSES* (at first review appointment):
- Blood acylcarnitines (dried blood spot or plasma): C8 (duplicate) and acylcarnitines full scan
- Qualitative urine organic acid (UOA) analysis
  In cases where a diagnostic increase in hexanoylglycine is not detected using qualitative methods, quantitation (measured using a sensitive, stable isotope dilution method e.g. gas chromatography-mass spectrometry employing a standard curve or equivalent) should be performed.
- Dried blood spot (or liquid blood) for DNA: c.985A>G mutation analysis and Extended Mutation Screening (EMS) if required – complete 3 separate blood spot cards, each sent for analysis at first review appointment (see Guidance notes)

CMSD

Yes

No

c.985A>G homozygous (2 copies)

Biochemically Abnormal*

EMS*

Other mutation identified (with c.985A>G)

Unaffected MCADD CARRIER

MCADD

MCADD UNLIKELY
Review and consider need for further biochemical investigations

Likely unaffected MCADD CARRIER
Review and consider need for further biochemical investigations

No

• Dried blood spot (or liquid blood) for DNA: c.985A>G mutation analysis and Extended Mutation Screening (EMS) if required – complete 3 separate blood spot cards, each sent for analysis at first review appointment (see Guidance notes)
Appendix H

Guidance notes

Sample requirements

Assumes mutation analysis (c.985A>G and EMS) is undertaken on dried blood spots.

Dried blood spot

Complete 3 blood spot cards with non-anticoagulated blood (if anticoagulant has to be used, please ensure this is EDTA, not Li Hep): 1 for C8 / full scan analysis*, 1 for c.985A>G analysis, 1 for EMS

Li Hep plasma

0.5 mL for C8 and acylcarnitines scan analysis*

Urine

5 mL minimum (no preservative, frozen immediately at –20 °C) (Equal share for qualitative and possible quantitative assay)

Extended Mutation Screening

Samples should be sent as indicated:

West Midlands, Sheffield, Leeds, Liverpool, Manchester, Newcastle and Oxford are to send samples for EMS to:-

Sheffield Laboratory

Service Lead: Richard Kirk or Dr. Ann Dalton
Sheffield Diagnostic Genetics Service
Sheffield Children’s NHS Foundation Trust
Western Bank
Sheffield
S10 2TH

Tel: 0114 271 7014
Fax: 0114 275 0629
Email: Richard.Kirk@sch.nhs.uk or Ann.Dalton@sch.nhs.uk

Bristol, Cambridge, GOSH, Portsmouth, SE Thames (Guy’s) and SW Thames are to send samples for EMS to:-

Guy’s Laboratory

Service lead: Kirsty Stewart or Dr. Stephen Abbs
DNA Laboratory
GSTS Pathology
Floor 5 Tower Wing
Guy’s Hospital
London SE1 9RT

Tel: 0207 188 2582
Fax: 0207 188 7273
Email: Kirsty.Stewart@gsts.com or Stephen.Abbs@gsts.com
Appendix H

All samples should be sent to the local metabolic diagnostic laboratory** for the following:

- DNA analysis for c.985A>G – send sample immediately to appropriate local laboratory (UKGTN Accredited)
- Biochemistry
- EMS – send sample immediately with biochemical dried blood spot to Metabolic Diagnostic laboratory who will arrange for EMS analysis if indicated.

Note: It is the responsibility of the Metabolic Diagnostic laboratory** to review results of c.985A>G analysis and biochemistry and, together with input from the clinician, arrange for EMS if required.

EMS is required in the following circumstances:

- 1 copy c.985A>G (regardless of biochemistry)
- No c.985A>G but with abnormal biochemistry
- Clinical reasons

*Sample requirement for C8 and acylcarnitine scan – dried blood spot or plasma – to be decided locally.

** If local arrangements differ from this for logistical reasons the responsibility for confirming whether EMS is required must be clear.

Results of follow-up samples

Biochemically ABNORMAL
Average of duplicate C8 \( \geq 0.50 \mu mol/L \) and / or abnormal UOA and / or abnormal HG (qualitative or quantitative analyses)

Biochemically NORMAL
Average of duplicate C8 \(< 0.50 \mu mol/L \) and normal UOA and normal HG (qualitative and quantitative analyses)

Note: A diagnostic increase in urine hexanoylglycine is defined as a clearly visible peak for hexanoylglycine with a confirmatory mass spectrum

EMS
Results of EMS, as performed by Sheffield or Guy’s laboratory (as above) are accompanied by an up-to-date report summary of the likely clinical significance of mutations identified (where known).

Timing of samples and results
All samples as listed above to be taken and sent for analysis at first review appointment, including sample for EMS. EMS analysis must be confirmed with Sheffield or Guy’s laboratory (as above) at 1st follow-up appointment following genetic / biochemical results.

Results of C8, qualitative UOA and 985A>G analysis to be available by 1st follow-up appointment (within 5 working days of first review). If diagnosis not confirmed, contact Sheffield or Guy’s laboratory (as above) immediately to proceed with analysis.

Results of quantitative UOA and EMS (if MCADD not previously confirmed) to be available by 2nd follow-up appointment (within 15 working days of 1st follow-up appointment).

See MCADD Screening and Clinical Management Protocols and MCADD Laboratory Handbook for further details. Available at [www.newbornbloodspot.screening.nhs.uk/mcadd](http://www.newbornbloodspot.screening.nhs.uk/mcadd)
Appendix 9 MCADD clinical management protocol*

Presumptive positive – MCADD**

Lab notifies MCADD Clinical Liaison Service (CLS) as per local protocol on the day of the result

ON THE SAME DAY
MCADD CLS contact designated (or specialist) team as per local protocol to arrange ‘first review’ appointment (to take place within 24hrs).
MCADD CLS contact GP and ensure appropriate face-to-face contact is made with family that day (nurse specialist / GP / HV / midwife as per local protocol).
MCADD CLS to fax / email following information to GP: GP letter, ‘MCADD is suspected’ leaflet, A&E letter, contact details for MCADD designated (or specialist) team, appointment time and location. Ensure family given above information (except GP letter) when first family contact is made.

FIRST REVIEW: FACE-TO-FACE - (within 24 hours of screening result)
Consent for DNA testing to be obtained
Take diagnostic samples***
Dietetic review / Emergency Regimen (ER) teaching
Clinician to ensure:
  i) Family have received - MCADD designated (or specialist) team contact details, A&E letter, appropriate dietary and ER guidelines
  ii) Letters have been sent to: GP, local paediatrician, local dietician as necessary

1st FOLLOW-UP VISIT - within 5 working days of 1st face-to-face review
Clinical review and results: - Octanoylcarnitine (C8), qual. urine organic acid (UOA), c.985A>G mutation analysis
Arrange sibling screening if MCADD confirmed****
If diagnosis not confirmed – see diagnostic protocol***

2nd FOLLOW-UP VISIT - within 15 working days of 1st follow-up visit
Clinical review and results: Extended mutation screening, quantitative UOA
Arrange sibling screening if MCADD confirmed****

MCADD CONFIRMED

NO FURTHER ACTION

WEANING FOLLOW-UP VISIT
Dietetic and clinical review (weaning) at 4 - 6 months of age

POST- WEANING FOLLOW-UP VISIT
Dietetic and clinical review - post-weaning

FURTHER FOLLOW-UP VISITS AS NEEDED

* See MCADD initial clinical referral guidelines and standards for further details

** See MCADD screening protocol for details

*** See MCADD diagnostic protocol for confirmatory test details

**** See MCADD sibling protocol for details
### Appendix 10 MCADD initial clinical referral guidelines and standards

<table>
<thead>
<tr>
<th>Stage of process</th>
<th>No.</th>
<th>Guidelines and standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defining a positive screening result</td>
<td>1</td>
<td>If a sample from a baby is found to have an octanoylcarnitine concentration equal to or greater than 0.40 μmol/L, repeat tests should be performed in duplicate on the original blood spot card.</td>
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<td></td>
<td>2</td>
<td>If the mean of triplicate results is equal to or greater than 0.50 μmol/L, AND the mean C8:C10 ratio ( \geq 1.0 ), this is a presumptive positive screening result.</td>
</tr>
</tbody>
</table>
| Referral of babies with positive screening result | 3   | The screening laboratory must inform the MCADD clinical liaison service* (CLS) – as per local protocol – of a positive screening result on the same day as the positive result (in 2 above) has been reported by the laboratory.  
**ON THE SAME DAY**  
The CLS must make contact, both verbally (by telephone) as well as in writing (by fax / email – use template – ref. below) with:  
- MCADD designated (or specialist) team – as per local protocol – to arrange appointment for family to be seen within 24 hours of receiving a positive screening result – ‘1st face-to-face review’ (see 6 below)  
- Family’s GP to coordinate first family contact (see 4 and 5 below)  
An MCADD designated team must include clinicians trained to receive MCADD referrals and have a paediatric dietitian.  
An MCADD specialist team should comprise:  
- A consultant Inherited Metabolic Disease paediatrician with relevant expertise  
- A paediatric dietitian with metabolic expertise  
- A clinical nurse specialist.  
Note. For those with mean triplicate C8 results reported on a Friday, provision must be made for ‘1st face-to-face review’ appointment to take place on same day (Friday) or Saturday as necessary.  
If a disorder / situation other than MCADD is suspected, referral must be made to the MCADD designated (or specialist) team as above.  
*CLS role may be undertaken by person(s) based in the screening laboratory (i.e. a screening clinical nurse specialist or duty biochemist), in the designated clinical team or in the community, depending on local arrangements.
## Appendix H

<table>
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<tr>
<th>Stage of process</th>
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</table>
| Communication flows | 4 | **Pre-family contact**  
**ON THE SAME DAY**  
The CLS must contact the GP and:  
- Co-ordinate local support  
- Obtain a telephone number for the family  
- Ensure that the family is seen by a health professional that day  
(e.g. GP / midwife / health visitor / clinical nurse specialist – as per local protocol)  
- Fax or email information about MCADD as follows:  
  - MCADD GP letter  
  - ‘MCADD is suspected’ leaflet (includes UK Newborn Screening Programme Centre website address and links to parent support group)  
  - MCADD A&E letter  
  - Contact numbers for the MCADD designated (or specialist) team  
  - Details of the time and location of an appointment with the MCADD designated (or specialist) team identified in 3 above. |

|  | 5 | **First family contact**  
**ON THE SAME DAY**  
Contact with the family must be made to inform them of the positive screening result. This contact must be face-to-face and, ideally, include a health professional known to the family. The person* contacting the family must be provided with information for the family as follows:  
- ‘MCADD is suspected’ leaflet (includes UK Newborn Screening Programme Centre website address and links to parent support group)  
- MCADD A&E letter  
- Contact numbers for the MCADD designated (or specialist) team  
- Details of the time and location of an appointment with the MCADD designated team identified in 3 above  
It is strongly recommended that the family has access to MCADD specialist team by telephone.  
*Local protocols may wish to consider utilising 24hr midwifery services |
<table>
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<tr>
<th>Stage of process</th>
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<tbody>
<tr>
<td>Clinical evaluation and confirmatory diagnostic tests</td>
<td>6</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; face-to-face review&lt;br&gt;(SAME OR) NEXT DAY&lt;br&gt;Pre-diagnosis management should include:</td>
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<td>• Explanation of the condition including introduction to inheritance</td>
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<td>• Introduction to dietary management (including maximum safe fasting times) and use of Emergency Regimen (ER) for illness – as per MCADD dietary guidelines (ref. below)</td>
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<td>• Contact with specialist dietitian</td>
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<td></td>
<td>• Clinician to ensure family have received – MCADD designated (or specialist) team contact details, A&amp;E letter, appropriate dietary and ER guidelines</td>
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<td></td>
<td></td>
<td>• Clinician to ensure letters have been sent to GP, local paediatrician, local dietitian as necessary (templates available – ref. below).</td>
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<td>7</td>
<td>Confirmatory diagnostic samples should be collected at 1&lt;sup&gt;st&lt;/sup&gt; face-to-face review (i.e. within 24 hours of receiving screening results). Consent for DNA testing to be obtained.</td>
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<td>8</td>
<td>When a baby is seen with a positive screening result, specimens for the following tests* should be collected to confirm diagnosis and exclude other possible metabolic disorders:</td>
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<td>• Repeat blood acylcarnitines: C8 and full scan</td>
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<td>• Urine organic acid (UOA) analysis</td>
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<td></td>
<td>• DNA analysis (c.985A&gt;G and Extended Mutation Screening (EMS – samples to be sent immediately)</td>
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<td>*see MCADD diagnostic protocol for details (ref. below)</td>
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<td>9</td>
<td>Follow-up visits&lt;br&gt;FURTHER APPOINTMENTS TO BE SCHEDULED AS FOLLOWS:&lt;br&gt;1&lt;sup&gt;st&lt;/sup&gt; follow-up visit within 5 working days of 1&lt;sup&gt;st&lt;/sup&gt; face-to-face review – for results of C8, qualitative UOA and DNA testing for the common mutation c.985A&gt;G.</td>
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<td>If diagnosis not confirmed – see diagnostic protocol*.</td>
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<td>2&lt;sup&gt;nd&lt;/sup&gt; follow-up visit if diagnosis not yet confirmed within 15 working days of 1&lt;sup&gt;st&lt;/sup&gt; follow-up visit – for results of quantitative UOA and EMS.</td>
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<td>*see MCADD diagnostic protocol for details (ref. below)</td>
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<tr>
<td>Upon confirmation of diagnosis</td>
<td>10</td>
<td>Dietary management and when to implement Emergency Regimen (ER) must be emphasised.</td>
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<td>11</td>
<td>Post-diagnosis discussion should ensure parents have good understanding of the condition, support information, correct contact numbers for MCADD team, information for A&amp;E, age appropriate dietary management / ER information.</td>
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<td>12</td>
<td>A specialist nurse should be available to provide advice and support.</td>
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<td>13</td>
<td>The specialist dietitian should make contact with the local dietitian if appropriate</td>
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</table>
|                  | 14  | Older siblings to be tested as appropriate – or arrangements made for testing. Family to be made aware of opportunities for early postnatal testing of subsequent siblings.  
  See MCADD sibling protocol for details (ref below) |
|                  | 15  | Post-confirmation of diagnosis appointments to be scheduled to meet needs of the family. This must include:  
  • A visit at 4-6 months of age for dietary review and advice on weaning  
  • Post-weaning clinical and dietetic review |
| Contact with dietitians | 16  | Parents should be given the opportunity to have ongoing access to a specialist dietitian and should be provided with appropriate contact details. |
|                  | 17  | Discussion between parents and the dietitian should cover:  
  • Age appropriate advice on feeding (including breastfeeding and weaning) and maximum safe fasting times  
  • ER for illness: including preparation and use of ER feeds |

**MCADD dietary management guidelines**  
MCADD Laboratory Handbook and Protocols (screening, diagnostic and sibling testing)  
MCADD letter / fax templates  
- all available at:  

www.newbornbloodspot.screening.nhs.uk/mcadd

Further information / protocols available at: www.bimdg.org.uk/mcadd.asp