



Public Health
England

NHS

NHS Newborn Blood Spot Screening Programme

A laboratory guide to newborn blood spot screening for inherited metabolic diseases

Updated September 2017

Public Health England leads the NHS Screening Programmes

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Public Health England, Wellington House, 133-155 Waterloo Road, London SE1 8UG

Tel: 020 7654 8000 www.gov.uk/phe

Twitter: [@PHE_uk](https://twitter.com/PHE_uk) Facebook: www.facebook.com/PublicHealthEngland

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Abbreviations

| | |
|---------------------------|--|
| C0 | free carnitine |
| C2 | acetylcarnitine |
| C5 | isovalerylcarnitine |
| C5-DC | glutarylcarnitine |
| C8 | octanoylcarnitine |
| C10 | decanoylcarnitine |
| CBS | cystathionine β -synthase |
| CDC | Centres for Disease Control and Prevention |
| CF | cystic fibrosis |
| CHT | congenital hypothyroidism |
| CLS | clinical liaison service |
| DHPR | dihydropteridine reductase |
| EQA | external quality assessment |
| GA1 | glutaric aciduria type 1 |
| GA2 | glutaric aciduria type 2 |
| GAL-1-PUT | galactose-1-phosphate-uridyl-transferase |
| GALP | galactose-1-phosphate |
| GCDH | glutaryl-CoA dehydrogenase |
| HCU | homocystinuria |
| IMD | inherited metabolic disease |
| IQC | internal quality control |
| IS | internal standards |
| IVA | isovaleric acidaemia |
| IVD | isovaleryl-CoA dehydrogenase |
| LCHADD | long-chain hydroxyacyl-CoA dehydrogenase deficiency |
| LC/MS/MS or UPLC-MS/MS | tandem mass spectrometer with liquid chromatography sample induction |
| Leu | leucine (when measured by MS/MS can include isoleucine and alloisoleucine) |
| MADD | multiple acyl-CoA dehydrogenase deficiency |
| MAT | methionine adenosyl transferase |
| MCADD | medium-chain acyl-CoA dehydrogenase deficiency |

| | |
|--------------|--|
| Met | methionine |
| MRM | multiple reaction monitoring |
| MS/MS | tandem mass spectrometry |
| MSUD | maple syrup urine disease |
| PAH | phenylalanine hydroxylase |
| Phe | phenylalanine |
| PHE | Public Health England |
| PKU | phenylketonuria |
| PPV | positive predictive value |
| SBCAD | short/branched chain acyl-CoA dehydrogenase deficiency |
| SCD | sickle cell disease |
| THcy | total homocysteine |
| TSH | thyroid stimulating hormone |
| Tyr | tyrosine |
| UKCSNS-MCADD | UK Collaborative Study of Newborn Screening for MCADD |
| UKNEQAS | UK National External Quality Assessment Service |
| UK NSC | UK National Screening Committee |

1. Introduction

This guide supports newborn screening laboratory service provision for inherited metabolic disease (IMD) screening and is available together with other relevant documents [online](#). In large part it is to be used as a guide and specifically not as a detailed manual of required practice or to provide a basis for standards forming the basis for regulation or accreditation. To make the distinction clear, the elements of regulatory standards to be maintained by all laboratories are shown separately in Appendix 15.

At the time of publication, every attempt has been made to provide correct and up-to-date information. If there are any errors or comments, please send them to the [NHS Newborn Blood Spot Screening Programme](#) for incorporation into the next version. Please note the guide will remain under regular review.

1.1 Background and aims of NHS Newborn Blood Spot Screening Programme

Newborn screening began in the late 1950s with locally organised screening initiatives for phenylketonuria (PKU) using ferric chloride solutions later developed into the Phenistix test. In 1969, following developments in the USA led by the late Robert Guthrie, the UK recommended changing to a blood-based screen, increased concentration of phenylalanine being a more sensitive and specific indicator of PKU than the urinary metabolites. Universal screening for PKU using dried blood spots collected onto a filter paper card (the Guthrie card) at around one week of age was in place throughout the UK by the early 1970s.

In 1980 screening for congenital hypothyroidism (CHT) based on thyroid stimulating hormone (TSH) was added and by 2007 laboratories in the UK were also offering screening for cystic fibrosis (CF). At around the same time, screening for sickle cell disease (SCD) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD) was introduced in England (and in the devolved nations during 2009–2013). The technology needed to undertake screening for MCADD, tandem mass spectrometry (MS/MS), simultaneously provided the potential to detect a range of rare metabolic conditions. In 2012/13 a study was undertaken to evaluate the utility of screening for five additional disorders: maple syrup urine disease (MSUD), isovaleric acidaemia (IVA), glutaric aciduria type 1 (GA1), homocystinuria (pyridoxine unresponsive) (HCU) and long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). In May 2014, the UK National Screening Committee recommended that four of these (MSUD, IVA, GA1 and HCU) should be included as part of the national programme with effect from January 2015.

The early detection offered by newborn screening is a significant benefit for patients with all of the conditions included within the programme and in some cases can be lifesaving. The successful introduction of screening depends upon the cohesive management of testing as a programme of care from pre-screening information to screening results and in the case of positive cases enrolment into appropriate treatment. It is important that parents are fully

informed at each stage and that only clinically significant disease is detected with a minimum number of false positive cases. Once a screen positive patient is identified, the period of uncertainty required for any associated confirmatory testing must be kept to a minimum and parents supported during this trying time.

While it is hoped that all parents take up the offer of screening for the IMDs, they are able to decline. It should be noted that parents can only decline screening for the six IMDs as a group rather than individually. Screening for SCD, CF and CHT can still be declined individually.

1.2 Scope and purpose

This handbook provides guidance to laboratories that provide a newborn blood spot screening service for IMDs. It defines a framework for the pre-analytical, analytical and post-analytical steps in the newborn screening process so that a consistent approach is maintained. Built into this framework is detailed guidance on achieving good quality by application of standards and audit.

1.3 Scientific background – common elements

The conditions included in this handbook share a common technological approach to their detection. MS/MS is used to detect all the key compounds of interest. The technique uses a triple quadrupole mass spectrometer. Ions produced by the source are selected by the first quadrupole to enter a second quadrupole which acts as a collision cell where molecular fragmentation takes place. The products, which are characteristic of the molecule, are then filtered by the final quadrupole before entering a detector. Complex mixtures can be resolved in this way, allowing untreated blood eluates to be analysed. The instrument can monitor a number of fixed transitions which restricts the compounds detected to a defined and pre-selected list including the amino acids and acylcarnitines of interest which are chosen as signature compounds related to the disorders to be detected. So, PKU may be detected by measuring phenylalanine (Phe), HCU by measuring methionine (Met), MSUD by measuring leucine (Leu), MCADD by measuring C8 acylcarnitine, GA1 by measuring C5-DC acylcarnitine and IVA by measuring C5 acylcarnitine. These analyses can be performed simultaneously in the same sample within two or three minutes and the process can be automated providing a useful basis for mass screening.

1.4 Screening laboratory organisation

IMD screening is fully integrated within the existing newborn blood spot screening programme and no additional blood spot sample is required. The screening tests are undertaken at the same time as the tests for other disorders (SCD, CF and CHT) using blood from the same heel-prick blood sample, collected on the standard newborn screening collection card.

C8, C5, C5-DC, Phe, Met and Leu are measured by electrospray 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. The IT and LIMS which supports laboratory operation and connectivity to Newborn Blood Spot Failsafe Solution and child health records departments should maintain adequate back-up and resilience with contingency and recovery plans in the event of system failure. There must also be a documented risk management policy for the laboratory aspects of the IMD screening programme as part of an overall newborn screening risk management policy.

2. The screening protocols – common elements

2.1 Aims

The screening protocols for each disorder are designed to maximise sensitivity while seeking to reduce the number of false positive results generated and deliver an acceptable positive predictive value for the test overall (PPV%). In some cases improved specificity is achieved by the use of secondary testing as part of the screening protocol (total homocysteine in the case of HCU and C10 in the case of MCADD). In all cases, an analytical cut-off is set approximately 20% below the referral cut-off and those samples which exceed the analytical cut-off are re-tested in duplicate on the same day. If the mean of the three results exceeds the referral cut-off then the patient is referred, or in the case of MCADD and HCU, the C8:C10 ratio or total homocysteine is assessed respectively and the decision to refer is based upon these results.

2.2 New sibling testing

A new sibling born to the same parents when an index case with PKU, MCADD, MSUD, IVA, GA1 or HCU has already been identified has a 1 in 4 risk of having the same disorder. In these circumstances it is good practice to test earlier than the 5–8 day timeframe for newborn screening to avoid delays in diagnosis and to allay parental anxiety. However, this does not remove the need for routine screening and it is essential that the routine blood spot collection is undertaken between 5 and 8 days to screen for the other conditions tested for as part of the newborn blood spot programme.

The decision about when to test depends upon the condition suspected; it is most pressing for disorders such as MSUD, MCADD and IVA which can potentially have an early neonatal presentation and may be at risk.

2.3 Older sibling testing

Older siblings of newly diagnosed screen detected cases, when born to the same parents, have a 1 in 4 chance of having been born with the same disorder. It may be that previous screening of these children or simply the fact that they are asymptomatic as an older child reduces this risk very considerably. Nevertheless, this possibility cannot be fully discounted and careful consideration should be given by the clinician investigating the baby referred via screening to the risks for other, older siblings in the family. Note that older sibling testing is undertaken by diagnostic laboratories.

2.4 Late testing

The cut-off values used at 5–8 days of age in relation to screening cannot automatically be assumed to be reliable in older infants and in the case of MSUD intermittent forms are known to occur. Similarly, GA1 patients with minimal excretion of key metabolites have been described and while screening should take place if a child is under a year of age, any pre-existing clinical concerns should be investigated thoroughly and separately.

If a child is under a year of age (up to but not including their first birthday) and has no documented results (or declines) for all five conditions screened for before the expansion of the programme, screening should be offered for all the untested conditions (including the four additional inherited metabolic diseases) only if the blood spot sample can be taken before they reach a year of age. See '[Procedures for newborn blood spot \(NBS\) screening of babies under a year of age, for whom screening results are not available](#)'.

The standard national cut-off should be applied. However, it is noted that rarely, older infants with MCADD may have C8 levels below the screening cut-off and there is a potential for false negative screening results (see section 9.4.2). If MCADD is suspected, the infant should be referred to a clinician for diagnostic testing. These effects, linked in part to carnitine depletion, might also be expected to reduce the sensitivity of testing for IVA and GA1. Again, if these conditions are suspected, the infant should be referred to a clinician for diagnostic testing.

2.5 Specimen requirements

As for all screening tests, good quality blood spots are essential. Specimens of inadequate size or that are compressed or damaged in other ways are likely to give erroneous / misleading results and may result in false negatives with babies being missed. National laboratory guidelines were published in 2015 to document agreed rejection criteria to be used by English newborn screening laboratories. These criteria are based on blood spot quality research and audit and are intended to improve the quality of blood spot samples and, in turn, the efficiency of the screening pathway.

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 guidelines in the '[Code of Practice for the Retention and Storage of Residual Spots](#)'. The Code of Practice is currently under review.

2.6 Receipt of 'not required' newborn screening cards

Occasionally laboratories may receive newborn screening cards which were not required and sent in error. Ideally laboratories should analyse and report **only** the first valid, day 5 sample, received. However, laboratory processes may mean that analysis is sometimes commenced before cards are identified as a 'not required sample'.

In all cases samples should be recorded on the system and a result code 0906 (not required, previous valid result) generated. If analysis can be avoided then it should not be performed. If it is performed and all the parameters are below the cut off for action in accord with the first valid sample then it should be reported as 0906.

If for any reason the results obtained require individual follow-up then this should be undertaken and reported appropriately. The samples should then be stored as usual in case further enquiries arise.

2.7 Genetic counselling

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

3. General analytical aspects

Newborn screening for the IMDs should be provided using the nationally agreed screening protocols with the screening tests performed using an underivatized multiple reaction monitoring (MRM) tandem mass spectrometric technique. Any proposal to introduce new analytical methods needs careful collective consideration and approval by the NHS Newborn Blood Spot Screening Programme.

Phenylalanine (Phe), tyrosine (Tyr), leucine (Leu), methionine (Met), octanoylcarnitine (C8), decanoylcarnitine (C10), isovalerylcarnitine (C5) and glutarylcarnitine (C5-DC) are measured in underivatized solvent extracts from dried blood spots using a tandem mass spectrometer with liquid chromatography sample induction (LC/MS/MS or UPLC-MS/MS). MRM acquisition mode must be used and analysis restricted to the specified analyte. Table 1 shows example MRM ion transitions used.

Table 1. MRM ion transitions for amino acids and acylcarnitines

| Metabolite | Transition* | Example IS | Transition* |
|------------|-------------|---|-------------|
| Phe | 166 → 120 | ² H ₅ - Phe | 171 → 125 |
| Tyr | 182 → 136 | ² H ₄ - Tyr | 186 → 140 |
| Leu | 132 → 86 | ² H ₃ - Leu | 135 → 89 |
| Met | 153 → 107 | ² H ₃ - Met | 156 → 110 |
| C8 | 288 → 85 | ² H ₃ - C8 | 291 → 85 |
| C10 | 316 → 85 | ² H ₃ - C8 or C10 | 291 → 85 |
| C5 | 246 → 85 | ² H ₉ - C5 | 255 → 85 |
| C5-DC | 276 → 85 | ² H ₃ - C5-DC | 279 → 85 |

*Please note specific transitions should be established by the tuning of each instrument in each individual laboratory.

Internal standards (IS) of stable isotopes of Phe, Tyr, Leu, Met, C8, C5, C5-DC are prepared in a suitable solvent (e.g. 80% methanol) and are used to elute Phe, Tyr, Leu, Met, C8, C10, C5, C5-DC from a punched dried blood spot disc in multiwell plates.

MS/MS sampling can be direct from the original plate (with blood spots in situ) or the eluates 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 Appendix 1 for guidance on discrepant replicates).

Calibration options include simple ratio to an 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 IS abundances by the analytical software since sensitivity requirements for C8 are 100 fold that of Phe. Inspection of individual flow profiles and ion abundances is also recommended.

4. Quality assurance

There are four foundational aspects to maintaining quality in the clinical laboratory:

1. The need to participate in a recognised laboratory accreditation process that addresses: structure, process and outcome characteristics when providing a clinical laboratory service.
2. A requirement to use and participate in: real time performance monitoring using carefully designed internal quality control procedures with clearly defined batch acceptability criteria and trend analysis; participation in approved regular external quality assurance scheme arrangements with a clearly defined poor performer policy used to identify and address inadequate performance; when available, population data monitoring and analysis to identify and report performance and trend analysis using real patient data.
3. The need to record and report incidents using this as a learning tool to improve service provision.
4. User feedback to ensure that the service provided is understood and meets user requirements. Again this can be used as a means of continuous improvement.

Laboratories must establish appropriate quality and performance monitoring procedures as set out below. Details on reporting performance and incidents are given in section 7.

4.1 Internal quality control and performance monitoring

It is recommended that relevant levels of dried blood spot internal quality control (IQC) are included at the beginning and end of each plate. Three levels of C8, C10, C5, C5-DC, Phe, Tyr, Met and Leu are recommended.

- i) normal
- ii) medium (level around initial cut off)
- iii) high

Suitable levels are not always available commercially so there may be a need for in-house preparations to be used on each plate.

4.1.1 Performance criteria

The performance criteria outlined are example data from **one** newborn screening laboratory. The results achieved from each newborn screening laboratory will vary, as there are many variable factors involved in the assay, therefore the data should only be referred to as a guide and not a standard to measure against.

Method validation should be carried out in accordance with the ISO 15189:2012 standards. Waters Xevo TQD was used to provide the following performance data.

Table 2. Performance data: between batch precision

| | | | | |
|----------------------|-------|-------|-------|-------|
| Low level | C5 | C5DC | C8 | C10 |
| Mean (µmol/L) | 0.099 | 0.115 | 0.041 | 0.011 |
| CV (%) | 7.9 | 19.9 | 6.0 | 22.9 |

| | | | | |
|----------------------|------|------|------|------|
| Medium level | C5 | C5DC | C8 | C10 |
| Mean (µmol/L) | 0.61 | 0.88 | 0.61 | 0.48 |
| CV (%) | 8.0 | 9.1 | 10.2 | 12.8 |

| | | | | |
|----------------------|-----|------|-----|-----|
| High level | C5 | C5DC | C8 | C10 |
| Mean (µmol/L) | 3.5 | 4.4 | 3.3 | 2.9 |
| CV (%) | 6.2 | 6.8 | 6.8 | 7.0 |

| | | | | |
|----------------------|-----|------|-----|-----|
| Low level | Met | Leu | Phe | Tyr |
| Mean (µmol/L) | 20 | 194 | 86 | 65 |
| CV (%) | 7.2 | 10.3 | 7.5 | 7.0 |

| | | | | |
|----------------------|-----|------|-----|-----|
| Medium level | Met | Leu | Phe | Tyr |
| Mean (µmol/L) | 70 | 322 | 211 | 285 |
| CV (%) | 9.5 | 11.4 | 7.5 | 7.2 |

| | | | | |
|----------------------|-----|------|------|-----|
| High level | Met | Leu | Phe | Tyr |
| Mean (µmol/L) | 264 | 690 | 406 | 654 |
| CV (%) | 9.6 | 11.6 | 10.4 | 7.4 |

Table 3. Performance data: sensitivity/linearity

| | |
|-------------|-------------|
| Met | 3.41 – 2091 |
| Leu | 8.19 – 2993 |
| Phe | 5.33 – 3098 |
| Tyr | 5.70 – 2757 |
| C5 | 0.07 – 24.7 |
| C5DC | 0.08 – 17.8 |
| C8 | 0.06 – 25.7 |
| C10 | 0.09 – 25.4 |

4.2 External quality assurance of screening metabolites

A UK external quality assessment (EQA) scheme is provided by UKNEQAS for the analytes phenylalanine (Phe), tyrosine (Tyr), octanoylcarnitine (C8) and decanoylcarnitine (C10). In addition to this scheme, UKNEQAS provide a scheme for the expanded newborn screening

analytes methionine (Met), leucine (Leu), isovalerylcarnitine (C5) and glutarylcarnitine (C5-DC). The purpose of these schemes is to provide a quality assessment of precision and accuracy for the analysis of Phe, Tyr, C8, C10, Met, Leu, C5 and C5-DC for laboratories that screen for PKU, MCADD and the expanded screening disorders (MSUD, IVA, GA1 and HCU).

Three specimens are circulated on a monthly basis for each scheme. All laboratories in the UK which screen for PKU, MCADD and the expanded screening disorders are expected to take part in this scheme. For more details please contact Finlay MacKenzie, UKNEQAS (Finlay.MacKenzie@uhb.nhs.uk).

Many laboratories also find that membership of the EQA scheme offered by the Centre for Disease Control (CDC) is valuable.

4.3 External quality assurance of other analytes involved in the screening pathway

Galactosaemia testing (PKU)

The Centres for Disease Control and Prevention (CDC) newborn screening proficiency programme includes testing for total galactose and galactose-1-phosphate uridylyltransferase (CDC lab standards).

Biopterin and DHPR (PKU)

There is currently no EQA scheme. ERNDIM commenced a pilot EQA scheme for blood spot pterins and dihydropteridine reductase (DHPR) in March 2014 but this has since ceased.

Homocysteine testing (HCU)

There is currently no EQA scheme in dried blood spots. Inter-laboratory comparisons are carried out between the three laboratories that provide this service. A pilot dried blood spot scheme is planned for 2017 to be organised by ERNDIM and laboratories are encouraged to enrol.

4.4 Normal population data

A programme for monitoring the performance of the C8 assay using whole population newborn screening data compared data from the six laboratories that took part in the expanded newborn screening pilot. This demonstrated good concordance in the population distribution of C8 values both within and between laboratories, and proved valuable as a quality assurance measure (Khalid et al, 2010).

This valuable approach has been re-instituted in 2015 to include the analytes that form part of screening for the additional disorders (MSUD, IVA, GA1 and HCU) together with C8 and Phe. The data returns made by laboratories (instructions to be provided) will be summarised and

discussed on a quarterly basis. Continuation of this scheme will be reviewed on an annual basis but is planned for 2017/18.

5. Stability of acylcarnitines and amino acids in dried blood spots

At room temperature acylcarnitines in blood spotted on filter paper are slowly hydrolysed to free carnitine, and their corresponding fatty acids and amino acids may also degrade (acylcarnitine profile images are available at [MetBioNet](#)).

For the acylcarnitines, 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 generally increases with the increasing chain-length of the acyl group. The decay appears to be biphasic with more rapid decay in the first few weeks at room temperature (Fingerhut et al, 2009). The stability of C5-DC (Johnson et al, 2004) appears particularly poor and at room temperature will degrade by at least 50% in six months. High humidity and ambient temperature will also increase the rate of degradation (Golbahar et al, 2014).

The amino acids Tyr, Leu, Phe and Met decrease at a rate of 1.7%, 3.1%, 5.7% and 7.3% per year respectively for the first five years in dried blood spots when stored at room temperature in a dry environment (Strnadova et al, 2007).

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%.

6. Clinical referral and follow up – common elements

The need for prompt and effective intervention in screen positive patients is important for all conditions that form part of the newborn screening programme. Some of the inherited metabolic diseases including MSUD, IVA, GA1 and HCU have particularly complex or urgent needs and evidence suggests that outcome may be strongly influenced by referral and treatment pathways. It is therefore important that the referral of all IMD screen positive patients is undertaken in co-ordination with a clinical service that complies with the service specification for a paediatric IMD centre; this is largely aimed at ensuring a continuity of specialist care.

It is essential that each screening laboratory, in collaboration with commissioners and local implementation groups, makes detailed local arrangements for the follow-up of presumptive positive cases for all districts covered by their laboratory. These arrangements should be updated regularly to reflect personnel and organisational changes.

7. Reporting and data collection

7.1 Reporting

7.1.1 Reporting results to child health records departments

Results need to be reported using the latest version of the screening status codes – see section 14 and Appendices 8–14. It is recommended that the screening report for a condition should be either ‘condition not suspected’ (code 04) or ‘condition suspected’ (code 08) (e.g. MCADD not suspected (code 04) or MCADD not suspected (08)). It is a requirement that all screening results are fed back to the child health records departments. It is recommended that child health records departments notify parents by **letter**.

Note that ‘PKU – other disorders follow up’ should be reported using status code 07.

7.1.2 Reporting positive cases to the NHS Newborn Blood Spot Screening Programme

Clinical information required to accompany the screening data should be requested from metabolic paediatricians by the laboratory directors on a case-by-case basis. Data on each case notified to the clinical referral services (‘condition suspected’ and ‘other disorders follow-up’) should be collated and anonymised before submission to the NHS Newborn Blood Spot Screening Programme using the annual data collection pro forma. It is the responsibility of the clinical services to provide this information to the laboratory directors.

7.1.3 Reporting affected not detected cases

It is important to notify the NHS Newborn Blood Spot Screening Programme when you are made aware of an affected baby that was not detected through the newborn screening programme. A **notification form** should be completed, anonymised and emailed from an nhs.net account to phe.screeninghelpdesk@nhs.net. Data on false negative cases will be collated by the NHS Newborn Blood Spot Screening Programme on an annual basis as an important part of the audit of the screening programme.

If a laboratory director is made aware of a case that has been reported as ‘condition not suspected’, in some cases (PKU and MCADD) this should be reported as a serious incident to the Trust and regional QA team in accordance with guidance on managing incidents (see section 7.1.4 – Incident reporting) on behalf of the regional directors of public health and to the NHS Newborn Blood Spot Screening Programme. Details must be collated by the clinical team in conjunction with the laboratory director and returned to the NHS Newborn Blood Spot Screening Programme.

Some cases (MSUD, IVA, GA1 and HCU) are not always detected by newborn screening and while the circumstances should be investigated it would not be appropriate to automatically treat these as a serious incident.

7.1.4 Incident reporting

The NHS Screening Programmes have clear guidance on the roles and responsibilities for reporting, investigating and managing screening incidents. The guidance is in addition to existing policies at local, regional and national level. For more details see '[Managing Safety Incidents in NHS Screening Programmes](#)'.

7.2 Data collection

Data collection to monitor the performance of the IMD screening programmes is essential to ensure that the screening protocols are detecting all clinically relevant cases and that the quality of the programmes is maintained.

The NHS Newborn Blood Spot Screening Programme collects data annually to measure UK performance against the quality standards (see section 15). The standards for IMD clinical referral will reflect the [IMD \(Paediatric\) Clinical Reference Group \(CRG\) Quality Dashboard](#) which states that newborn babies with a positive metabolic NBS result for PKU, IVA, GA1, MCADD and MSUD should be seen by day 14 of life in a designated clinic and that diagnostic testing should be completed within 5 working days of receipt of the diagnostic samples into the laboratory (some exceptions apply – see individual condition-specific chapters).

Each screening laboratory is expected to submit specific data – results are then reported in the data collection and performance analysis reports. The laboratory-based data should be collected by the directors of newborn screening for each area and submitted annually, on a retrospective basis, to the NHS Newborn Blood Spot Screening Programme by 15 July for the previous financial year (1 April to 31 March). For more information please contact phe.screeninghelpdesk@nhs.net.

Laboratories should also provide data on screening performance to regional and local audit / quality management groups as required.

The results and performance of the IMD 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). Normally this will be a combined report covering all blood spot screens, dealing also with common issues such as specimen quality, timeliness, etc. There should also be periodic multidisciplinary review of local policies for IMD screening in the light of accumulated results, technical developments and any local changes in health care provision.

8. Phenylketonuria (PKU)

8.1 Scientific background

Phenylketonuria was first described by Asbjørn Følling in 1934 and is one of the most common inherited metabolic disorders with an overall incidence in Europe and the USA of 1:10,000-15,000 live births (Scriver et al, 1995). The average incidence across the UK is approximately 1 in 10,000 births although there is geographical variance. PKU is an autosomal recessively inherited disorder of amino acid metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH). The enzyme is required to metabolise phenylalanine to tyrosine. A deficiency of the enzyme results in an accumulation of phenylalanine and associated metabolites in blood and tissues. The infant brain is sensitive to high phenylalanine levels and if left untreated, patients with PKU develop severe mental retardation and microcephaly, and a proportion of patients develop epilepsy. Older patients have behavioural problems and some suffer from psychiatric illnesses. A few patients develop movement disorders with extrapyramidal signs (Brenton and Pietz, 2000).

In PKU / hyperphenylalaninaemia due to PAH deficiency the blood tyrosine level is normal / low and tyrosine becomes an essential amino acid. In babies with a high phenylalanine on screening due to liver dysfunction the blood tyrosine level is usually high, an important measure to differentiate this from PKU / hyperphenylalaninaemia.

The clinical consequences of the metabolic defect are dependent on the degree of elevation of phenylalanine which is determined by the residual activity of PAH. PKU is heterogeneous and a number of phenotypic variants are recognised. The following definition of screening outcomes is recommended in the 2010 report of the PKU Expert Group (UK Newborn Screening Programme Centre, 2010):

- cases that require treatment for PKU
- cases that require ongoing monitoring (who may or may not at some point require treatment for PKU)
- non-PKU conditions

In PKU, the blood phenylalanine level at the time of newborn screening is usually in excess of 1000 $\mu\text{mol/L}$. This, however, depends on the baby having received normal feeds. In some cases the initial level may be less than this but will increase in the following weeks / months; such cases will require frequent monitoring of the plasma / blood phenylalanine level in the early stages following referral before deciding whether treatment is required.

Children with PKU requiring treatment are treated with a phenylalanine-restricted diet to reduce the flux through the affected metabolic pathway, thereby preventing the accumulation of toxic metabolites. Dietary therapy utilises a phenylalanine-free synthetic amino acid mixture as a substitute for natural protein and requires careful management to ensure appropriate vitamins and trace elements together with small amounts of natural protein are provided. Blood phenylalanine levels must be closely monitored and diet adjusted so that blood phenylalanine levels stay within accepted ranges to enable normal growth and intellectual function. Treatment should begin with minimum delay. Newborn Blood Spot Screening Standard 11 (NHS Newborn Blood Spot Screening Programme, 2017) states that clinical referral should be initiated by 14 days of age.

8.2 Other conditions potentially identified

An increased blood phenylalanine is not specific for PKU. In addition to disorders of phenylalanine hydroxylase deficiency, increased phenylalanine on newborn screening at 5 days may occur in several other situations; in some cases the phenylalanine increase may also be associated with an increase in tyrosine. In these latter cases PKU is **not** suspected (although not impossible if there are two co-existing disorders) and it is misleading to report screening results as 'PKU suspected'. These babies with an associated increase in tyrosine require different investigation and management and urgent referral to an appropriate specialist clinician is essential. An associated increase in tyrosine is used to categorise the screening result (see Figure 1 – PKU newborn screening protocol).

8.2.1 Non-specific causes of increased phenylalanine

See also section 8.5.2 – Potential for false positive results.

There are a range of non-specific causes which include:

- major illness e.g. organ failure (see below for disorders associated with liver dysfunction)
- transient illness*
- premature – liver maturity*
- diet / feed*
- parenteral nutrition
- analytical error – unlikely with triplicate testing*
- contamination of card* (see also section 8.5.2 – Potential for false positive results)

*These are the causes that might result in an increased phenylalanine on the screening specimen but normal on follow-up diagnostic testing, i.e. false positives.

In some of the above situations the phenylalanine increase may be associated with an increase in tyrosine.

8.2.2 Other disorders suspected

These are clinically significant 'by products' of screening for PKU and as such should be followed-up for further investigation. Detection of these other disorders is not the objective of the PKU screening programme and all cases will not be reliably detected.

Disorders of pterin metabolism

There are several different rare disorders of pterin synthesis / recycling which may be associated with an isolated increase in phenylalanine at screening and therefore detected by the PKU programme. They are disorders due to defects in the synthesis and recycling of the biopterin cofactor required for normal PAH activity, in particular deficiency of DHPR. These are very rare with a combined estimated incidence of 1–3% of all cases referred with hyperphenylalaninaemia.

Patients with these disorders may develop severe neurological dysfunction and require treatment and management which is different to that for PKU. It is not possible to exclude these disorders from the blood phenylalanine level and for this reason all babies with a PKU suspected screening result are tested for pterin disorders at follow-up (see section 8.8 – Diagnostic protocol).

Disorders associated with liver dysfunction

An increased blood phenylalanine **and** tyrosine may occur in babies with disorders associated with liver dysfunction.

Aetiologies include several causes of liver disease in the neonate e.g. hepatitis, biliary atresia, cytomegalovirus (CMV) and some inherited disorders, in particular galactosaemia and tyrosinaemia type 1. It is not possible to differentiate the cause of the liver dysfunction from the phenylalanine and tyrosine concentrations.

Galactosaemia

Galactosaemia requires urgent diagnosis and treatment (lactose-free diet) to avoid severe liver failure and death in the neonatal period.

It has been well established that an elevated phenylalanine (usually greater than 240 $\mu\text{mol/L}$) and tyrosine (usually greater than 240 $\mu\text{mol/L}$) on newborn screening are a feature of galactosaemia and for this reason it is important to consider as part of the differential diagnosis of a raised phenylalanine (Pollitt et al, 1982; Shakespeare et al, 2010). The incidence of galactosaemia in the UK is approximately 1 in 40,000 births.

Tyrosinaemia type 1

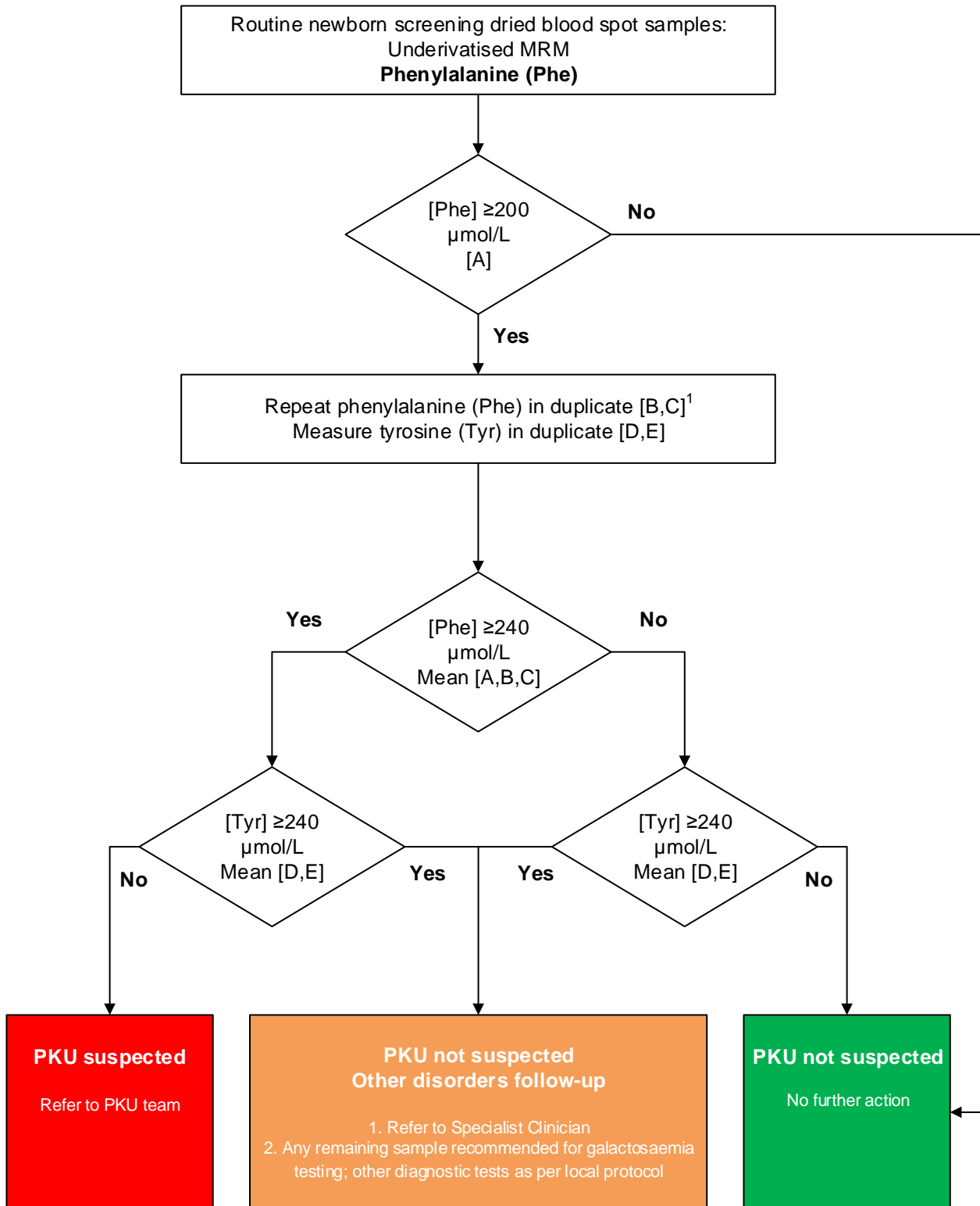
Tyrosinaemia type 1 if untreated usually results in rapidly progressive liver dysfunction with

significant morbidity and mortality and may require liver transplantation if not treated early with a specific drug. It is rare but more likely in certain ethnic groups.

It is important these disorders associated with liver dysfunction are excluded / diagnosed at an early stage and for this reason testing for galactosaemia using the screening specimen (see section 8.6.2) is recommended in those cases with an elevated phenylalanine associated with an increase in tyrosine. The clinical follow-up needs to consider these disorders where indicated from the screening results.

8.3 Screening protocol

Figure 1. PKU newborn screening protocol



¹ if insufficient blood to re-test and [Phe] ≥ 200 μmol/L arrange urgent referral to PKU team

8.4 Sibling testing

The pregnancy should be discussed with the specialist metabolic team caring for the proband early in pregnancy to ensure a plan for early testing is put in place.

When to test and samples to be taken

48–72 hours: Phenylalanine test (dried blood spot screening test)
Write on blood spot card 'Family history of PKU'

Day 5: Routine newborn screen
Indicate on the screening card that this is a second sample and write on the blood spot card 'Family history of PKU'

The baby should be established on a normal milk intake (bottle or breast milk) until the results become available.

8.5 Pre-analytical aspects

8.5.1 Potential for false negatives

False negative results arising within the laboratory may occur for the following reasons:

- 1. Missing sample spot in the plate well** should result in total absence of phenylalanine and a several fold increase in internal standard MS/MS signal abundance due to lack of ion suppression as a result of the missing blood sample. Procedures in the laboratory should be in place to detect this and could include visual checking that all designated wells contain a blood spot as processing progresses and a thorough examination of any suspicious results e.g. those with a very low result [Phe] <20 µmol/L and / or double normal internal standard abundance on MS/MS. Procedures should also be in place to detect any inappropriate addition of internal standard, which may lead to a falsely reduced phenylalanine concentration.
- 2. Transfusions** could result in a false negative result, as for other screening tests, and a repeat sample should be taken after a reasonable time has elapsed. At least 72 hours is recommended, as for the other screening tests, to allow pre-transfusion levels to be reached.
- 3. Delays in transit / sample deterioration** – it is possible that a sample may deteriorate due to adverse transport conditions and result in 'loss' of measured phenylalanine in the blood spot.
- 4. Physiological reasons** – false negative results may be of physiological origin, although this is in practice exceedingly rare.

The potential exists for false negative results to arise for physiological reasons e.g. in patients with mild mutations who are rapidly growing. There are no practical steps the laboratory can take to ensure detection of such cases but complete failure to detect a case of PKU is less likely in the UK due to the later sampling age at 5–8 days and the immediate referral of all cases with [Phe] ≥ 240 $\mu\text{mol/L}$.

Historically, using the Guthrie method for PKU newborn screening, there was a requirement for established milk feeding before sample collection to guarantee sufficient elevation of Phe levels for detection of abnormal cases. With MS/MS methodology and the UK protocols this is no longer necessary (UK Newborn Screening Programme Centre, 2005).

8.5.2 Potential for false positives

A false positive is where the phenylalanine result is confirmed on repeat analysis of the screening specimen (triplicate testing) as elevated (screen positive) but is not confirmed on follow-up i.e. confirmatory diagnostic testing result is normal. In practice it may be impossible to differentiate an incorrect / artefactual result on the screening specimen from a genuine increase of phenylalanine which is transient and not present at diagnostic follow-up. Possible causes of a 'false positive' include:

1. **Contamination of the sample** – a potential exogenous source of phenylalanine is the artificial sweetener Aspartame (a methylester of phenylalanine / aspartic acid dipeptide) that may cause contamination if drinks containing it are spilt onto the screening card.
2. **Non-sample source contamination** with phenylalanine e.g. contamination during the analytical process from standards, or with mass 166.

Both of these situations (1 and 2) could also manifest as discrepant results (see Appendix 1 for guidance on discrepant replicates).

3. **Physiological reasons** – see section 8.2.1.

8.6 Analysis

8.6.1 Guidance on discrepant phenylalanine and tyrosine replicates

If a sample has an initially raised phenylalanine (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial phenylalanine is suspected. The same should apply to tyrosine values. See Appendix 1 for guidance on discrepant replicates.

8.6.2 Galactosaemia testing

It is recommended that screening laboratories or associated metabolic laboratories are able to undertake an appropriate test for galactosaemia in situations where Phe and Tyr are both elevated – see section 8.2. This is required **urgently** so that the result can be provided for the

clinical referral. This may be on the blood spot card or an urgently obtained follow-up specimen depending on the local circumstances.

Detailed laboratory methods are not provided but the section below outlines three possible approaches.

Measurement of blood spot galactose-1-phosphate-uridylyl-transferase (GAL-1-PUT)

Most cases of galactosaemia are due to deficiency of GAL-1-PUT. A reaction mixture containing galactose-1-phosphate, UDP-galactose, NADP, digitonin and dried blood spot is incubated at 37°C. The digitonin in the reaction mixture lyses the cells and releases the enzymes. Drops from the mixture are spotted on to chromatography paper at zero time and at 1 hour intervals for 3 hours. The spots are visually inspected under long-wave UV light. Development of fluorescence indicates the presence of GAL-1-PUT activity (Beutler and Baluda, 1966; Personal communication from Calvin, J., 2010).

Thin-layer chromatography of sugars (galactose) using dried blood spots

Sugars can be estimated qualitatively by thin-layer chromatography. The separation of both monosaccharides and disaccharides is achieved by double ascending development with good separation of glucose and galactose. The bands are visualised by staining with p-aminobenzoic acid reagent and sensitivity is enhanced when the thin-layer plate is examined under UV (Menzies and Seakins, 1976; Pollitt et al, 1982; Personal communication from Downing, M., 2010).

Measurement of blood spot galactose-1-phosphate (GALP)

Total hexose monophosphates (galactose-1-phosphate, glucose-1-phosphate, fructose-1-phosphate and fructose-6-phosphate) can be used as a surrogate marker of galactose-1-phosphate. Analysis is performed by liquid chromatography tandem mass spectrometry in negative ionisation mode. Although the HMPs fragment to produce common daughter ions, the aldose monophosphates (such as GALP) produce a predominant daughter ion at m/z 79 whereas the ketose monophosphates produce a predominant daughter ion at m/z 97. By optimising the production of these daughter ions and utilising the ratio of AMP:KMP as an indirect measure of GALP, patients with classical galactosaemia can be discriminated from control subjects (Jensen et al, 2001).

8.7 Clinical referral and follow up

See [PKU clinical management guidelines](#) and Appendix 2 (PKU initial clinical referral guidelines and standards).

8.7.1 Follow up of presumptive/suspected positive cases

These are babies with mean of triplicate phenylalanine ≥ 240 $\mu\text{mol/L}$ and tyrosine ≤ 240 $\mu\text{mol/L}$ (Figure 1 – PKU newborn screening protocol):

- all babies with a 'PKU suspected' screening result should be referred to the specialist clinical team (or designated local team) via the CLS (as per local arrangements) **on the same working day** that the 'PKU suspected' screening result is available. This referral with detailed screening results must be reported both verbally as well as in writing (a **template** is available)
- the family will be instructed by the specialist or designated team to take the baby to an appropriate hospital where the first review appointment will take place on the same or next working day

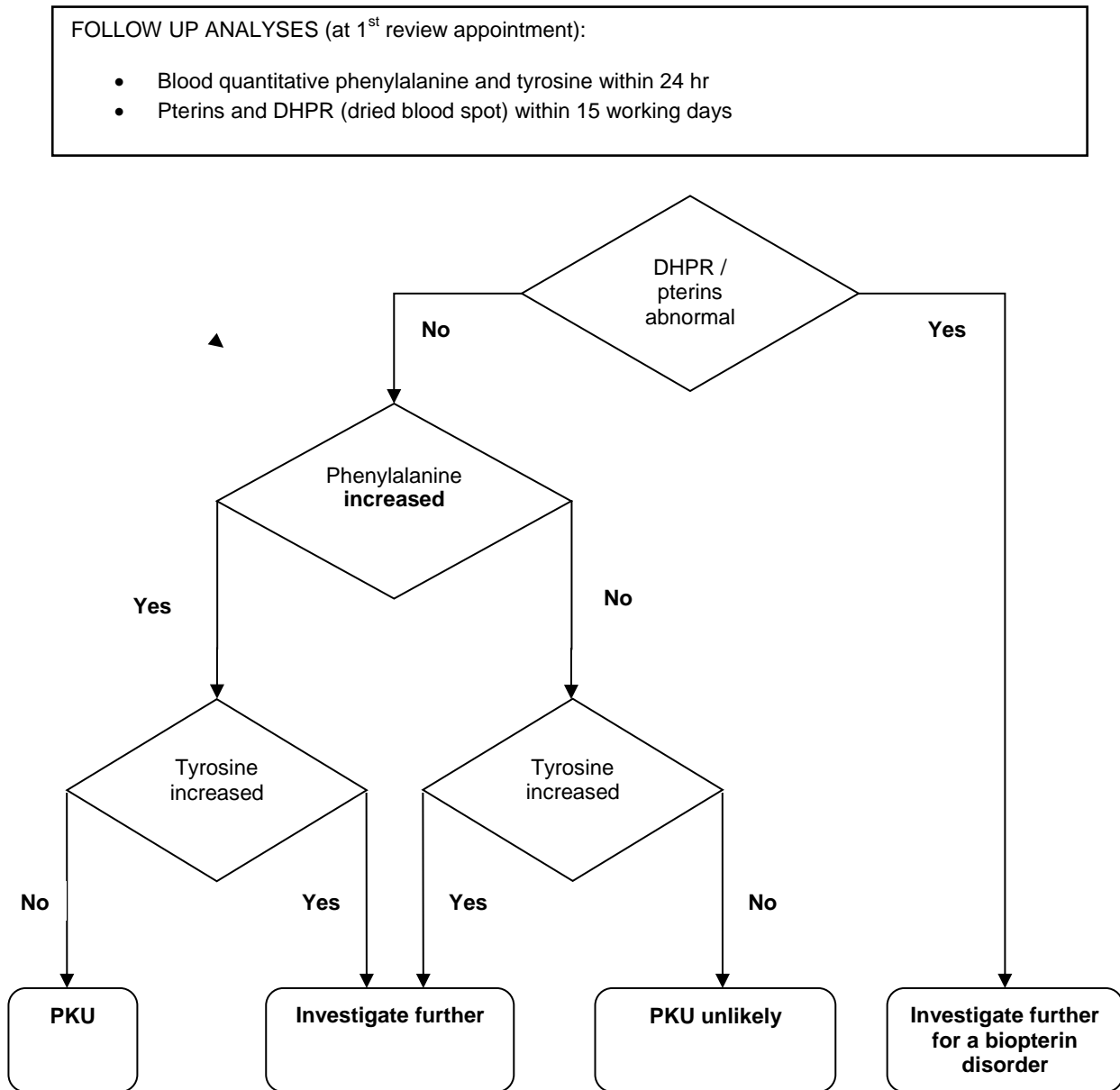
8.7.2 Other disorders follow up

These are babies with a mean triplicate phenylalanine ≥ 240 $\mu\text{mol/L}$ and tyrosine ≥ 240 $\mu\text{mol/L}$ also babies with a mean triplicate phenylalanine < 240 $\mu\text{mol/L}$ and tyrosine ≥ 240 $\mu\text{mol/L}$ (when galactosaemia testing has been initiated because the first phenylalanine was ≥ 200) (Figure 1 – PKU newborn screening protocol):

- referral must be made to the metabolic or other appropriate specialist team (a **template** is available), and will depend on the specific local situation e.g. baby may already be under the care of a medical consultant
- it is recommended that a galactosaemia test be carried out on the screening specimen as an **urgent** investigation or arrangements are made for urgent testing at diagnostic follow-up
- the decision to carry out other investigations, on the screening specimen, to aid differential diagnosis is a matter for the local service

8.8 Diagnostic protocol

Figure 2. PKU diagnostic protocol



8.8.1 'PKU suspected'

This applies to babies with a presumptive positive newborn screening test, i.e. phenylalanine concentration $\geq 240 \mu\text{mol/L}$ and tyrosine $< 240 \mu\text{mol/L}$ who are reported as 'PKU suspected'.

This protocol does not include guidance for investigation if other disorders are suspected (see section 8.2.2 – Other disorders suspected and 8.7.2 – Other disorders follow up).

8.8.2 Diagnostic specimen requirements

Table 4. PKU diagnostic specimen requirements

| Requirements | Timing of specimen and results |
|--|---|
| <p>Amino acids phenylalanine and tyrosine using an analytical technique that is different to the screening method. The specimen can be plasma (Lithium Heparin - 0.5 mL) or dried blood spots dependent on local arrangements</p> | <p>All follow-up diagnostic blood specimens should be taken before commencement of a phenylalanine restricted diet</p> <p>All tests should be measured on the same blood specimen to aid interpretation</p> <p>Results of the phenylalanine and tyrosine measurements should be available within 1 working day of specimen collection</p> |

Pterins analysis

Complete two blood spot cards with non-anticoagulated blood for DHPR and pterins analysis (and additional card if phenylalanine and tyrosine are to be measured in blood spots)

The DHPR and pterins analysis results should be available within 15 working days of specimen receipt in the Birmingham Women's and Children's Hospital laboratory

DHPR and pterins analyses are available at:

Newborn Screening and Biochemical Genetics Department
 Paediatric Laboratory Medicine
 Birmingham Women's and Children's NHS Foundation Trust
 Steelhouse Lane
 Birmingham, B4 6NH

8.9 Laboratory standards

See section 15 for generic laboratory standards.

8.9.1 Performance standards, including diagnostic tests

- laboratory services should be configured to enable the PKU newborn screening protocol (a raised phenylalanine confirmed in triplicate and where required tyrosine analysis completed in duplicate) to be completed within 3 working days from receipt of an adequate sample as core standard
- 'PKU suspected' results should be referred by the laboratory to the appropriate PKU clinical liaison team on the day they become available. If a disorder other than PKU is suspected the referral must be made to the metabolic or other specialist team within 24 hours. If there is insufficient sample to repeat / confirm an initial phenylalanine concentration of $\geq 200 \mu\text{mol/L}$, urgent referral to the designated / specialist metabolic team should be arranged
- confirmatory blood specimens for quantitative phenylalanine and tyrosine (diagnostic protocol) should be received by the metabolic laboratory and results available within 1 working day of the child being seen and ideally, on the same day
- follow-up diagnostic tests (pterins and DHPR analyses) must be undertaken in line with the diagnostic protocol by an accredited laboratory that must participate and demonstrate acceptable performance in appropriate quality arrangements (if available accredited EQA schemes). Provision should be made to ensure these tests are completed within 15 working days of specimen collection; the responsibility for this must be defined locally

9. Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)

9.1 Scientific background

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.

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 develop 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 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.

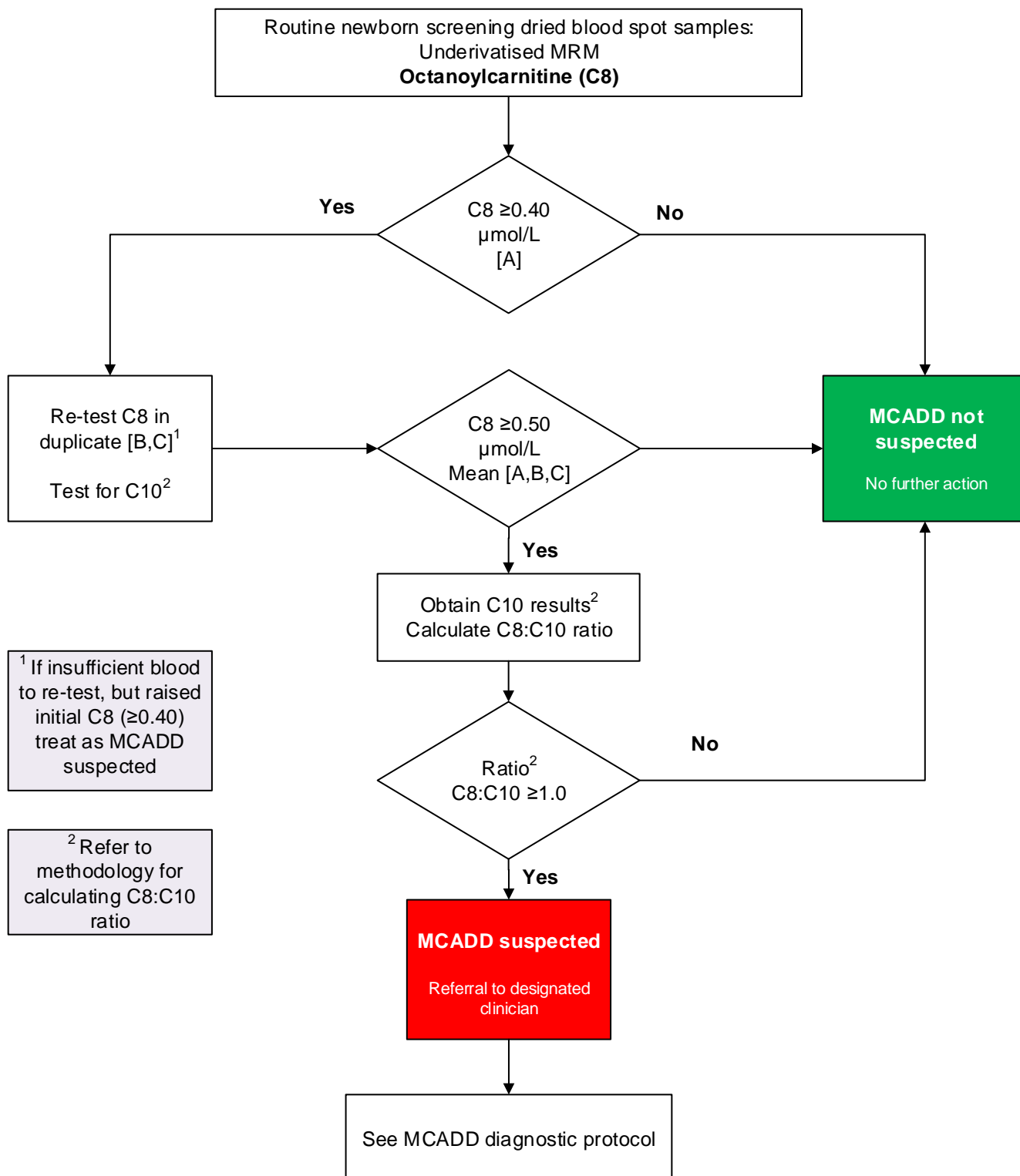
The gene for MCADD is located on chromosome 1p31. About 88% of clinically diagnosed MCADD cases are homozygous for the common c.985A>G mutation (Pollitt and Leonard, 1998), however this percentage is lower (45–55%) in cases picked up through newborn screening in the UK. MCADD affects between one in 10,000 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 et al, 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 (Oerton et al, 2005).

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.

9.2 Screening protocol

Figure 3. MCADD newborn screening protocol



9.3 Sibling testing

9.3.1 Protocol for the management of an at risk delivery – neonatal testing for siblings born after proband diagnosis

It is extremely important that any baby at risk of having MCADD due to a relevant family history is tested at the earliest opportunity. In 2011 the National Patient Safety Agency issued **guidance** to all NHS organisations in England and Wales to ensure that pathways for testing are put in place for any at risk pregnancy.

The pregnancy should be discussed with the specialist metabolic team caring for the proband and local Genetic Service early in the pregnancy to discuss the option of prenatal testing and to plan careful management of the birth to minimise the risk of decompensation.

Management at birth will depend on the presentation of the previous sibling. If a previous sibling became ill shortly after birth, consider transferring the mother before birth to a centre with all facilities for managing an affected baby.

When to test and samples to be taken

24–48 hours: C8 (blood spot), qualitative urinary organic acids and genotyping.
Write on blood spot card 'Family history of MCADD'. Send to specialist centre laboratory by courier.

NB Genotyping is only advised if the baby being investigated is the full sibling of the proband in the family, and if the proband has two recognised disease causing mutations. In cases where one or both of the disease causing mutations are not the common c.985A>G mutation, genotyping will need to be carried out by one of the two UK EMS laboratories (see section 9.7.3).

Please contact the metabolic laboratory undertaking the C8 and urinary organic acid analysis in advance of sending the genotyping sample in order to clarify the testing required and where this sample should be sent.

Day 5: Routine newborn screen
Write on blood spot card 'Family history of MCADD'

Management

Prior to results: **Management guidelines** for the prospective management for a baby at risk of MCADD at birth are available.

If results indicate the baby is affected with MCADD, follow the MCADD clinical management and dietetic management guidelines*.

9.3.2 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 **or** two recognised disease causing mutations on genotyping.

Sibling samples

- i. C8 (blood spot) and qualitative urinary organic acids
- ii. DNA – send for genotyping once definite MCADD diagnosis secured in proband (two disease causing mutations identified)

Management

Before the results are available and thereafter if MCADD confirmed: follow the clinical management and dietetic management guidelines*.

*For more information please refer to [clinical management and dietetic management guidelines](#). Also see section 2.6 – Genetic counselling.

9.4 Pre-analytical aspects

9.4.1 Factors affecting the screening results

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 demonstrated 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.

9.4.2 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 72 hours 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 levels
- 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 approximately 1 month of age) and infants with MCADD may have C8 levels below the screening cut-off (see section 2.4 – Late testing)

9.4.3 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 are very rare
- early sampling in the immediate postnatal period may give higher results as discussed above
- card contamination of unknown cause resulted in three false positive results during the pilot study; a protocol for dealing with this is outlined in Appendix 1 (guidance on discrepant replicates)

9.5 Analysis

9.5.1 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. The same should apply to C10 values. See Appendix 1 for guidance on discrepant replicates.

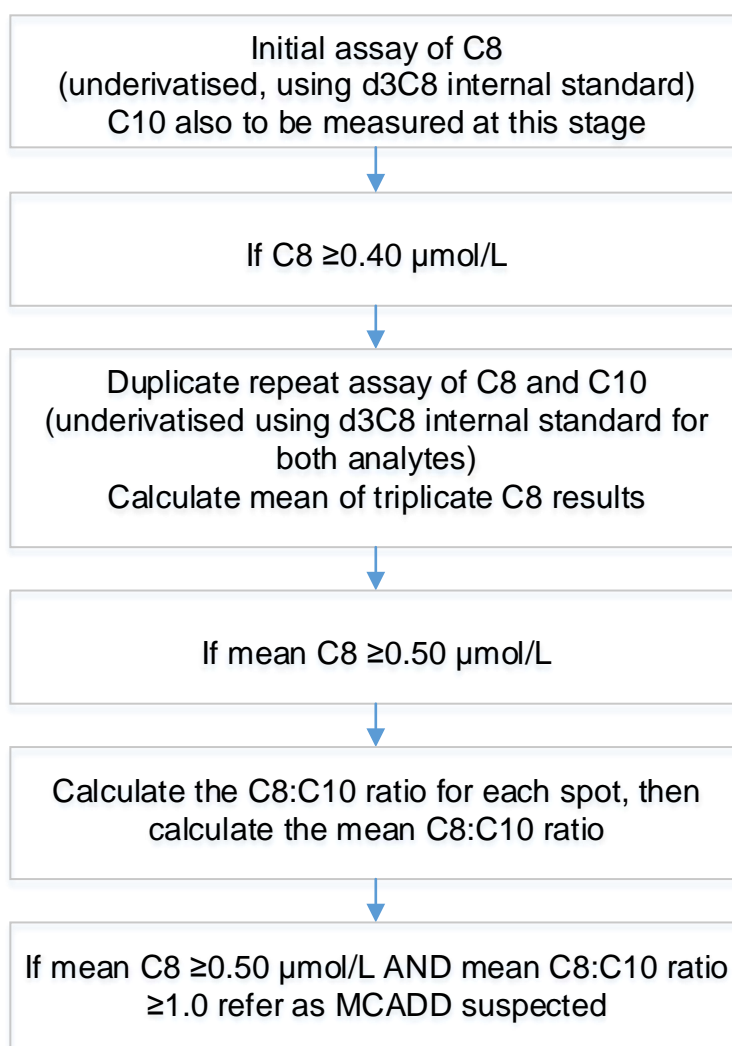
9.5.2 Calculation of C8:C10 ratio

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 $\mu\text{mol/L}$). If the triplicate C8 result is ≥ 0.50 $\mu\text{mol/L}$ then calculate the mean C8:C10 ratio.

The methodology used in the original MCADD pilot study for calculating the C8:C10 ratio i.e. C10 measured using the d3C8 internal standard, is outlined in Figure 4. It is recognised that many laboratories include d3C10 in the internal standard to calculate C10. It would be of value if those laboratories could continue to collect comparative data for C10 and C8:C10 ratios obtained from using the d3C10 internal standard to enable validation of its use.

This is summarised in Figure 4.

Figure 4. Calculation of C8:C10 ratio



9.6 Clinical referral and follow up

See [MCADD clinical management guidelines](#) and Appendix 3 (MCADD initial clinical referral guidelines and standards).

9.6.1 Follow up of presumptive/suspected positive cases

These are babies with mean of triplicate C8 ≥ 0.5 $\mu\text{mol/L}$ AND C8:C10 ratio ≥ 1.0 (Figure 3 – MCADD newborn screening protocol):

all babies with a 'MCADD suspected' screening result should be referred to the specialist or designated clinical team via the CLS (as per local arrangements) **on the same day** that the 'MCADD suspected' screening result is available. This referral with detailed screening results must be reported both verbally as well as in writing – a [template](#) is available

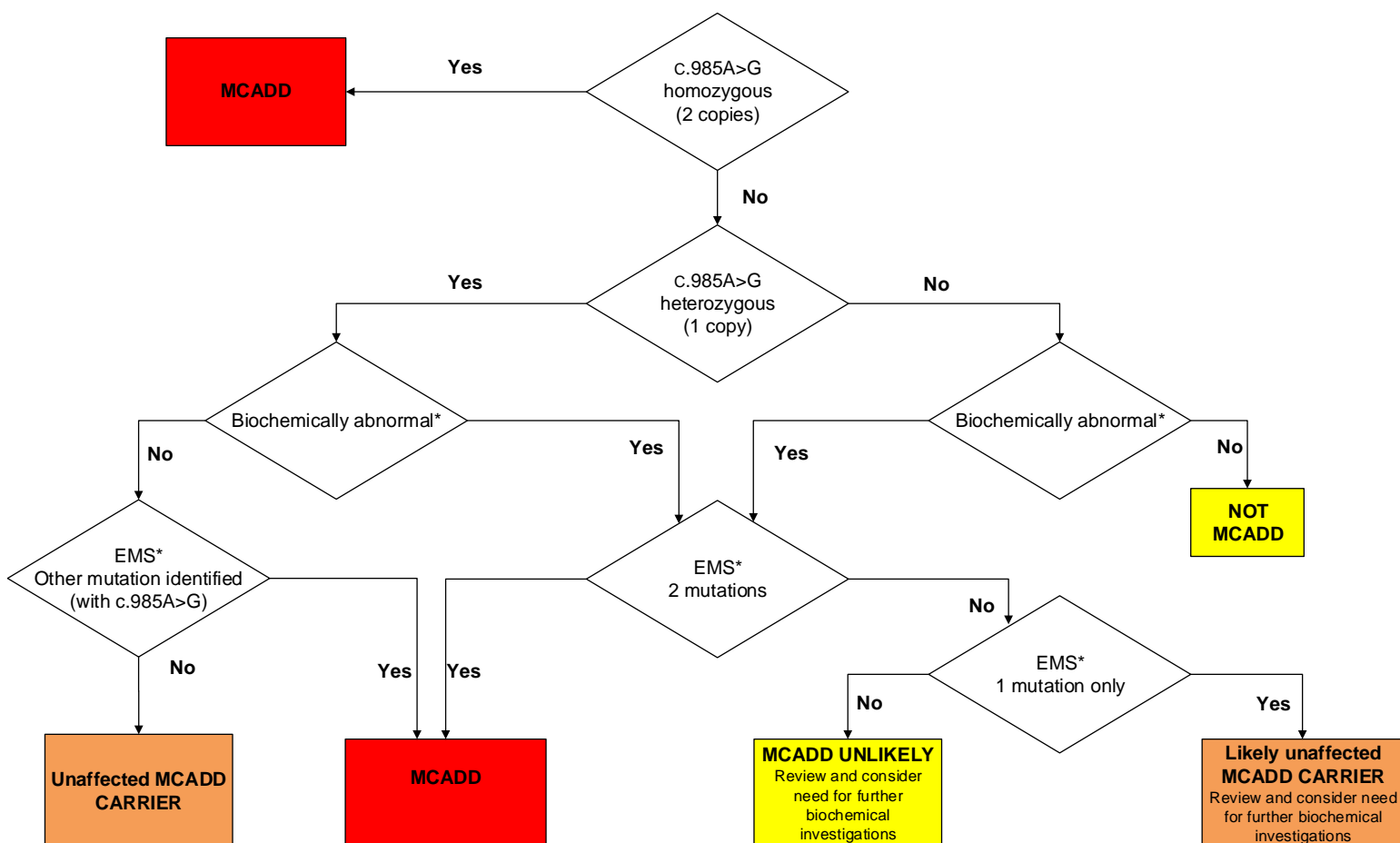
- the family will be instructed by the specialist or designated team to take the baby to an appropriate hospital (if not an inpatient already) where initial assessment will take place within 24 hours of the screening referral

9.7 Diagnostic protocol

Figure 5. MCADD diagnostic protocol

FOLLOW-UP ANALYSES* (at first review appointment):

- Blood acylcarnitines (dried blood spot or plasma): C8 (duplicate) and acylcarnitine 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 2 separate blood spot cards, each sent for analysis at first review appointment (see Table 8).



9.7.1 Diagnostic specimen requirements

All samples should be sent to the local metabolic diagnostic laboratory for the following: Note this assumes mutation analysis (c.985A>G and EMS) is undertaken on dried blood spots.

Table 5. MCADD diagnostic specimen requirements

| Requirements | Timing of specimen and results |
|--|--|
| Blood acylcarnitines Dried blood spot or 0.5 ml Li Hep plasma: C8 duplicate and acylcarnitine full scan | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |
| Qualitative urine organic acids 5ml minimum (no preservative, frozen immediately at -20°C). (Equal share for qualitative and possible quantitative hexanoylglycine assay) | Results of qualitative organic acids should be available within 5 working days of receipt of the diagnostic samples into the laboratory. Results of quantitative hexanoylglycine if required should be available within 15 working days from receipt of sample |
| DNA analysis Two blood spot cards Card 1 is for c.985A>G testing Card 2 is held by the metabolic diagnostic laboratory until c.985A>G results have been obtained and is then sent to EMS laboratory if required | DNA analysis should be completed within 15 working days from receipt of sample |

9.7.2 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 at [MetBioNet](#).

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.

9.7.3 Extended mutation screen (EMS)

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

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 are available by contacting phe.screeninghelpesk@nhs.net.

Table 6. EMS referral contact details

| Screening laboratory | EMS laboratory contact details |
|--|---|
| West Midlands, Sheffield, Leeds, Liverpool, Manchester, Newcastle and Oxford are to send samples for EMS to: | <p>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</p> <p>Tel: 0114 271 7014 Fax: 0114 275 0629</p> <p>Email: Richard.Kirk@sch.nhs.uk or Ann.Dalton@sch.nhs.uk</p> |
| Bristol, Cambridge, GOSH, Portsmouth, SE Thames (St Thomas’) and SW Thames are to send samples for EMS to: | <p>Guy’s Laboratory Service Lead: Nicholas Parkin Molecular Genetics Laboratory Genetics Department Viapath at Guy’s Hospital Floor 5, Tower Wing, Guy’s Hospital London SE1 9RT</p> <p>Tel: 0207 188 2582 / 1696 Fax: 0207 188 7273 Email: DNAdutyScientist@viapath.co.uk</p> |

- samples for EMS screening should be sent with the appropriate EMS referral form (available by contacting phe.screeninghelpdesk@nhs.net) 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

9.8 Laboratory standards

See section 15 for generic laboratory standards.

9.8.1 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) to be completed within 3 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 Saturdays in those cases where a result can be reported from samples already analysed overnight on Friday. If there is insufficient sample to repeat / confirm an initial raised octanoylcarnitine ($\geq 0.40 \mu\text{mol/L}$), urgent referral to the designated / specialist metabolic team should be arranged
- follow-up diagnostic tests must be undertaken in line with the diagnostic protocol by accredited laboratories that must participate and demonstrate acceptable performance in the relevant / accredited EQA schemes. Provision should be made to ensure these tests are completed within 5 working days of receipt of the diagnostic samples into the laboratory; the responsibility for this must be defined locally
- results of further follow-up diagnostic tests (EMS and quantitative hexanoylglycine) should be available within 15 working days from receipt of diagnostic sample

10. Maple syrup urine disease (MSUD)

10.1 Scientific background

Maple syrup urine disease (MSUD) is an autosomal recessive disorder caused by a deficiency of the branched chain alpha keto acid dehydrogenase complex which consists of four subunits, E₁ α , E₁ β , E₂ and E₃. MSUD occurs in approximately 1 in 200,000 live births.

The resulting metabolic block leads to an increased concentration of the branched chain amino acids leucine, valine, isoleucine and alloisoleucine and their corresponding keto acids. These compounds accumulate in tissues resulting in a life threatening metabolic decompensation in some affected individuals and are elevated in the blood and urine.

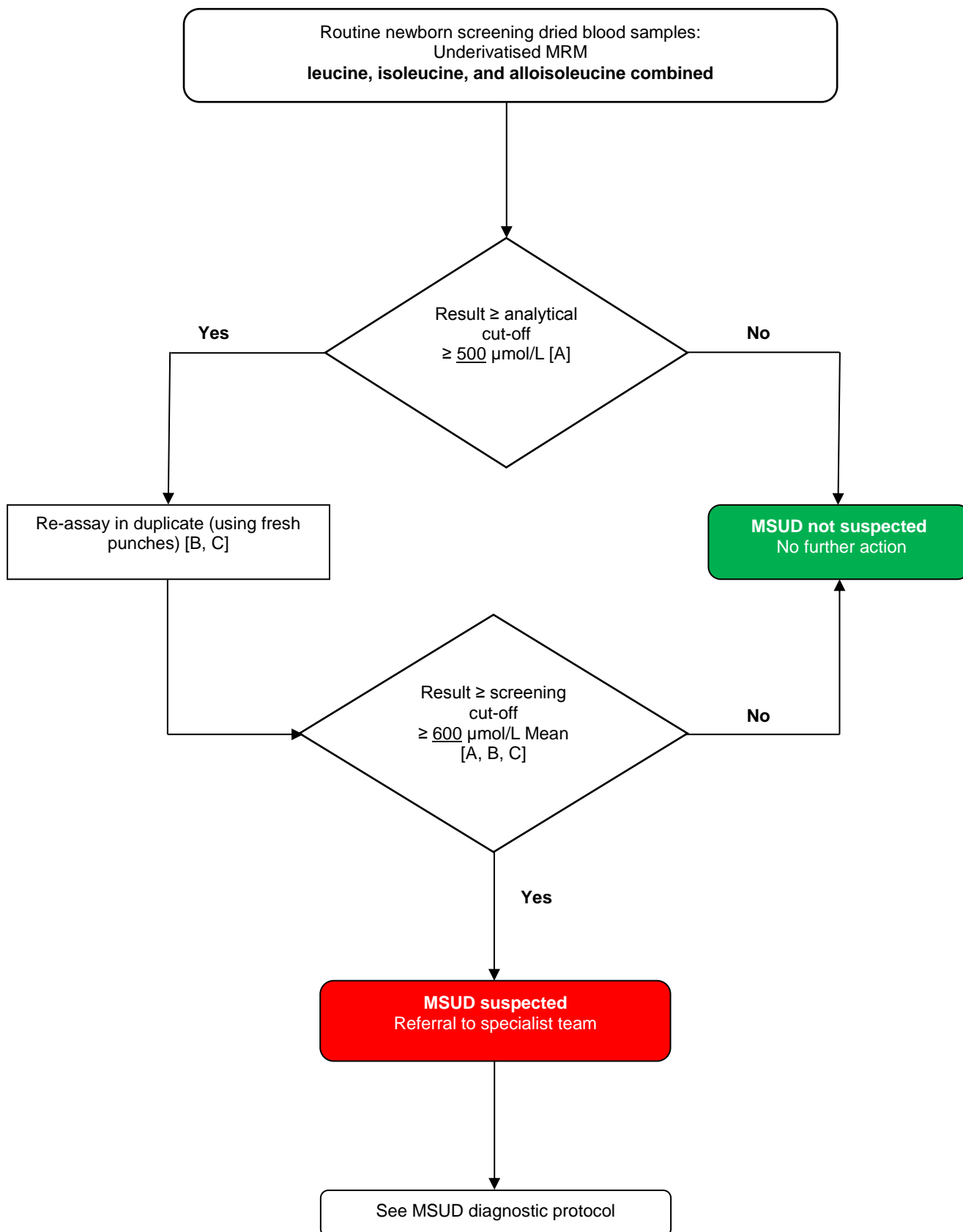
The name of the condition derives from the sweet smelling urine sometimes produced by affected individuals which some have likened to the smell of maple syrup.

The classic form of the disorder presents shortly after birth, often in the first two weeks of life. Vomiting or difficulty feeding are often early symptoms accompanied by lethargy and progressive neurological deterioration. Intermediate and intermittent forms of the condition are also described. Patients with the intermediate form may present with developmental delay although the characteristic elevation of branched chain amino acids is still present. The intermittent form of the disease may only manifest at times of stress or infection and branched chain amino acids may not be continuously elevated. Rarer thiamine-responsive disease has been described together with the E₃ variant which also affects the pyruvate dehydrogenase complex resulting in marked lactic acidosis.

It is likely that newborn screening will detect patients with the classic condition but may not detect individuals with intermediate or intermittent forms which have a spectrum of clinical and biochemical severity.

10.2 Screening protocol

Figure 6. MSUD newborn screening protocol



10.3 Sibling testing

10.3.1 Protocol for management of at risk delivery – neonatal testing for siblings born after proband diagnosis

The pregnancy should be discussed with the specialist metabolic team caring for the proband and local genetic service early in the pregnancy to discuss the option of prenatal testing and to plan careful management of the birth to minimise the risk of decompensation.

Management at birth will depend on the presentation of the previous sibling. If a previous sibling became ill shortly after birth, consider transferring the mother before birth to a centre with all facilities for managing an affected baby.

When to test and samples taken

12–24 hours: Alloisoleucine (plasma) and qualitative urinary organic acids
Write on request 'Family history of MSUD'. Send to specialist centre laboratory by courier

Day 5: Routine newborn screen
Write on blood spot card 'Family history of MSUD'

Management

Prior to results: **Management guidelines** for the prospective management of a baby at risk of MSUD at birth are available.

If results indicate the baby is affected with MSUD, follow the MSUD clinical management guidelines and dietetic management guidelines*.

10.3.2 Testing siblings born before proband diagnosis

When to test

Offer to test if sibling has not been previously screened for MSUD and if proband has abnormal biochemistry at follow-up visit.

Sibling samples

- i. Alloisoleucine (plasma) and qualitative urinary organic acids
- ii. DNA – send for genotyping if mutation in proband known

Management

Before the results are available and thereafter if MSUD confirmed: follow the MSUD clinical management guidelines and dietetic management guidelines*.

*For more information please refer to **clinical management and dietetic management guidelines**. Also see section 2.6 – Genetic counselling.

10.4 Pre-analytical aspects

10.4.1 Potential for false negatives

- 1. Transfusions** could result in a false negative result, as for other screening tests, and a repeat sample should be taken after a reasonable time has elapsed. At least 72 hours is recommended, as for the other screening tests, to allow pre-transfusion levels to be reached.
- 2. Delays in transit / sample deterioration** It is possible that a sample may deteriorate due to adverse transport conditions and result in 'loss' of measured leucine in the blood spot.
- 3. Physiological reasons** Patients with intermediate enzyme activity may not all be detected by newborn screening but may still manifest some features of the disease during times of stress or illness or may present with a more progressive form of the condition accompanied by developmental delay.

10.4.2 Potential for false positives

- MS/MS analysis does not differentiate leucine from isoleucine or hydroxyproline. While elevation of leucine and isoleucine both result from MSUD, increased hydroxyproline may indicate the rare benign condition hydroxyprolinaemia
- an increased leucine concentration can sometimes be observed in ketotic babies and in babies with galactosaemia or other severe liver disease in the newborn period

10.5 Analysis

10.5.1 Guidance on discrepant Leu replicates

If a sample has an initially raised Leu (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial Leu is suspected. See Appendix 1 for guidance on discrepant replicates.

10.6 Clinical referral and follow up

See **MSUD clinical management guidelines** and Appendix 4 (MSUD initial clinical referral guidelines and standards).

10.6.1 Follow up of presumptive/suspected positive cases

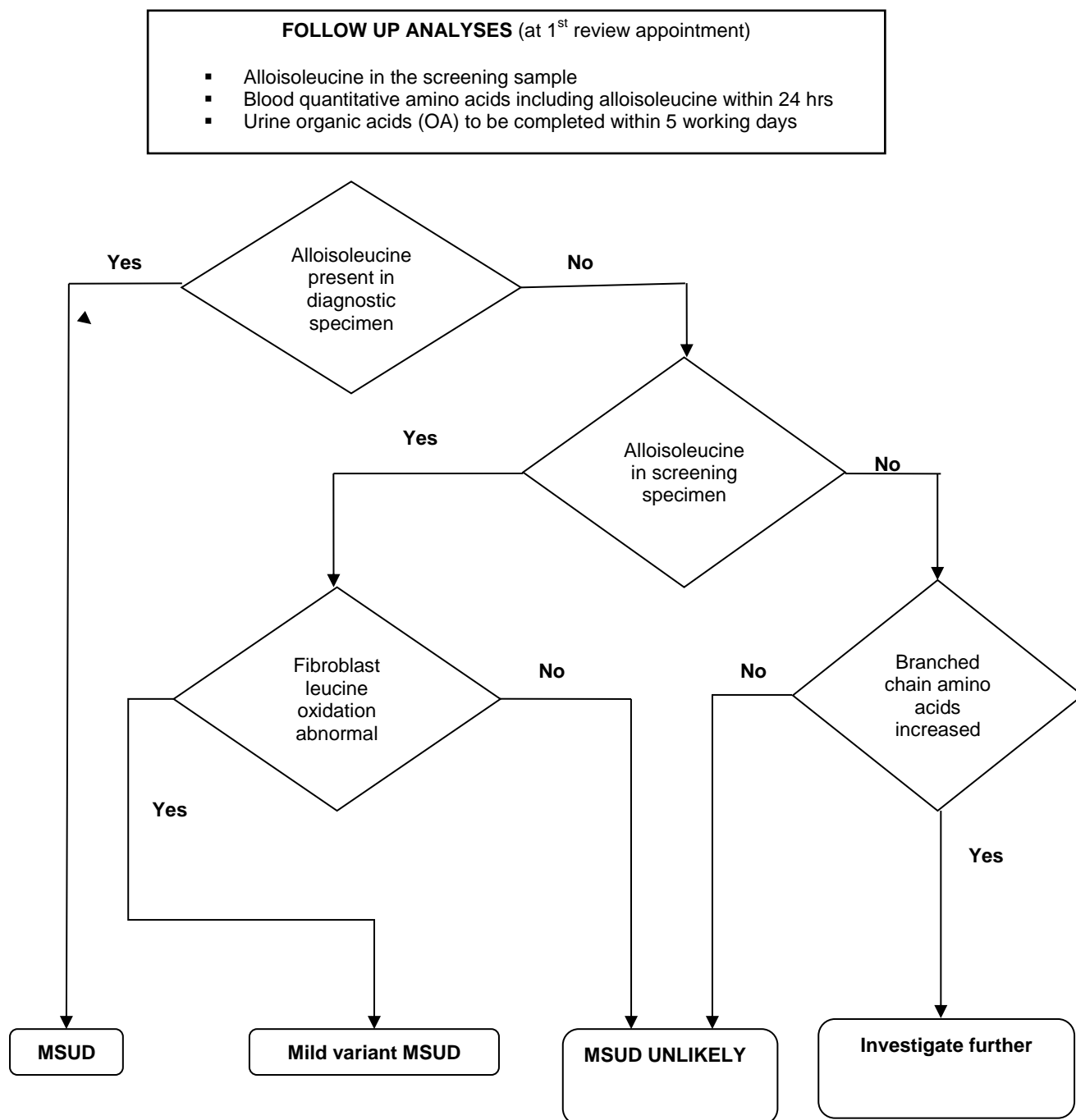
These are babies with mean of triplicate Leu ≥ 600 $\mu\text{mol/L}$ (Figure 6 – MSUD newborn screening protocol):

- all babies with a 'MSUD suspected' screening result should be referred to the specialist clinical team via the CLS (as per local arrangements) **on the same day** that the 'MSUD suspected' screening result is available. This referral with detailed screening results must be reported both verbally as well as in writing – a **template** is available
- the family will be instructed by the specialist team to take the baby to an appropriate hospital (if not an inpatient already) where initial assessment will take place. The baby will be transferred to the specialist IMD centre on the same day of hearing about a positive screening result
- if transfer is not available, the specialist team will liaise with the local hospital to arrange diagnostic testing and supply of dietary supplements

10.7 Diagnostic protocol

This applies to babies with a presumptive positive newborn screening test, i.e. leucine concentration $\geq 600 \mu\text{mol/L}$ that are reported as 'MSUD suspected'.

Figure 7. MSUD diagnostic protocol



10.7.1 Diagnostic specimen requirements

Table 7. MSUD diagnostic specimen requirements

| Requirements | Timing of specimen and results |
|---|--|
| Amino acids including alloisoleucine The specimen can be plasma (Lithium Heparin - 0.5 mL) or dried blood spots dependent on local arrangements | Results should be available within 24 hours |
| Urine organic acids 5ml minimum (no preservative, frozen immediately at -20°C) | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |

10.8 Laboratory standards

See section 15 for generic laboratory standards.

10.8.1 Performance standards, including diagnostic tests

- the laboratory analytical service should be configured to enable the MSUD newborn screening protocol (a raised leucine confirmed in triplicate) to be completed within 3 working days from receipt of an adequate sample as core standard
- presumptive positive results (average of triplicate leucine) should be referred by the laboratory to the appropriate clinical team on the day they become available. This includes referral on Saturdays in those cases where a result can be reported from samples already analysed overnight on Friday. If there is insufficient sample to repeat / confirm an initial raised leucine ($\geq 500 \mu\text{mol/L}$), urgent referral to the designated / specialist metabolic team should be arranged
- follow-up diagnostic tests must be undertaken in line with the diagnostic protocol by accredited laboratories that must participate and demonstrate acceptable performance in the relevant / accredited EQA schemes. Results of blood quantitative amino acids will need to be available within 24 hours. The remaining tests should be completed within 5 working days of receipt of the diagnostic samples into the laboratory; the responsibility for this must be defined locally

11. Isovaleric acidaemia (IVA)

11.1 Scientific background

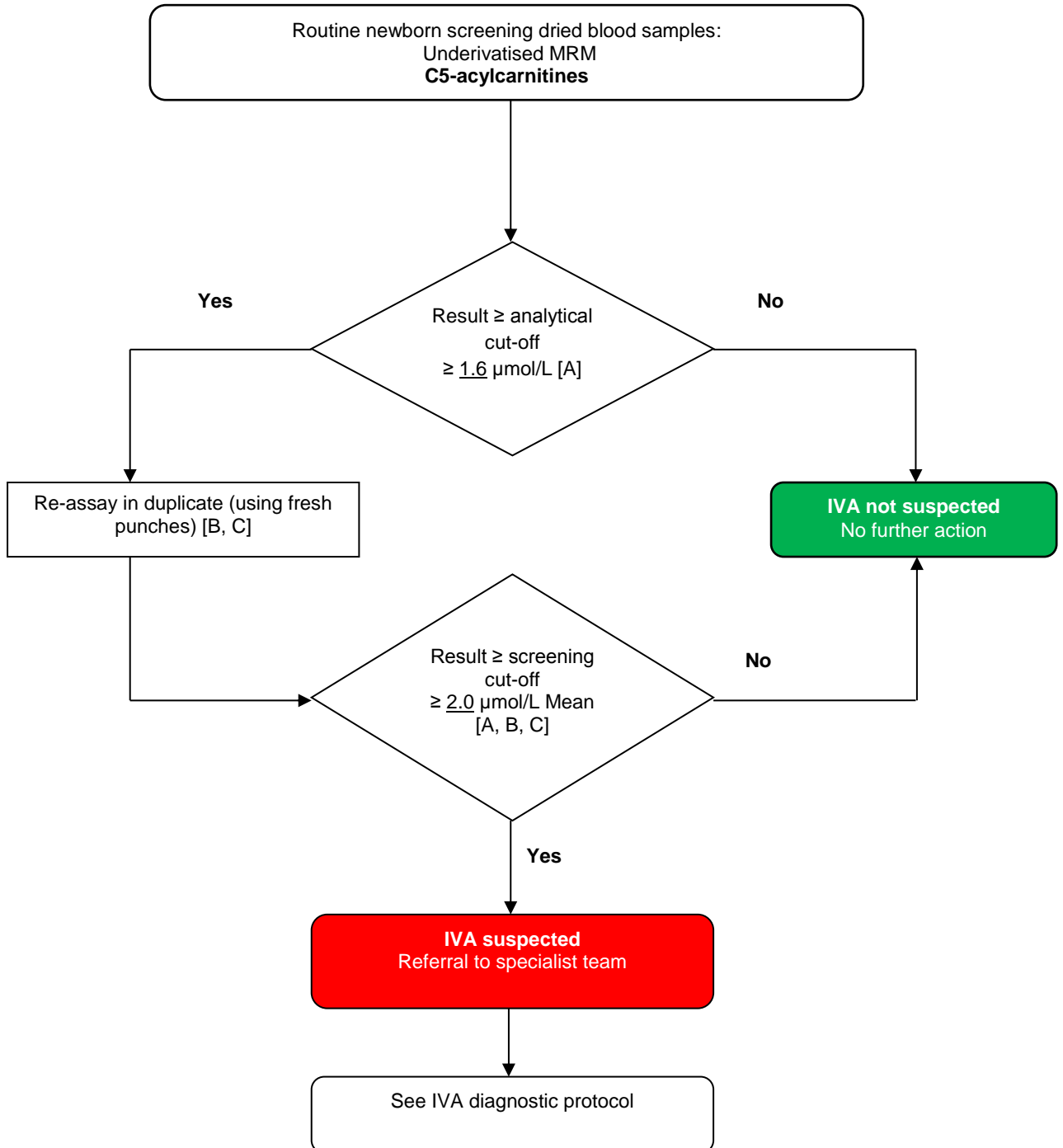
Isovaleric acidaemia (IVA) is caused by a deficiency in isovaleryl-CoA dehydrogenase (IVD), involved in the catabolism of the amino acid leucine. It is an autosomal recessive disease, with an estimated incidence of around 1 in 100,000 with higher incidence in some locations and ethnic groups.

Loss of function of the enzyme leads to the toxic build-up of metabolites including isovaleric acid and its glycine and carnitine derivatives. Over 25 mutations in the IVD gene have been associated with disease, a number of which lead to complete lack of the enzyme. Although a firm phenotype/genotype correlation has not been identified, recent research suggests that the 932C>T mutation in the IVD gene may be associated with a milder phenotype. The disease has a spectrum of clinical phenotypes which might include acute neonatal presentations, acute presentations at a later age and chronic intermittent presentations. The acute neonatal presentation is characteristically in the first two weeks after birth. Infants are initially well, then develop vomiting and lethargy, progressing to coma. Patients may also present with similar symptoms at a later age, usually precipitated by an infection. Other patients present with chronic symptoms – failure to thrive and/or developmental delay, usually within the first year.

Newborn screening has identified individuals with partial as well as complete IVD deficiency.

11.2 Screening protocol

Figure 8. IVA newborn screening protocol



11.3 Sibling testing

11.3.1 Protocol for management of at risk delivery – neonatal testing for siblings born after proband diagnosis

The pregnancy should be discussed with the specialist metabolic team caring for the proband and local genetic service early in the pregnancy to discuss the option of prenatal testing and to plan careful management of the birth to minimise the risk of decompensation.

If a previous sibling became ill shortly after birth, consider transferring the mother before birth to a centre with all facilities for managing an affected baby.

Management at birth will depend on the presentation of the previous sibling.

1. If the affected sibling was not biochemically mild, or became ill in the neonatal period, and there has not been a negative prenatal diagnosis:

When to test and samples taken

| | |
|--------------|---|
| 24–48 hours: | C5 (blood spot), qualitative urinary organic acids and genotyping. Write on blood spot card 'Family history of IVA'. Send to specialist centre laboratory by courier |
| Day 5: | Routine newborn screen Write on blood spot card 'Family history of IVA' |

Management

Prior to results: Manage as in the BIMDG emergency treatment guideline: 'Management of a baby at risk of an organic acidaemia at birth' section A (found at [Emergency Guidelines, Prospective Neonatal Management of baby at Risk](#)). Transfer to specialist centre as soon as possible.

If results indicate baby is affected with IVA, follow the IVA clinical management guidelines 'Unwell baby' pathway, and IVA dietary management guidelines*.

2. If the affected sibling was biochemically mild (\pm 932C>T mutation) and was not unwell in the neonatal period:

When to test and samples taken

| | |
|--------------|---|
| 24–48 hours: | C5 (blood spot), qualitative urinary organic acids and genotyping (blood spot) Write on blood spot card 'Family history of IVA'. Send to specialist centre laboratory by courier |
|--------------|---|

Day 5: Routine newborn screen
 Write on blood spot card 'Family history of IVA'

Management

Prior to results: Manage as in the BIMDG emergency treatment guideline: 'Management of a baby at risk of an organic acidaemia at birth' section B (found at [Emergency Guidelines, Prospective Neonatal Management of baby at Risk](#)).

If results indicate the baby is affected with biochemically mild IVA or the 932C>T variant, follow the clinical management protocol, 'Well baby' and 'biochemically mild / 932C>T variant' pathways if well; and the IVA dietary management guidelines (mild variant section).

11.3.2 Testing siblings born before proband diagnosis

When to test

Offer to test if sibling has not been previously screened for IVA and if proband has abnormal biochemistry at follow-up visit.

Sibling samples

- i. C5 (blood spot) and qualitative urinary organic acids
- ii. DNA – send for genotyping once definite IVA diagnosis secured in proband

Management

Before the results are available and thereafter if IVA confirmed: follow the IVA clinical and dietary management guidelines* if the proband was not biochemically mild and did not have the 932C>T mutation. If the proband was biochemically mild or had the 932C>T mutation, follow the 'Dietetic Management Pathway mild IVA (932C>T)'.

*For more information please refer to [clinical management and dietary management guidelines](#). Also see section 2.6 – Genetic counselling.

11.4 Pre-analytical aspects

11.4.1 Potential for false negatives

1. **Transfusions** could result in a false negative result, as for other screening tests, and a repeat sample should be taken after a reasonable time has elapsed. At least 72 hours is recommended, as for the other screening tests, to allow pre-transfusion levels to be reached.

2. **Delays in transit / sample deterioration** – it is possible that a sample may deteriorate due to adverse transport conditions and result in 'loss' of measured isovalerylcarnitine in the blood spot.
3. **Physiological reasons** – later onset isovaleric acidaemia presenting with failure to thrive and developmental delay has been described. It is unclear whether all such cases would be detected by newborn screening.

11.4.2 Potential for false positives

- pivaloylcarnitine is isobaric with isovaleryl carnitine and can result in false positive results. Antibiotics containing pivalate are in use in the UK. In addition, there are nipple creams that contain pivalic derivatives. This has been a cause of some false positive results for C5 in the UK programme. It is recommended that antibiotic and full drug history is taken at the first appointment and confirmatory testing to identify pivalate, subsequent to patient referral, is available from Viapath on a cost per test basis
- glutaric aciduria type 2 is often associated with an increase in C5, C8 and C5-DC acylcarnitines, all detected as part of the newborn screening programme. Screen positive results for any of these metabolites should prompt consideration of glutaric aciduria type 2
- 2-methylbutyryl carnitine is elevated in short/branched chain acyl-CoA dehydrogenase deficiency (SBCAD) and is isobaric with isovalerylcarnitine and causes a positive screening result. SBCAD is a rare condition, probably harmless, but is known in the UK population

11.5 Analysis

11.5.1 Action on discrepant replicates

If a sample has an initially raised C5 (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial C5 is suspected. See Appendix 1 for guidance on discrepant replicates.

11.6 Clinical referral and follow up

See [IVA clinical management guidelines](#) and Appendix 5 (IVA initial clinical referral guidelines and standards).

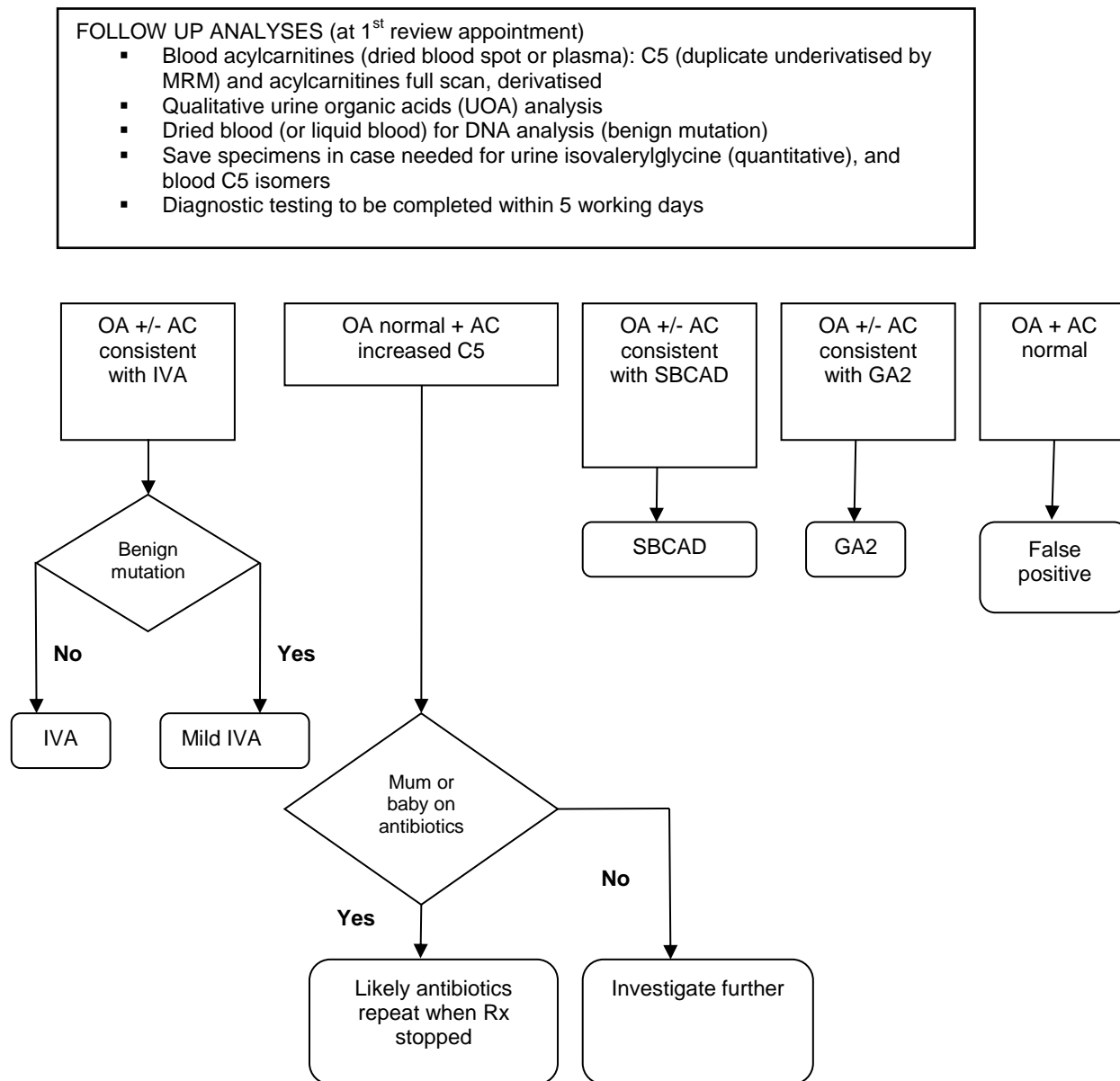
11.6.1 Follow up of presumptive/suspected positive cases

Please refer to the screening protocol (Figure 8). These are babies with mean of triplicate C5 ≥ 2.0 $\mu\text{mol/L}$.

- All presumptive positives should be referred to the specialist IMD 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 available
- The family will be instructed by the specialist team to take the baby to an appropriate hospital (if not an inpatient already) where initial assessment will take place. The baby will be transferred to the specialist IMD centre on the same day of hearing about a positive screening result
- If transfer is not available, the specialist team will liaise with the local hospital to arrange diagnostic testing and supply of dietary supplements

11.7 Diagnostic protocol

Figure 9. IVA diagnostic protocol



Key

GA2 Glutaric aciduria type 2 (multiple acyl-CoA dehydrogenase deficiency, MADD)

SBCAD Short/branched chain acyl-CoA dehydrogenase deficiency

11.7.1 Diagnostic specimen requirements

Table 8. IVA diagnostic specimen requirements

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

| Requirements | Timing of specimen and results |
|---|--|
| Blood acylcarnitines Dried blood spot or plasma: C5 duplicate and acylcarnitine full scan | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |
| Urine organic acids 5ml minimum (no preservative, frozen immediately at -20°C) | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |
| DNA analysis for 932C>T Send sample immediately to appropriate laboratory (UKAS accredited) (dried blood spot) | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |

Specimens should be saved in case needed for urine isovalerylglycine and blood C5 isomers.

11.8 Laboratory standards

See section 15 for generic laboratory standards.

11.8.1 Performance standards, including diagnostic tests

- the laboratory analytical service should be configured to enable the IVA newborn screening protocol (a raised C5 acylcarnitine confirmed in triplicate) to be completed within 3 working days from receipt of an adequate sample as core standard
- presumptive positive results (average of triplicate C5 acylcarnitine) should be referred by the laboratory to the appropriate clinical team on the day they become available. This includes referral on Saturdays in those cases where a result can be reported from samples already analysed overnight on Friday. If there is insufficient sample to repeat / confirm an initial raised C5-acylcarnitine ($\geq 1.6 \mu\text{mol/L}$), urgent referral to the designated / specialist metabolic team should be arranged
- follow-up diagnostic tests must be undertaken in line with the diagnostic protocol by accredited laboratories that must participate and demonstrate acceptable performance in the relevant / accredited EQA schemes. Provision should be made to

ensure these tests are completed within 5 working days of receipt of the diagnostic samples into the laboratory; the responsibility for this must be defined locally

12. Glutaric aciduria type 1 (GA1)

12.1 Scientific background

Glutaric aciduria type 1 (GA1) is an autosomal recessive condition caused by a deficiency of the enzyme glutaryl-CoA dehydrogenase (GCDH). The estimated incidence in the UK is around 1 in 100,000 live births.

GCDH is involved in the dehydration and subsequent decarboxylation of glutaryl-CoA, which is an intermediate in the breakdown of the amino acids lysine, hydroxylysine and tryptophan. Defective catabolism causes the toxic accumulation of glutaric acid, 3-hydroxyglutaric acid, glutaconic acid, and glutaryl carnitine. Over 150 disease causing mutations have been identified; of these the R402W mutation is the most prevalent among Caucasians. Most mutations, including the R402W mutation, are associated with undetectable GCDH activity and excretion of high amounts of glutaric acid. However, mutations that lead to varying levels of residual GCDH activity and low excretion of glutaric acid have also been reported. Consequently, patients with GA1 can be divided into two biochemically defined subgroups based on the levels of glutaric acid present in the urine: low excretors are those with less than 100 mmol/mol creatinine and high excretors are more than 100 mmol/mol creatinine. Although these subgroups are clinically similar, confirmatory testing in low excretors requires more complex follow-up, with either determination of GCDH enzyme activity or by mutation analysis of the GCDH gene.

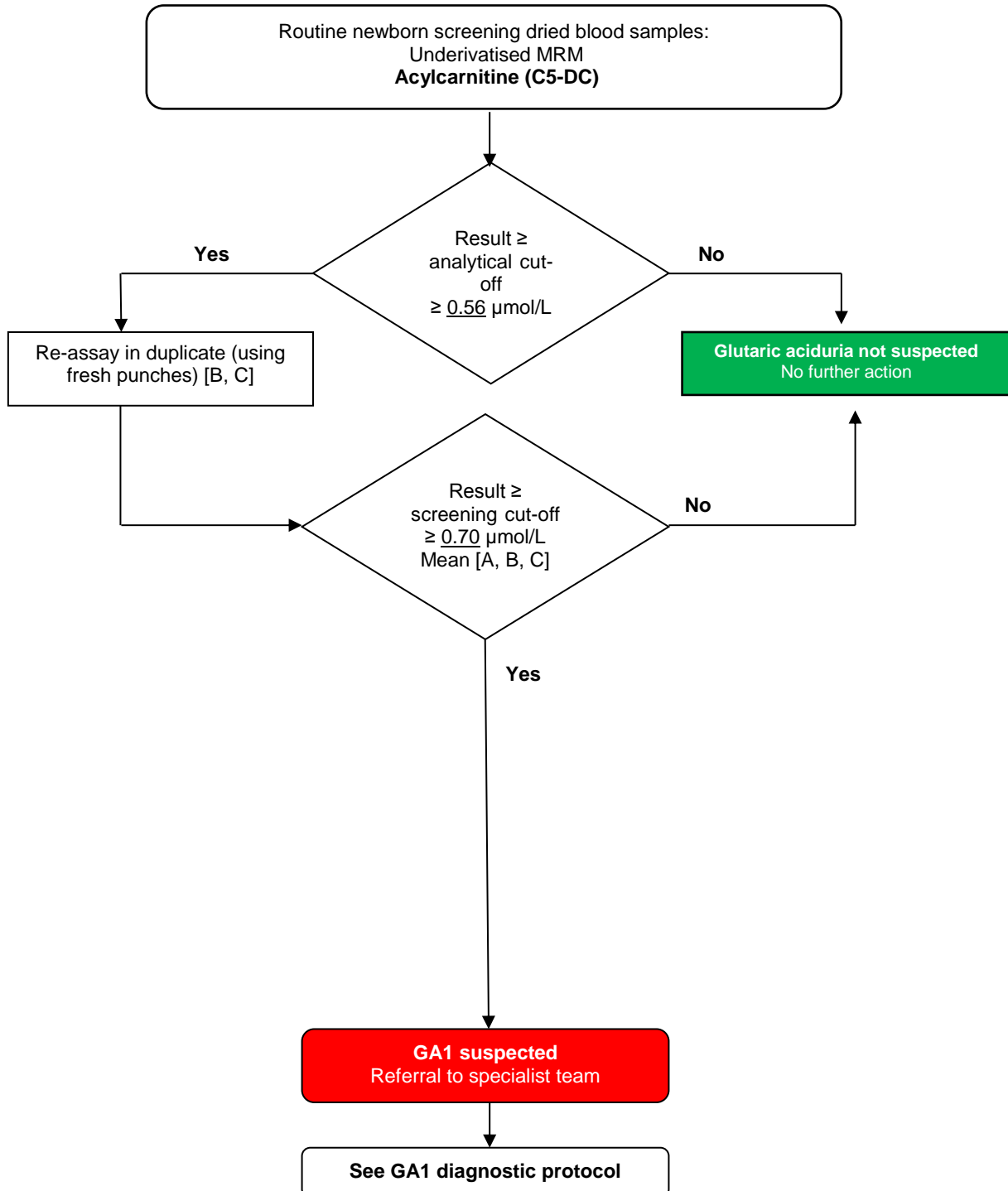
The clinical features and natural history of GA1 are now very well understood following the publication by Kolker et al (2006) of an international cross-sectional observational study of 279 patients from 35 metabolic centres. About 70% of patients (including both high and low excretors) have an encephalopathic crisis, which is most commonly at around 9 months, with 90% by age 2 years. These are usually precipitated about 1–3 days after onset of a non-specific intercurrent illness, gastrointestinal infection or pneumonia and lead to dystonia and dyskinesia as permanent sequelae but with relative preservation of the intellect. Only 6% of patients had no neurological abnormalities following encephalopathy. Of symptomatically diagnosed patients, about 50% die before the age of 25 years. For those who have had an encephalopathic crisis, the average handicap score was 2.7 (representing moderate to severe handicap) and the morbidity score was 2 indicating problems in at least two areas (relating to loss of mobility, feeding problems, respiratory problems and seizures). There is also evidence for two clinically defined sub-groups with insidious or later onset and occasionally for a neonatal onset with non-specific symptoms including irritability and transient lactic acidosis. These infants go on to show delayed motor development. Very occasionally an individual might be asymptomatic.

The clinical features and natural history of GA1 are now very well understood following the publication by Kolker et al (2006) of an international cross-sectional observational study of 279

patients from 35 metabolic centres. About 70% of patients (including both high and low excretors) have an encephalopathic crisis, which is most commonly at around 9 months, with 90% by age 2 years. These are usually precipitated about 1–3 days after onset of a non-specific intercurrent illness, gastrointestinal infection or pneumonia and lead to dystonia and dyskinesia as permanent sequelae but with relative preservation of the intellect. Only 6% of patients had no neurological abnormalities following encephalopathy. Of symptomatically diagnosed patients, about 50% die before the age of 25 years. For those who have had an encephalopathic crisis, the average handicap score was 2.7 (representing moderate to severe handicap) and the morbidity score was 2 indicating problems in at least two areas (relating to loss of mobility, feeding problems, respiratory problems and seizures). There is also evidence for two clinically defined sub-groups with insidious or later onset and occasionally for a neonatal onset with non-specific symptoms including irritability and transient lactic acidosis. These infants go on to show delayed motor development. Very occasionally an individual might be asymptomatic.

12.2 Screening protocol

Figure 10. GA1 newborn screening protocol



12.3 Sibling testing

12.3.1 Protocol for management of at risk delivery – neonatal testing for siblings born after proband diagnosis

When to test and samples taken

24–48 hours: Blood spot acylcarnitines (C5-DC), qualitative urinary organic acids and genotyping

Write on blood spot card 'Family history of GA1'

Day 5: Routine newborn screen

Write on blood spot card 'Family history of GA1'

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 metabolic paediatrician for review and consideration of nasogastric tube feeds or commencing intravenous glucose*.

If GA1 confirmed: Follow the standard GA1 clinical management guidelines and dietary management guidelines*.

12.3.2 Testing siblings born before proband diagnosis

When to test

Offer to test if sibling has not been previously screened for GA1.

Sibling samples

- i. Blood spot acylcarnitine profile (C5-DC) and qualitative urinary organic acids
- ii. DNA – send for genotyping once definite GA1 diagnosis secured in proband

Management

Before the results are available and thereafter if GA1 confirmed: Follow the standard GA1 clinical and dietary management guidelines*.

*For more information please refer to [clinical management and dietary management guidelines](#). Also see section 2.6 – Genetic counselling.

12.4 Pre-analytical aspects

12.4.1 Potential for false negatives

1. **Transfusions** could result in a false negative result, as for other screening tests, and a repeat sample should be taken after a reasonable time has elapsed. At least 72 hours is recommended, as for the other screening tests, to allow pre-transfusion levels to be reached.
2. **Delays in transit / sample deterioration** – it is possible that a sample may deteriorate due to adverse transport conditions and result in ‘loss’ of measured glutarylcarnitine the blood spot.
3. **Physiological reasons** – patients with glutaric aciduria type 1 who excrete a very low concentration of glutarate and related metabolites are well described. It is not clear whether these patients would be detected by measuring C5-DC acylcarnitine in the newborn period.

12.4.2 Potential for false positives

- C6OH acylcarnitine is isobaric with C5-DC acylcarnitine. An elevated C6OH acylcarnitine is seen in association with ketosis in some patients and may produce a false positive result.
- Glutaric aciduria type 2 is often associated with an increase in C5, C8 and C5-DC acylcarnitines, all detected as part of the newborn screening programme. Screen positive results for any of these metabolites should prompt consideration of glutaric aciduria type 2.

12.5 Analysis

12.5.1 Action on discrepant replicates

If a sample has an initially raised C5-DC (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial C5-DC is suspected. See Appendix 1 for guidance on discrepant replicates.

12.6 Clinical referral and follow up

See [GA1 clinical management guidelines](#) and Appendix 6 (GA1 initial clinical referral guidelines and standards).

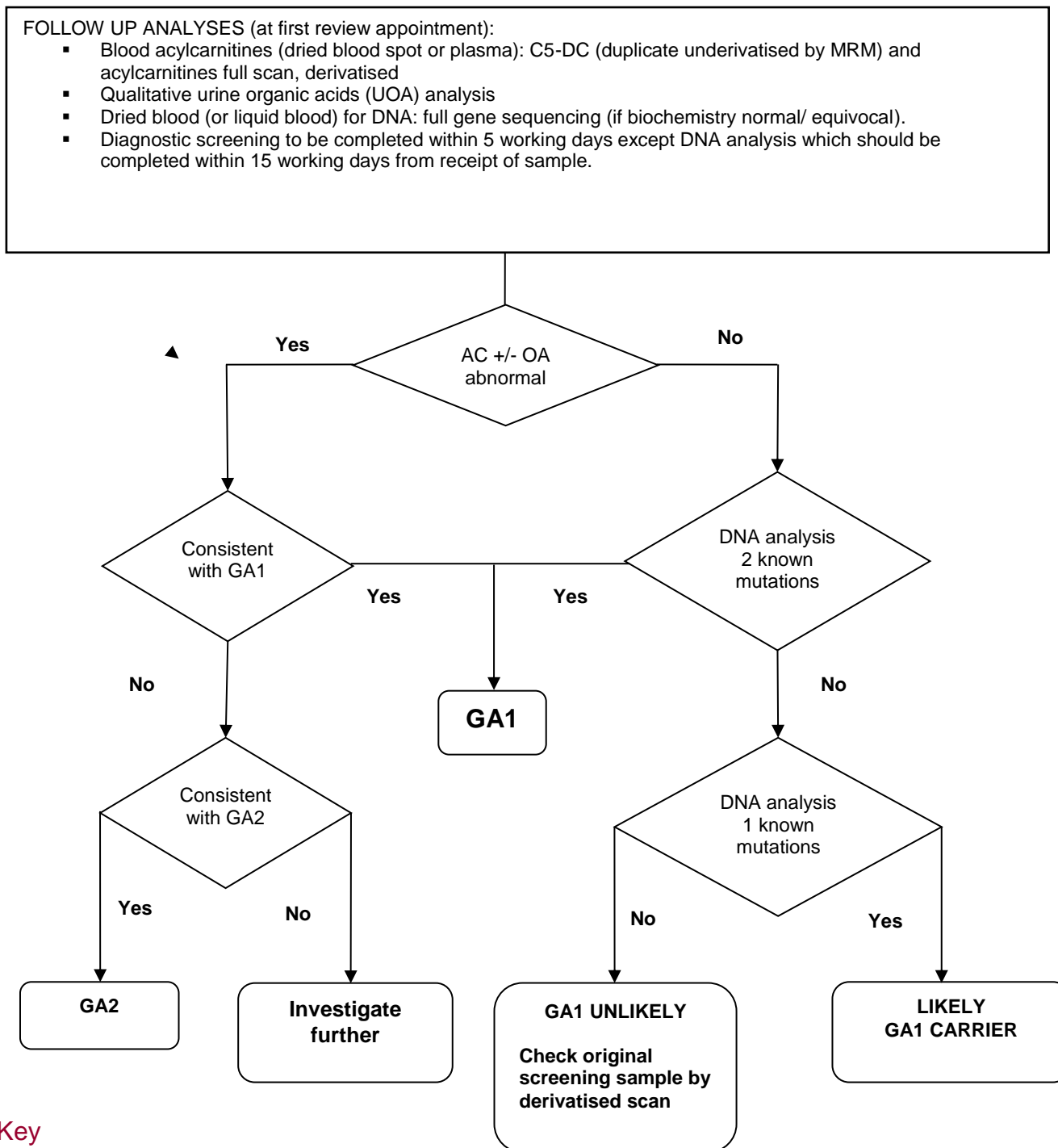
12.6.1 Follow up of presumptive/suspected positive cases

Please refer to the screening protocol (Figure 10). These are babies with mean of triplicate C5-DC ≥ 0.70 $\mu\text{mol/L}$.

- All presumptive positives should be referred to the specialist IMD 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 available
- The family will be instructed by the specialist team to take the baby to an appropriate hospital (if not an inpatient already) where initial assessment will take place. The baby will be transferred to the specialist IMD centre on the same or next working day of hearing about a positive screening result.
- If transfer is not available, the specialist team will liaise with the local hospital to arrange diagnostic testing and supply of dietary supplements.

12.7 Diagnostic protocol

Figure 11. GA1 diagnostic protocol



Key

GA2 Glutaric aciduria type 2

12.7.1 Diagnostic specimen requirements

Table 9. GA1 diagnostic specimen requirements

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

| Requirements | Timing of specimen and results |
|---|--|
| Blood acylcarnitines Dried blood spot or 0.5 ml Li Hep plasma: C5-DC duplicate and acylcarnitine full scan | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |
| Qualitative urine organic acids 5ml minimum (no preservative, frozen immediately at -20°C) | Results should be available within 5 working days of receipt of the diagnostic samples into the laboratory |
| DNA analysis If the biochemistry is normal or equivocal, liquid blood should be taken for DNA: full genome sequencing | DNA analysis should be completed within 15 working days from receipt of sample |

12.8 Laboratory standards

See section 15 for generic laboratory standards.

12.8.1 Performance standards, including diagnostic tests

- The laboratory analytical service should be configured to enable the GA1 newborn screening protocol (a raised C5-DC acylcarnitine confirmed in triplicate) to be completed within 3 working days from receipt of an adequate sample as core standard
- Presumptive positive results (average of triplicate C5-DC acylcarnitine) 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. If there is insufficient sample to repeat / confirm an initial raised C5-DC acylcarnitine ($\geq 0.56 \mu\text{mol/L}$), urgent referral to the designated / specialist metabolic team should be arranged
- Follow-up diagnostic tests must be undertaken in line with the diagnostic protocol by accredited laboratories that must participate and demonstrate acceptable performance in the relevant / accredited EQA schemes. Diagnostic screening to be

completed within 5 working days of receipt of the diagnostic samples into the laboratory except DNA analysis which should be completed within 15 working days from receipt of sample; the responsibility for this must be defined locally

13. Homocystinuria (pyridoxine unresponsive) (HCU)

13.1 Scientific background

The most common cause of homocystinuria (HCU) is a defect in the enzyme cystathionine β -synthase (CBS); this is referred to as “classical” homocystinuria. The overall incidence in the UK is reported to be around 1 in 100,000 live births. The mode of inheritance of classical homocystinuria is autosomal recessive. Classical homocystinuria is associated with a number of clinical and pathological abnormalities. Infants are usually normal at birth and without screening the diagnosis is not usually made until the first 2–3 years of life. Myopia followed by dislocation of the lens, osteoporosis, thinning and lengthening of the long bones, mental retardation and thromboembolism affecting large and small arteries and veins are the commonest clinical features. Without treatment, 25% of patients will die before the age of 30, usually as a result of arterial thromboembolism. There is a great deal of clinical heterogeneity, with some patients displaying all clinical symptoms whilst others display very few or none. The concentration of plasma total homocysteine can be measured to assess the clinical severity of disease and can be monitored to determine the response to treatment.

Homocystinuric patients can be sub-divided into two important biochemical phenotypes:

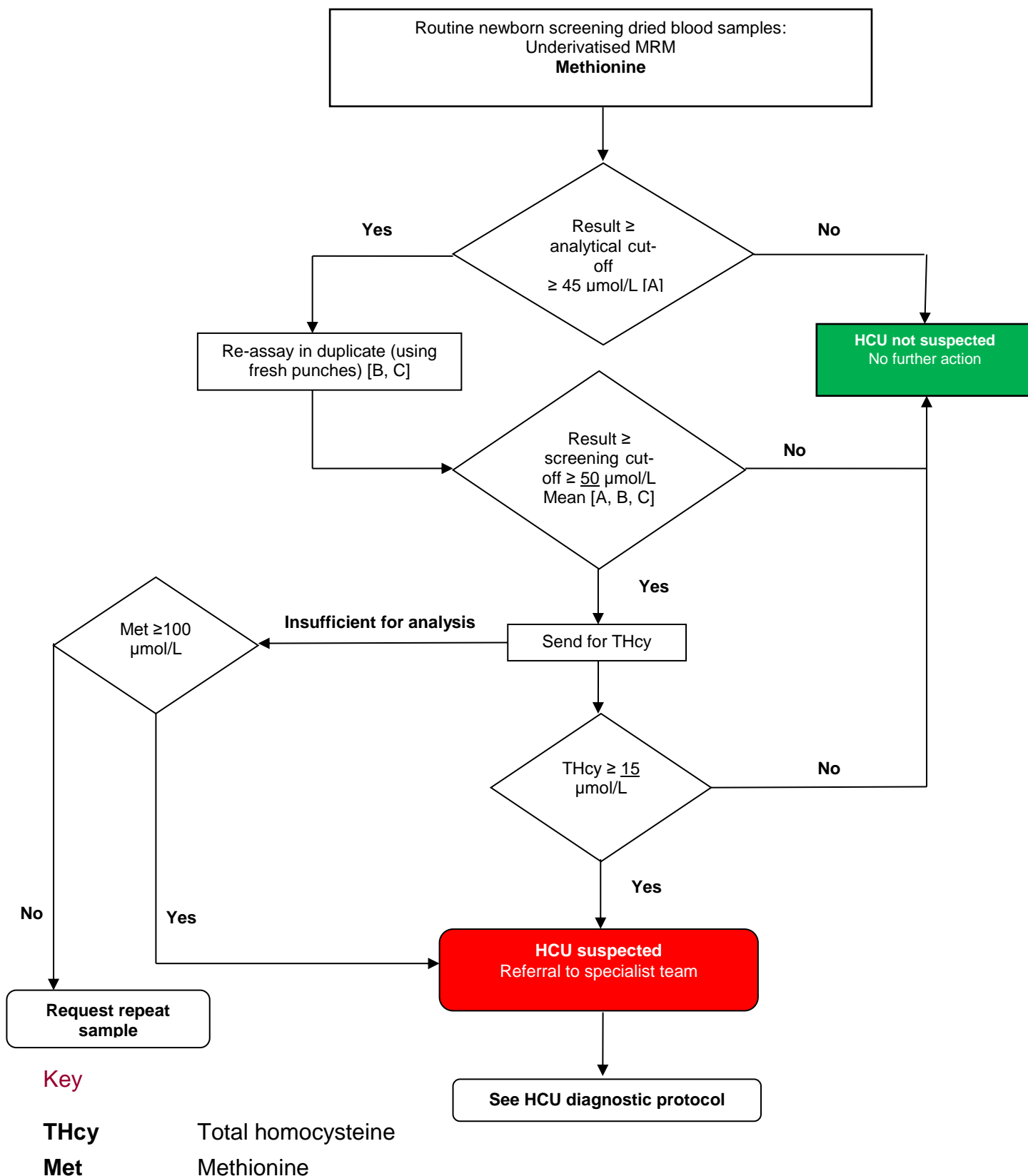
- Pyridoxine responsive (screen undetectable)
- Pyridoxine unresponsive (screen detectable)

In the UK approximately 50% of patients with classical homocystinuria are classified as pyridoxine responsive; these patients usually have milder symptoms and disease progression is slower and slowed further by oral pyridoxine (Vitamin B6) supplementation. They are very unlikely to be detected by newborn screening.

Screening for homocystinuria is based on quantitation of methionine followed by the measurement of total homocysteine in dried blood spots (note that dried blood spot total homocysteine is lower than in plasma). Confirmatory testing for classical homocystinuria involves the measurement of plasma and amino acids and homocysteine using a standard amino acid analyser.

13.2 Screening protocol

Figure 12. HCU newborn screening protocol



13.3 Sibling testing

13.3.1 Protocol for management of at risk delivery - neonatal testing for siblings born after proband diagnosis

When to test and samples taken

Day 5: Routine newborn screen
 Write on blood spot card 'Family history of HCU'

If the family want a result sooner, send liquid blood at no less than 3 days of age for plasma amino acids and total homocysteine. The sample should be taken in hospital and separated promptly and the laboratory should be informed that the sample needs to be prioritised.

Management

Prior to results: no special management of the baby is required.

If results indicate baby is affected with HCU, follow the HCU clinical management guidelines*.

13.3.2 Testing siblings born before proband diagnosis

When to test

This should generally be at the next clinic visit but it can be deferred for a week or two if the siblings are asymptomatic. Check whether the siblings are taking preparations containing pyridoxine as a negative result under these circumstances might not completely exclude HCU.

Sibling sample

Liquid blood should be taken and separated promptly for measurement of plasma amino acids and total homocysteine. The results should be available within 1 week.

Management

No special management is required.

*For more information please refer to [clinical management and dietary management guidelines](#). Also see section – 2.6 Genetic counselling.

13.4 Pre-analytical aspects

13.4.1 Potential for false negatives

1. **Transfusions** could result in a false negative result, as for other screening tests, and a repeat sample should be taken after a reasonable time has elapsed. At least 72 hours is

recommended, as for the other screening tests, to allow pre-transfusion levels to be reached.

2. **Delays in transit / sample deterioration** – it is possible that a sample may deteriorate due to adverse transport conditions and result in 'loss' of measured methionine in the blood spot.
3. **Physiological reasons** – patients with pyridoxine responsive homocystinuria are very unlikely to be detected.

13.4.2 Potential for false positives

- Liver disease (for example due to tyrosinaemia type I or galactosaemia), parenteral nutrition, and methionine adenosyl transferase (MAT) deficiency can give rise to an elevated methionine concentration in the newborn period.
- Raised total homocysteine concentrations are also seen in some rarer inborn errors of metabolism (MTHFR deficiency and defects of vitamin B12 metabolism) and in maternal B12 deficiency but these would not be detected by screening as they are associated with low, rather than high, methionine concentrations.

13.5 Analysis

13.5.1 Guidance on discrepant Met replicates

If a sample has an initially raised Met (above the screening protocol cut-off), which is clearly normal on duplicate repeat, a falsely elevated initial Met is suspected. See Appendix 1 for guidance on discrepant replicates.

13.6 Clinical referral and follow up

See [HCU clinical management guidelines](#) and Appendix 7 (HCU initial clinical referral guidelines and standards).

13.6.1 Follow up of presumptive/suspected positive cases

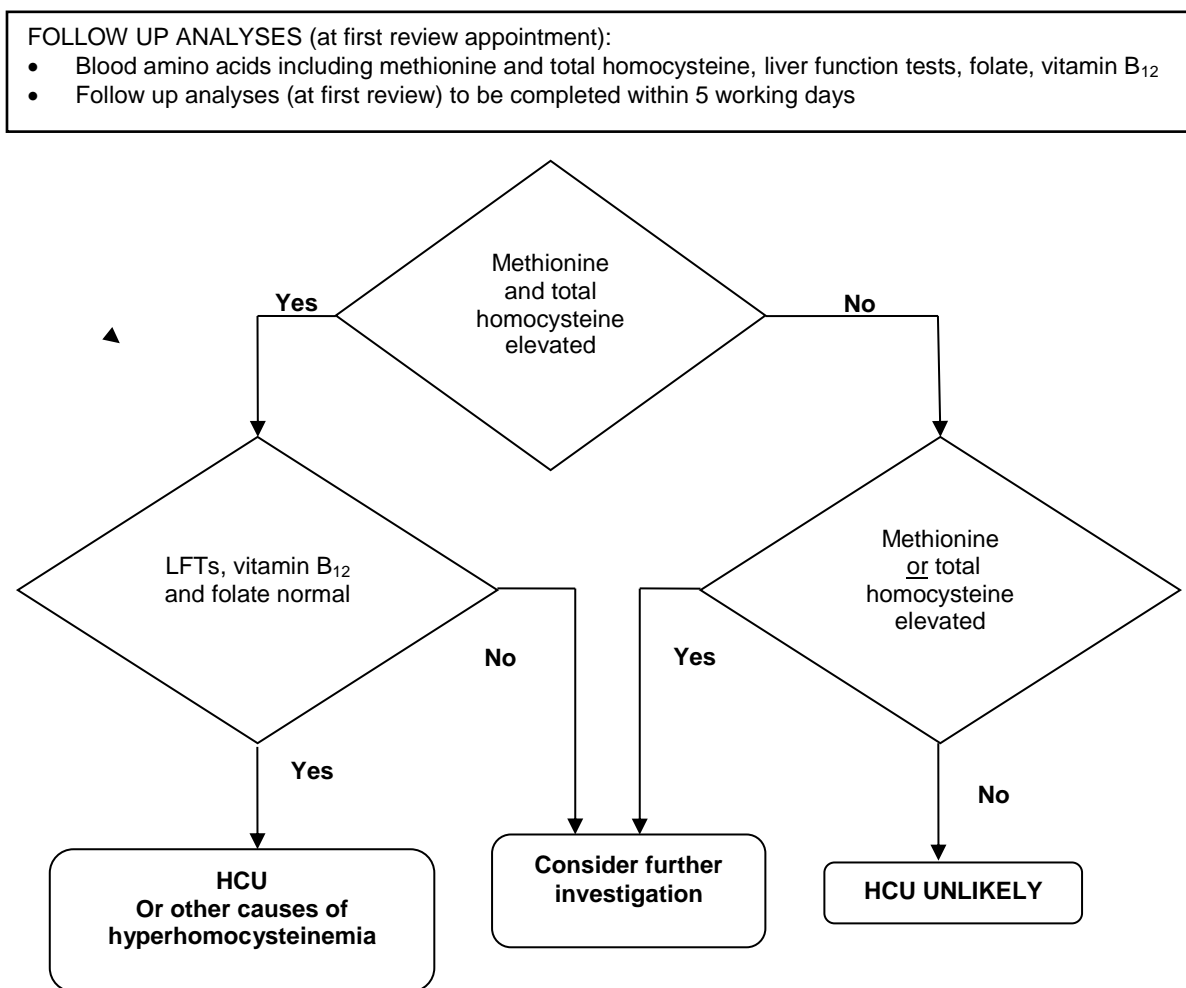
These are babies with mean of triplicate Met ≥ 50 $\mu\text{mol/L}$ (Figure 12 – HCU newborn screening protocol) AND a THcy ≥ 15 $\mu\text{mol/L}$.

- All babies with a 'HCU suspected' screening result should be referred to the specialist clinical team (or designated local team) via the CLS (as per local arrangements) **on the same working day** that the 'HCU suspected' screening result is available. This referral with detailed screening results must be reported both verbally as well as in writing – a [template](#) is available

13.7 Diagnostic protocol

This applies to babies with a presumptive positive newborn screening test, i.e. Methionine concentration $\geq 50 \mu\text{mol/L}$ AND THcy $\geq 15 \mu\text{mol/L}$ who are reported as 'HCU suspected'.

Figure 13. HCU diagnostic protocol



13.7.1 Diagnostic specimen requirements

Table 10. HCU diagnostic specimen requirements

| Requirements | Timing of specimen and results |
|--|---|
| Amino acids including methionine and total homocysteine The specimen can be plasma (Lithium Heparin - 0.5 mL) or dried blood spots dependent on local arrangements | Follow up analyses (at 1st review appointment) to be completed within 5 working days of receipt of the diagnostic samples into the laboratory |

13.8 Laboratory standards

See section 15 for generic laboratory standards.

13.8.1 Performance standards, including diagnostic tests

- The laboratory analytical service should be configured to enable the HCU newborn screening protocol (a raised methionine confirmed in triplicate, followed by a raised total homocysteine) to be completed within 9 working days from receipt of an adequate sample as core standard. This would imply a turnaround time of 5 working days for total homocysteine analysis from sample receipt
- Presumptive positive results 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 must be undertaken in line with the diagnostic protocol by accredited laboratories that 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 of receipt of the diagnostic samples into the laboratory; the responsibility for this must be defined locally

14. Status codes

See Appendix 8 for a complete list of status codes and sub codes for newborn blood spot screening and Appendices 9 to 14 for the relevant status codes for each condition.

15. Standards

15.1 Generic standards

The NHS Newborn Blood Spot Screening Programme has generic standards for blood spot screening relating to completeness of coverage, timely identification of babies with a null or incomplete result, use of the NHS number as a unique identifier, timely sample collection and receipt, quality of the blood spot sample, timely taking of a repeat, laboratory accreditation, processing of screen positives, timely receipt into clinical care and timeliness of results to parents. See '[Standards for Newborn Blood Spot Screening](#)' (NHS Newborn Blood Spot Screening Programme, 2017).

15.2 Laboratory standards

Laboratories screening for IMDs must be accredited by United Kingdom Accreditation Service (UKAS). There must be a member of staff at consultant level responsible for IMD 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 in to account audit data, accumulating results, technical developments and local changes in healthcare provision.

See sections 8.9, 9.8, 10.8, 11.8, 12.8 and 13.8 for condition-specific laboratory performance standards and Appendix 15 for the elements of regulatory standards to be maintained by all laboratories.

16. References

- Beutler, E. and Baluda, M.C. (1966) 'A simple spot screening test for galactosaemia', *Journal of Lab and Clinical Medicine*, 68, 137-141.
- Brenton, D. and Pietz, J. (2000) 'Adult care in phenylketonuria and hyperphenylalaninaemia: the relevance of neurological abnormalities', *Eur J Pediatr*, 159(Suppl. 2), S114-120.
- Fingerhut, R., Ensenauer, R., Röschinger, W., Arnecke, R., Olgemöller, B. and Roscher, A.A. (2009) 'Stability of acylcarnitines and free carnitine in dried blood samples: Implications for retrospective diagnosis of inborn errors of metabolism and neonatal screening for carnitine transporter deficiency', *Anal Chem*, 81, 3571-75.
- Golbahar, J., Altayab, D.D. and Carreon, E. (2014) 'Short-term stability of amino acids and acylcarnitines in the dried blood spots used to screen newborns for metabolic disorders', *J Med Screen*, 21(1): 5-9.
- Gregersen, N., Winter, V., Curtis, D., Deufel, T., Mack, M., Hendrickx, J., Willems, P.J., Ponzzone, A., Parrella, T., Ponzzone, R., et al. (1993) 'Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: the prevalent mutation G985 (K304E) is subject to a strong founder effect from northwestern Europe', *Hum Hered*, 43(6), 342-350.
- Jensen, U.G., Brandt, N.J., Christensen, E., Skovby, F., Nørgaard-Pedersen, B. and Simonsen, H. (2001) 'Neonatal Screening for Galactosemia by Quantitative Analysis of Hexose Monophosphates Using Tandem Mass Spectrometry: A Retrospective Study', *Clinical Chemistry*, 47(8), 1364-1372.
- Johnson, D.W. and Trinh, M.U. (2004) 'Stability of malonylcarnitine and glutarylcarnitine in stored blood spots', *J Inher Metab Dis*, 27(6), 789-90.
- Khalid, J.M. Oerton, J., Besley, G., Dalton, N., Downing, M., Green, A., Henderson, M., Krywawych, S., Wiley, V., Wilcken, B., Dezateux, C., UK Collaborative Study of Newborn Screening for MCADD (2010) 'Relationship of Octanoylcarnitine Concentrations to Age at Sampling in Unaffected Newborns Screened for MCADD', *Clinical Chemistry*, 56(6), 1015-21.
- Kolker, S., Garbade, S.F., Greenberg, C.R., Leonard, J.V., Saudubray, J.M., Ribes, A. et al. (2006) 'Natural history, outcome, and treatment efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency', *Pediatric Research*, 59(6):840-847.
- Menzies, I.S. and Seakins, J.W.T. (1976) 'Liquid sample sugars'. In Smith, I. and Seakins, J.W.T. (eds.) *Chromatographic and Electrophoretic Techniques*, Vol. 1, Second ed., London, Heinemann.

NHS Newborn Blood Spot Screening Programme (2017) *Newborn blood spot screening standards: implementation date 1 April 2017* [Online] Available at www.gov.uk/government/publications/standards-for-nhs-newborn-blood-spot-screening (accessed 27 March 2017).

Oerton, J., Downing, M., Andresen, B.S., Champion, M., Cleary, M., Chakrapani, A., Sharrard, M., Walter, J.H., Phillips, P., Dezateux, C., UK Collaborative Study of Newborn Screening for MCADD (2005) 'Predictive value, clinical status and genotype of medium-chain acyl-CoA dehydrogenase deficiency (MCADD) ascertained by screening at one week of age using electrospray tandem mass spectrometry of underivatized blood spots: findings from a UK multicentre study', *J Inherit Metab Dis*, 28(Suppl.1), 9.

Phillips, P., Dalton, N., Besley, G., Bonham, J., Foo, Y., Green, A., Henderson, M., Oerton, J., Dezateux, C., UK Collaborative Study of Newborn Screening for MCADD (2005) 'Newborn screening for medium-chain acyl-CoA dehydrogenase deficiency at one week of age: octanoylcarnitine distributions from a multicentre study using electrospray tandem mass spectrometry of underivatized blood spot samples', *J Inherit Metab Dis*, 28(Suppl.1), 9.

Pollitt, R.J. and Leonard, J.V. (1998) 'Prospective surveillance study of medium-chain acyl-CoA dehydrogenase deficiency in the UK', *Arch Dis Child*, 79, 116-119.

Pollitt, R.J., Worthy, E. and Green, A. (1982) 'Galactosaemia detection as a bonus from screening for phenylketonuria', *J Inherit Metab Dis*, 5, 51-52.

Scriver, C.R., Kaufman, S., Eisensmith, R.C. and Woo, S.L.C. (1995) 'The hyperphenylalaninaemias'. In Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D. (eds.) *The metabolic and molecular basis of inherited disease*. New York, McGraw-Hill, 1015-1075.

Seddon, H.R., Green, A., Gray, R.G., Leonard, J.V. and Pollitt, R.J. (1995) 'Regional variations in medium-chain acyl-CoA dehydrogenase deficiency', *Lancet*, 345(8942), 135-136.

Shakespeare, L., Downing, M., Allan, J., Casbolt, A., Ellin, E., Maloney, M., Race, G. and Bonham, J. (2010) 'Elevated phenylalanine on newborn screening: follow up testing may reveal undiagnosed galactosaemia', *Annals Clin Biochem*, 47, 567-569.

Strnadova, K.A., Holub, M., Muhl, A., Heinze, G., Ratschmann, R., Mascher, H., Stockler-Ipsiroglu, S., Waldhauser, F., Votava, F., Lebl, J. and Bodamer, O.A. (2007) 'Endocrinology and Metabolism: Long-Term Stability of Amino Acids and Acylcarnitines in Dried Blood Spots', *Clinical Chemistry*, 53(4), 717-22.

UK Newborn Screening Programme Centre (2005) *Newborn blood spot screening in the UK – Policies and Standards*. London, UK Newborn Screening Programme Centre.

UK Newborn Screening Programme Centre (2010) *PKU Expert Group Report: Newborn Blood Spot Screening in the UK 2010*.

Wilcken, B., Carpenter, K.H. and Hammond, J. (1993) 'Neonatal symptoms in medium-chain acyl coenzyme A dehydrogenase deficiency', *Arch Dis Child*, 69, 292-294.

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This second revised edition was prepared by Professor Jim Bonham, Dr Philippa Goddard and Dr Helena Kemp.

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18. Appendices

Appendix 1: Guidance on discrepant replicates

This guidance applies if a sample has an initially raised analyte result (above screening protocol analytical cut-off) which is clearly normal on retesting (duplicate repeat) the original card.

Laboratories should use their discretion on investigation of discrepant results; the following is a suggested approach:

Visually inspect the plate* and the screening card for any obvious contamination which may have affected the spots.

A. Acylcarnitine analysis

| Disorder | Analyte (acylcarnitine) | Underivatised | Derivatised |
|----------|----------------------------|---------------|---------------|
| | | Mass <i>x</i> | Mass <i>y</i> |
| MCADD | C8 | 288 | 344 |
| IVA | C5 | 246 | 302 |
| GA1 | C5-DC | 276 | 388 |

Check persistence of ‘acylcarnitine analyte’ (relevant *underivatised mass x*, see Table above) by performing an underivatised acylcarnitine full scan on the well* that gave the raised result plus two normal samples and high QC from the same plate for comparison. (If necessary reconstitute the well):

If mass *x* persists:

Derivatise the transfer plate well that gave the raised result plus the same two normal and high QC samples from the plate to check for the respective *derivatised mass y*.

Perform a derivatised acylcarnitine full scan (or product ion scan).

If no mass *x*:

Contamination not of sample origin, **no further testing of samples** in batch necessary but other non-sample sources of contamination may be sought.

| If acylcarnitine confirmed i.e. mass y detected * | If acylcarnitine not confirmed i.e. no mass y |
|--|--|
|--|--|

| | |
|--|--|
| Re-test the plate/whole batch to eliminate the possibility of sample mix up. | Contamination is not due to genuine analyte, no further testing of samples in batch necessary but should investigate other non-sample sources of mass x contamination. |
|--|--|

When method involves eluate transfer from elution to transfer plate:

* visually inspect both elution and transfer well and perform scan on both

- Can identify any sample mix up at transfer stage by performing an underivatised acylcarnitine scan of the whole elution plate (reconstituted with 75 ul methanol). If any elution well shows increased **x** – retest that sample. However, re-analysis of the elution plate is often not successful due to insufficient residual sample

B. Amino acid analysis

| Disorder | Analyte (amino acid) | Underivatised Mass x |
|-----------------|-----------------------------|-----------------------------|
| PKU | Phe | 166 |
| | Tyr | 182 |
| MSUD | xLeu | 132 |
| HCU | Met | 153 |

Note: if an amino acid full scan method is available then this can be employed but as it does not form any part of the screening protocols it may not be available, in which case information derived from the other amino acid screening results may be sufficient to draw conclusions.

Check persistence of ‘amino acid analyte’ (relevant **underivatised mass x**, see Table above) by performing an underivatised amino acid full scan **or** MRM on the well* that gave the raised result plus two normal samples and high QC from the same plate for comparison. (If necessary reconstitute the well):

Possible causes and suggested further action for discrepant amino acid results:

| If mass x persists AND other screen amino acid results or the full scan result suggest contamination | If mass x persists AND other screen amino acid results or the full scan result do not suggest contamination | If mass x no longer increased AND other screen amino acid results or the full scan result are normal |
|--|---|--|
| <p>Possible causes could be biological contamination: generalised increase in amino acids including phenylalanine, proline, branched-chains (valine, leucine / isoleucine) and histidine usually without significant increase in tyrosine.</p> <p>Aspartame contamination: increased phenylalanine and aspartic acid.</p> <p>No further testing of samples is necessary.</p> | <p>Possible sample mix up at punching / transposition on plate.</p> <p>This must be investigated in the context of local sample preparation procedures. Adjacent samples or possibly the whole plate should be re-punched and re-analysed**.</p> <p>Request repeat samples as required.</p> | <p>Amino acid or mass contamination was from a non-sample source.</p> <p>No further testing of samples is necessary but origin of the contamination will need to be investigated in the context of local procedures.</p> |

When method involves eluate transfer from elution to transfer plate:

* visually inspect both elution and transfer well and perform scan or MRM on both

** sample mix up / transposition could have occurred at the transfer stage, which would be revealed if the elution well does not show increased mass **x**. In this case before re-punching samples the potential transfer error could be investigated by re-analysing adjacent elution wells (or possibly the whole plate) by the screening method. If any elution well shows increased mass **x** the responsible sample should be re-analysed. However, re-analysis of the elution plate is often not successful due to insufficient residual sample, in which case a repeat sample may be necessary.

Appendix 2: PKU initial clinical referral guidelines and standards

| Stage of process | No. | Guidelines and standards |
|---|----------|--|
| Defining a 'PKU suspected' screening result | 1 | <p>1.1 If a sample from a baby is found to have a phenylalanine concentration equal to or greater than 200 $\mu\text{mol/L}$, a repeat phenylalanine test and initial tyrosine test should be performed in duplicate on the original blood spot card as soon as possible and within 1 working day.</p> <p>1.2 If the mean of the phenylalanine results are equal to or above 240 $\mu\text{mol/L}$ (and mean of tyrosine <240 $\mu\text{mol/L}$) this should be reported as a 'PKU suspected' screening result. Other disorder is suspected if tyrosine is increased.</p> |
| Referral of babies with a 'PKU suspected' screening result | 2 | <p>2.1 The screening laboratory must inform the PKU clinical liaison service (CLS)* of a 'PKU suspected' screening result on the same day as the result (in 1.2 above) has been reported by the laboratory.</p> <p>2.2 <i>*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 specialist clinical team or in the community, based on local arrangements.</i></p> <p>2.3 If a disorder other than PKU is suspected referral must be made to the PKU or other appropriate specialist team.</p> |
| PKU specialist clinical team | 3 | <p>3.1 Each screening laboratory should link with a specialist clinical team who are currently managing at least 20 cases.</p> <p>3.2 A PKU specialist team must comprise as a minimum:</p> <ul style="list-style-type: none"> • A consultant Inherited Metabolic Disease paediatrician • A paediatric dietitian with metabolic expertise • A paediatric nurse with metabolic expertise <p>3.3 A PKU designated team, who work with a specialist team, must include clinicians trained to receive PKU referrals and have a paediatric dietetic service with metabolic training.</p> |

3.4 ON THE SAME DAY AS SCREENING RESULT AVAILABLE

The PKU CLS must make contact, both verbally (by telephone) as well as in writing (by fax / email – [template](#) available) with:

- PKU team* to arrange appointment for family to be seen on the same or next working day# following receiving a positive screening result – 1st face-to-face review
- Notify family's GP (see 4 below)

*PKU team is defined as either specialist or designated as per local protocol

#Note: Parents should be offered an appointment with the clinical team on the same or next day after hearing about a positive screening result. Parents should NOT be informed of a positive result if an appointment cannot be given for the same or next day e.g. parents should not be informed on a Friday unless an appointment is for that day or the Saturday. In this case contact should be deferred until after the weekend to the next working day.

Communication flows **4** **Pre-family contact**

The PKU CLS must:

4.1 Co-ordinate local support.

4.2 Obtain a telephone number for the family.

4.3 Ensure that the family is seen by a health professional who has had direct contact with the PKU team and is trained to give this information to the family.

4.4 Fax or email the GP information about PKU / screening test result as follows:

- PKU GP letter
- PKU is suspected leaflet
- Contact telephone numbers for the PKU team

- Details of the time and location of the appointment with the PKU team

5 First family contact (usually at home)

5.1 Contact with the family must be made to inform them of the positive screening result by a member of the PKU team.

5.2 The person contacting the family must be provided with information for the family as follows:

- PKU is suspected leaflet
- Contact numbers for the PKU team
- Details of the time and location of an appointment with the PKU team

To facilitate the above, the contacting person should:

- Contact the GP
- Fax to the GP the following:
 - GP letter
 - 'PKU is suspected' leaflet
 - contact details for PKU designated (or specialist) team
 - appointment time and location
- Ask the GP to provide the family with:
 - 'PKU is suspected' leaflet
 - contact details for PKU designated (or specialist) team
 - appointment time and location

Clinical evaluation and confirmatory tests

6 1st face-to-face review (appointment with PKU specialist clinical team)

(SAME) OR NEXT WORKING DAY AFTER FIRST FAMILY CONTACT

Management should include:

6.1 Explanation of the condition including introduction to inheritance.

6.2 Introduction to dietary management and monitoring (if clinically required) (**dietary management information** is available).

6.3 Contact with specialist dietitian (if clinically required).

6.4 Ensure family has received available written information including PKU team contact details and PKU is suspected leaflet.

6.5 The report of the first review should be communicated via letter to the GP, with a copy to midwife, health visitor and screening laboratory.

6.6 When a baby is seen with a positive screening result, the following must be performed to confirm diagnosis and exclude other possible metabolic disorders:

- a. Confirmatory blood specimen for quantitative phenylalanine and tyrosine analyses
- b. Tests to exclude / confirm other disorders that may be associated with raised phenylalanine. This includes galactosaemia, tyrosinaemia and bipterin defects where appropriate

Detection of these disorders is not the objective of the PKU screening programme and all such cases will not be reliably detected.

6.7 Results of confirmatory phenylalanine test to be available within one working day of being seen and ideally on the same day.

6.8 Diet should be commenced on this initial visit if clinically appropriate.

Achievable standard: by 14 days of age (100% of infants)

Acceptable standard: by 17 days of age (100% of infants)

6.9 Arrangements should be made for follow-up blood phenylalanine monitoring samples to be collected.

6.10 Arrangements should be made by specialist metabolic nurses and dietitians to provide on-going support.

7 Follow-up visit

Follow-up management should include:

7.1 A review of the condition and inheritance, supported with written information.

7.2 Review of dietary management, blood monitoring requirements, growth and developmental and general health.

7.3 Support information – including contact details of parent support organisations.

7.4 A specialist nurse should be available if possible to provide advice and support.

7.5 As appropriate parents should be taught how to perform the heel-prick on their baby.

7.6 The specialist dietitian may make contact with the local dietitian as appropriate.

Appendix 3: MCADD initial clinical referral guidelines and standards

| Stage of process | No. | Guidelines and standards |
|--|-----|---|
| Defining an 'MCADD suspected' screening result | 1 | <p>If a sample from a baby is found to have an octanoylcarnitine concentration equal to or greater than 0.40 $\mu\text{mol/L}$, repeat tests should be performed in duplicate on the original blood spot card.</p> <p>If the mean of triplicate results is equal to or greater than 0.50 $\mu\text{mol/L}$, AND the mean C8:C10 ratio ≥ 1.0, this is a presumptive positive screening result.</p> |
| Referral of babies with a 'MCADD suspected' screening result | 2 | <p>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.</p> <p>ON THE SAME DAY</p> <p>The CLS must make contact, both verbally (by telephone) as well as in writing (by fax / email – template available) with:</p> <ul style="list-style-type: none"> • 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) <p>An MCADD designated team must include clinicians trained to receive MCADD referrals and have a paediatric dietitian.</p> |
| | 3 | <p>An MCADD specialist team should comprise:</p> <ul style="list-style-type: none"> • A consultant Inherited Metabolic Disease paediatrician with relevant expertise • A paediatric dietitian with metabolic expertise • A clinical nurse specialist with metabolic expertise <p>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.</p> |

*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.

Communication flows **4**

Pre-family contact

ON THE SAME DAY

The specialist / designated team must inform the GP (as soon as is practicable) 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
- 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.

If it is not possible for the baby to be reviewed at the specialist / designated hospital, the specialist designated team should co-ordinate review at an appropriate hospital with 24 hr paediatric cover and discuss with the on-call paediatric consultant or registrar. Information

should be sent by fax/email to the hospital for clinicians and parents, GP letter, MCADD A&E letter, 'MCADD is suspected' leaflet, contact numbers for the MCADD specialist / designated team and location

5

First family contact

ON THE SAME DAY

The person contacting the family must be provided with information for the family as follows:

- 'MCADD is suspected' leaflet
- 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.

Clinical evaluation and confirmatory tests

6

1st face-to-face review

(SAME OR) NEXT DAY

This should be with the specialist or designated team but in exceptional circumstances may be with an hospital with 24 hour paediatric cover with support from the specialist / designated team including written information as above

Pre-diagnosis management should include:

- Explanation of the condition including introduction to inheritance
- Introduction to dietary management (including maximum safe fasting times) and use of Emergency Regimen (ER) for illness – as per **MCADD dietary guidelines**

- Contact with specialist or designated dietitian
- Clinician to ensure family have received – MCADD designated (or specialist) team contact details, A&E letter, appropriate dietary and ER guidelines, ‘MCADD is suspected’ leaflet
- Clinician to ensure letters have been sent to GP, local paediatrician, local dietitian as necessary ([template available](#))

Confirmatory diagnostic samples should be collected at 1st face-to-face review (i.e. within 24 hours of receiving screening results).

Consent for DNA testing to be obtained.

7

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:

- Repeat blood acylcarnitines: C8 and full scan
- Urine organic acid (UOA) analysis
- DNA analysis (c.985A>G and Extended Mutation Screening (EMS – samples to be sent immediately)

*see MCADD diagnostic protocol for details

8

Follow-up visits

FURTHER APPOINTMENTS TO BE SCHEDULED AS FOLLOWS:

Follow up visits should be with the specialist or designated MCADD team

9

1st follow-up visit within 5 working days of 1st face-to-face review – for results of C8, qualitative UOA and DNA testing for the common mutation c.985A>G.

If diagnosis not confirmed – see diagnostic protocol*.

2nd follow-up visit if diagnosis not yet confirmed within 15

working days of 1st follow-up visit – for results of quantitative UOA and EMS.

*see MCADD diagnostic protocol for details

Upon confirmation of diagnosis

- 10** Dietary management and when to implement Emergency Regimen (ER) must be emphasised.
- 11** Post-diagnosis discussion should ensure parents have good understanding of the condition, support information, correct contact numbers for MCADD team, information for A&E, age appropriate dietary management / ER information.
- 12** A specialist nurse should be available to provide advice and support.
- 13** The specialist dietitian should make contact with the local dietitian if appropriate
- 14** Older siblings to be tested as appropriate (if not previously tested or screened) – 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

- 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 on-going 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

Appendix 4: MSUD initial clinical referral guidelines and standards

| Stage of process | No. | Guidelines and standards |
|--|-----|---|
| Defining a positive screening result | 1 | If a sample from a baby is found to have a leucine, isoleucine, and alloisoleucine combined result equal to or greater than an analytical cut-off equal to or greater than 500 µmol/L, repeat tests should be performed in duplicate on the original blood spot card. |
| | 2 | If the mean of triplicate results is equal to or greater than 600 µmol/L, this is a presumptive positive screening result. |
| Referral of babies with positive screening result | 3 | The screening laboratory must inform the MSUD 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 – [template](#) available) with MSUD specialist team.

MSUD specialist team to:

1. CONTACT FAMILY
2. Arrange urgent admission of the baby to a hospital with 24 hr paediatric cover, and offer to arrange an ambulance
3. Specialist team to liaise with the local hospital (on call Paediatric Consultant, or registrar or equivalent grade if unable to contact)
 - a. Fax/email information to the hospital for clinicians and parents, BIMDG MSUD guidelines, 'MSUD is suspected' leaflet, contact numbers for the MSUD specialist team
 - b. Clinical assessment and admission to hospital regardless of clinical status. Obtain blood gases, U&E, LFT, FBC, cultures, urine ketones dipstick. Site IV cannula
 - c. Hospital to liaise with specialist centre regarding clinical status
 - d. Commence clinical management:
 - i. IV 10% dextrose/0.45% saline +added potassium infusion
 - ii. Transfer to specialist centre. If Glasgow Coma

- Scale ≤ 8 – intubate and ventilate and organise paediatric intensive care unit retrieval
- iii. If transfer not possible same day, obtain diagnostic samples** and courier urgently to specialist centre laboratory. Specialist team to liaise with laboratory to expect samples from admitting hospital
 - iv. If transfer not possible same day, specialist team to organise supplies of MSUD Anamix Infant, Isoleucine and Valine sachets and feeding plan***
 - e. Continue liaison between specialist and local hospital until transferred
4. Inform GP (as soon as practicable), send MSUD GP letter via fax / email
 5. Inform maternity services and health visiting services

An MSUD specialist team should comprise:

- A consultant Inherited Metabolic Disease paediatrician with relevant expertise
- A paediatric dietitian with metabolic expertise
- A clinical nurse specialist

If a disorder / situation other than MSUD is suspected, referral must be made to the MSUD 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), depending on local arrangements.

**See MSUD diagnostic protocol for confirmatory test

***See MSUD dietetic management pathway for details on [BIMDG website](#)

Communication flows

4 First family contact

ON THE SAME DAY

Contact with the family must be made by the specialist team to inform them of the positive screening result. The family must be provided with information as follows:

- 'MSUD is suspected' leaflet.
- Contact numbers for the MSUD specialist team.
- Outline plan for admission and assessment at local hospital

with subsequent interhospital transfer to the specialist centre

It is strongly recommended that the family has access to MSUD specialist team by telephone.

5 GP contact

ON THE SAME DAY

The MSUD specialist team must contact the GP (as soon as practicable):

- Co-ordinate local support
- Fax or email information about MSUD as follows:
 - MSUD GP letter
 - 'MSUD is suspected' leaflet
 - MSUD A&E letter
 - Contact numbers for the MSUD specialist team

Clinical evaluation and confirmatory diagnostic tests

6 **FIRST REVIEW within 24 hours of screening result. If not in specialist centre, speak directly via telephone or other communication**

- Assess infant to determine clinical state and commence MSUD treatment.

Pre-diagnosis management should include:

- Explanation of the condition including introduction to inheritance.
- Introduction to dietary management and use of Emergency Regimen (ER) for illness – as per **MSUD dietary guidelines**)
- Contact with specialist dietitian
- Clinician to ensure family have received – MSUD specialist team contact details, A&E letter, appropriate dietary and ER guidelines
- Review available test results
- (If at DGH, do not discharge until agreed by specialist team)

7 Confirmatory diagnostic samples should be collected and analysed ideally within 24 hours of receiving screening results.

8 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:

- Blood quantitative amino acids including alloisoleucine within 24 hours
- Urine organic acid (UOA) analysis

*See MSUD diagnostic protocol for details

9 Follow-up visits

FURTHER APPOINTMENTS TO BE SCHEDULED Based on clinical and Biochemical findings

1st follow-up visit within 5 working days of 1st face-to-face review if fit for discharge prior to results available– for results of blood quantitative amino acids including alloisoleucine, and UOA. Review original blood spot card alloisoleucine result*, if positive send for genetics/enzymology and manage as MSUD until results known

If clinical presentation/unwell, follow up appointment organised on discharge.

2nd follow-up visit if MSUD diagnosis not confirmed on 1st visit, confirm if intermittent MSUD or further investigations required (to exclude liver disease including galactosaemia)

*See MSUD diagnostic protocol for details

| | | |
|---------------------------------------|-----------|---|
| Upon confirmation of diagnosis | 10 | Dietary management and when to implement Emergency regimen (ER) must be emphasised (www.bimdg.org.uk/site/guidelines-enbs.asp) |
| | 11 | Post diagnosis discussion should ensure parents have good understanding of the condition, support information, correct contact numbers for MSUD team, information for A& E, age appropriate dietary management / ER information |
| | 12 | A specialist nurse should be available to provide advice and support |
| | 13 | The specialist dietitian should make contact with the local dietitian |

if appropriate

- 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 MSUD sibling protocol for details

- 15** Post-confirmation of diagnosis appointments to be scheduled to meet needs of the family. This must include:

- 5-7 day diagnostic review (may be an inpatient)
- Weekly home monitoring – branched-chain amino acids, urine ketones as required during inter-current illness, weekly or fortnightly weighing until 1st outpatient appointment
- 4-8 weeks review
- A visit at 4-6 months of age for dietary review and advice on weaning
- Post-weaning clinical and dietetic review

See [dietary management pathway](#)

Contact with dietitians

- 16** Parents will have ongoing access to a specialist dietitian and should be provided with appropriate contact details

- 17** Discussion between parents and dietitian should cover:

- Age appropriate advice on feeding (including breastfeeding and weaning)
- ER for illness: including preparation and use of ER feeds

Appendix 5: IVA initial clinical referral guidelines and standards

| Stage of process | No. | Guidelines and standards |
|--|-----|--|
| Defining a positive screening result | 1 | If a sample from a baby is found to have C5 acylcarnitines equal to or greater than 1.6 $\mu\text{mol/L}$, repeat tests should be performed in duplicate on the original blood spot card. |
| | 2 | If the mean of triplicate results is equal to or greater than 2.0 $\mu\text{mol/L}$, this is a presumptive positive screening result. |
| Referral of babies with positive screening result | 3 | <p>The screening laboratory must inform the IVA 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 and send original dried blood spot sample for C5 isobars</p> <p>ON THE SAME DAY</p> <p>The CLS must make contact, both verbally (by telephone) as well as in writing (by fax / email – template available) with IVA specialist team:</p> <p>IVA specialist team to:</p> <ol style="list-style-type: none"> 1. CONTACT FAMILY 2. Arrange urgent admission of the baby to a hospital with 24 hr paediatric cover, and offer to arrange an ambulance 3. Liaise with the hospital on call Paediatric Consultant (or Registrar if unable to contact) for assessment 4. Fax/email information to the hospital for clinicians and parents, IVA A&E letter, 'IVA is suspected' leaflet, contact numbers for the IVA specialist team <p><u>If well baby</u></p> <ol style="list-style-type: none"> 5. Commence clinical management <ol style="list-style-type: none"> a. Obtain diagnostic samples** and send urgently to specialist centre laboratory (courier) b. Ensure adequate feeding and obtain history of maternal antibiotics, nipple cream use and full drug history (cause of false positive) c. Discharge home with BIMDG emergency |

guidelines and glucose polymer. Instruct to take to hospital if unwell (when baby is discharged)

If unwell baby

- a. Clinical assessment and admission to hospital regardless of clinical status
 - b. Obtain history of maternal antibiotics, nipple cream use and full drug history (cause of false positive)
 - c. Obtain blood gases, U&E, LFT, FBC, cultures, urine ketones dipstick. Site IV cannula
 - d. Hospital to liaise with specialist team regarding clinical status
 - e. Obtain diagnostic samples** and send urgently to specialist centre laboratory (courier)
 - f. IV 10% dextrose/0.45% saline infusion
 - g. Carnitine – specialist team to organise supply and send to local hospital if necessary
 - h. Reintroduce natural protein within 24-48 hours (refer to dietetic management pathway***)
 - i. Transfer to specialist centre as soon as appropriate
6. Specialist team to liaise with diagnostic laboratory – inform lab to expect samples (including transport arrangement) and which hospital child has gone to in case samples need following up
 7. Hospital to feedback to specialist team with a review within 2 hours of admission if not already transferred to specialist centre
 8. Inform GP (as soon as practicable), send IVA GP letter via fax / email
 9. Inform maternity services and health visiting services

An IVA specialist team should comprise:

- A consultant Inherited Metabolic Disease paediatrician with relevant expertise
- A paediatric dietitian with metabolic expertise

- A clinical nurse specialist

If a disorder / situation other than IVA is suspected, referral must be made to the IVA 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

**See IVA diagnostic protocol for confirmatory test

***See IVA dietetic management pathway for details on [BIMDG website](#)

Communication flows 4 First family contact

ON THE SAME DAY

Contact with the family should be made by the specialist team to inform them of the positive screening result. The family should be provided with information as follows:

- 'IVA is suspected' leaflet
- Contact numbers for the IVA specialist team
- Outline plan for admission and assessment at local hospital with subsequent interhospital transfer to the specialist centre

It is strongly recommended that the family has access to IVA specialist team by telephone.

5 GP contact

ON THE SAME DAY

The IVA specialist team must contact the GP (as soon as practicable):

- Co-ordinate local support
- Fax or email information about IVA as follows:
 - IVA GP letter
 - 'IVA is suspected' leaflet

- IVA A&E letter
- Contact numbers for the IVA specialist team

Clinical evaluation and confirmatory diagnostic tests

6 FIRST REVIEW with parents within 24 hours of screening result. If not already seen in specialist centre, speak directly via telephone or other communication

- Assess infant to determine clinical state and commence IVA treatment.

Pre-diagnosis management should include:

- Explanation of the condition including introduction to inheritance
- Introduction to dietary management and use of Emergency Regimen (ER) for illness – as per **IVA dietary guidelines**
- Contact with specialist dietitian
- Clinician to ensure family have received – IVA specialist team contact details, A&E letter, appropriate dietary and ER guidelines
- Review available test results
- Reintroduce natural protein within 24-48 hours, refer to **dietetic management pathway**
- (If at DGH, do not discharge until agreed by specialist team)

7 Confirmatory diagnostic samples should be collected within 24 hours of receiving screening results.

Consent for DNA testing to be obtained.

8 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:

- Blood acylcarnitines (dried blood spot or plasma): C5 and acylcarnitines full scan
- Qualitative urine organic acids (UOA) analysis
- Dried blood (or liquid blood) for DNA analysis (benign mutation)

*See IVA diagnostic protocol for details

9 Follow-up visits

FURTHER APPOINTMENTS TO BE SCHEDULED AS FOLLOWS:

If well baby

Specialist team to arrange **1st follow-up visit within 5 working days** of diagnostic sampling – for results of C5, UOA and testing for the common mutation 932C>T.

If diagnosis not confirmed – see diagnostic protocol*

If unwell baby

Specialist team to arrange **1st follow-up visit as soon as practicable** of 1st face-to-face review – for available results. Arrange to feedback remaining results by 5 working days of diagnostic sampling.

*See IVA diagnostic protocol for details

Upon confirmation of diagnosis

- 10 Dietary management and when to implement Emergency regimen (ER) must be emphasised (www.bimdg.org.uk/site/guidelines-enbs.asp).
- 11 Post diagnosis discussion should ensure parents have good understanding of the condition, support information, correct contact numbers for IVA team, information for A&E, age appropriate dietary management / ER information.
- 12 A specialist nurse should be available to provide advice and support.
- 13 The specialist dietitian should make contact with the local dietitian if appropriate.
- 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 IVA sibling protocol for details

- 15** Post-confirmation of diagnosis appointments to be scheduled to meet needs of the infant and family.

See [dietary management pathway](#)

Contact with dietitians **16** Parents should be given the opportunity to have on-going access to a specialist dietitian and should be provided with appropriate contact details.

- 17** Discussion between parents and dietitian should cover:
- Age appropriate advice on feeding (including breastfeeding and weaning)
 - ER for illness: including preparation and use of ER feeds
-

Appendix 6: GA1 initial clinical referral guidelines and standards

| Stage of process | No. | Guidelines and standards |
|--|-----|--|
| Defining a positive screening result | 1 | If a sample from a baby is found to have a C5-DC concentration equal to or greater than 0.56µmol/L, repeat test should be performed in duplicate on the original blood spot card. |
| | 2 | If the mean of triplicate results is equal to or greater than 0.70 µmol/L AND the derivatised C5-DC is elevated, this is a presumptive positive screening result. |
| Referral of babies with positive screening result | 3 | <p>The screening laboratory must inform the GA1 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.</p> <p>ON THE SAME DAY AS SCREENING RESULT AVAILABLE</p> <p>The CLS must make contact, both verbally (by telephone) as well as in writing (by fax/email – template) with the GA1 specialist team – as per local protocol.</p> <p>CLS team to arrange appointment for family to be seen on the same or next working day# following receiving a positive screening result</p> <p>#Note: Parents should be offered an appointment with the clinical team on the same or next day after hearing about a positive screening result. Parents should NOT be informed of a positive result if an appointment cannot be given for the same or next day e.g. parents should not be informed on a Friday unless an appointment is for that day or the Saturday. In this case contact should be deferred until after the weekend to the next working day.</p> <p>GA1 specialist team to:</p> <ol style="list-style-type: none"> 1. CONTACT FAMILY 2. Instruct family to go to specialist centre – if not possible go to appropriate local hospital¹ |

3. Inform GP, send GA1 GP letter via fax / email
4. Inform maternity services and health visiting services
5. Commence clinical management
 - i. Consent for DNA testing to be obtained.
 - ii. Take diagnostic samples** and send to specialist centre laboratory (courier)
 - iii. Provide BIMDG emergency guidelines and glucose polymer. Instruct to take to hospital if unwell.
6. Specialist team to liaise with diagnostic laboratory – inform lab to expect samples

A GA1 specialist team should comprise:

- A consultant Inherited Metabolic Disease paediatrician with relevant expertise.
- A paediatric dietitian with metabolic expertise.
- A clinical nurse specialist.

If a disorder / situation other than GA1 is suspected, referral must be made to the GA1 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), depending on local arrangements

**See GA1 diagnostic protocol for confirmatory test

***See GA1 dietetic management pathway for details on [BIMDG website](#)

Communication flows 4 First family contact

ON THE SAME DAY

Contact with the family must be made by the specialist team to inform them of the positive screening result. The family should be provided with information as follows:

- 'GA1 is suspected' leaflet.
- Contact numbers for the GA1 specialist team.

- Details of the time and location of an appointment with the GA1 specialist team identified in 3 above

It is strongly recommended that the family has access to GA1 specialist team by telephone.

5 GP contact

ON THE SAME DAY that the family are informed

The specialist team must contact the GP and:

- Co-ordinate local support
- Fax or email information about GA1 as follows:
 - GA1 GP letter
 - 'GA1 is suspected' leaflet
 - GA1 A&E letter
 - Contact numbers for the GA1 specialist team

Clinical evaluation and confirmatory diagnostic tests

6 FIRST REVIEW: FACE-TO-FACE

Pre-diagnosis management should include:

- Explanation of the condition including introduction to inheritance
- Introduction to dietary management and use of Emergency Regimen (ER) for illness – as per **GA1 dietary guidelines**
- Contact with specialist dietitian
- Clinician to ensure family have received – GA1 specialist team contact details, appropriate dietary and ER guidelines
- Ensure adequate feeding

7 Confirmatory diagnostic samples should be collected at 1st face-to-face review (i.e. within 24 hours of receiving screening results if possible).

Consent for DNA testing to be obtained.

8 When 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:

- Repeat blood glutaryl acylcarnitines: (C5-DC) and

full scan

- Urine organic acid (UOA) analysis
- DNA analysis (full gene sequencing only if biochemistry normal or equivocal).

*See GA1 diagnostic protocol for details

9 Within 5 working days of diagnostic samples

Review and communicate to family diagnostic results available.

10 Follow-up visits

FURTHER APPOINTMENTS TO BE SCHEDULED based on clinical and biochemical findings:

Biochemistry abnormal

Arrange 1st follow-up visit

Biochemistry normal or equivocal

Send dried blood (or liquid blood) for full gene sequencing*

1st follow-up visit within 15 working days of diagnostic sampling (see 8 above) – for results of full genome sequencing.

*See GA1 diagnostic protocol for details

Upon confirmation of diagnosis

- | | |
|-----------|--|
| 11 | Dietary management and when to implement Emergency Regimen (ER) must be emphasised (www.bimdg.org.uk/site/guidelines-enbs.asp). |
| 12 | Post-diagnosis discussion should ensure parents have good understanding of the condition, support information, correct numbers for GA1 team, information for A&E, age appropriate dietary management and ER information. |
| 13 | A specialist nurse should be available to provide advice and support. |

14 The specialist dietitian should make contact with the local dietitian if appropriate.

15 Older siblings to be tested as appropriate – or arrangements made for testing. Family to be made aware of opportunities for early post-natal testing of subsequent siblings.

See GA1 sibling protocol for details

16 Post-confirmation of diagnosis appointments to be scheduled to meet needs of the family. This must include:

- Dietetic follow-up every 2 months up until the age of 12 months
- Post-weaning clinical and dietetic review

Contact with dietitians **17** Parents should be given the opportunity to have on-going access to a specialist dietitian and should be provided with appropriate contact detail.

18 Discussion between parents and the dietitian should cover:

- Age appropriate advice on feeding (including breastfeeding and weaning)
- ER for illness: including preparation and use of ER feeds

Appendix 7: HCU initial clinical referral guidelines and standards

| Stage of process | No. | Guidelines and standards |
|---|-----|--|
| Defining a positive screening result | 1 | If a sample from a baby is found to have a methionine concentration equal to or greater than 45µmol/L, repeat test should be performed in duplicate on the original blood spot card. |
| | 2 | If the mean of triplicate results is equal to or greater than 50µmol/L AND the total homocysteine is more than or equal to 15µmol/L, this is a presumptive positive screening result. |
| | 3 | If insufficient for total homocysteine analysis review methionine screening result. If methionine is equal to or greater than 50 µmol/L and less than 100µmol/L request repeat for insufficient sample (for methionine). If methionine equal to or greater than 100 µmol/L this is a presumptive positive screening result. |

| | | |
|--|---|--|
| Referral of babies with positive screening result | 4 | The screening laboratory must inform the HCU clinical liaison service* (CLS) – as per local protocol – of a positive screening result on the same day as the positive result (in 2 and 3 above) has been reported by the laboratory. |
|--|---|--|

The CLS must make contact, both verbally (by telephone) as well as in writing (by fax/email – [template](#) available) with the HCU specialist team – as per local protocol.

HCU specialist team to:

1. Contact family and arrange appointment next working day#
2. Provide family with 'HCU is suspected leaflet' either via web link or in person
3. Inform GP, send HCU GP letter via fax/email
4. Inform maternity services and health visiting services

A HCU specialist team should comprise:

- A consultant Inherited Metabolic Disease paediatrician with relevant expertise
- A paediatric dietitian with metabolic expertise
- A clinical nurse specialist

If a disorder / situation other than HCU is suspected, referral must be made to the HCU 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, depending on local arrangements

#Note: Parents should be offered an appointment with the clinical team on the same or next day after hearing about a positive screening result. Parents should NOT be informed of a positive result if an appointment cannot be given for the same or next day e.g. parents should not be informed on a Friday unless an appointment is for that day or the Saturday. In this case contact should be deferred until after the weekend to the next working day.

Communication flows

5 First family contact

Contact with the family must be made by the specialist team, to inform them of the positive screening result. The family should be provided with information as follows:

- 'HCU is suspected' leaflet
- Contact numbers for the HCU specialist team
- Details of the time and location of an appointment with the HCU specialist team identified in 4 above

It is strongly recommended that the family has access to HCU specialist team by telephone.

6 GP contact

ON THE SAME DAY

The specialist team must contact the GP and:

- Co-ordinate local support
- Fax or email information about HCU as follows:
 - HCU GP letter
 - 'HCU is suspected' leaflet
 - Contact numbers for the HCU specialist team
 - Details of the time and location of an appointment with the HCU specialist team identified in 4 above

Clinical evaluation and confirmatory diagnostic tests

7 FIRST REVIEW: FACE-TO-FACE

SAME DAY OR NEXT WORKING DAY

Pre-diagnosis management should include:

- Explanation of the condition including introduction to inheritance
- Clinician to ensure family have received – HCU specialist team contact details, appropriate dietary guidelines

- 8 Confirmatory diagnostic samples should be collected at 1st face-to-face review (i.e. same day or next working day).
- 9 When 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:
 - Plasma amino acids and total homocysteine
 - Liver function test
 - Folate
 - Vitamin B₁₂

*See HCU diagnostic protocol for details

Give supply of pyridoxine 50mg bd & folic acid (5mg/day) to be started if diagnostic tests confirm positive for HCU.

- 10 Telephone the family with the diagnostic results when available (within 5 working days of diagnostic sampling).

If HCU is confirmed, repeat plasma total homocysteine (local hospital or specialist centre) and then start pyridoxine 100mg per day & folic acid 5mg/day. Review patient in metabolic clinic, ideally after approximately one week and after two weeks (see 11 below).

11 **Follow-up visits if homocystinuria is confirmed**

Review the patient and repeat plasma amino acids and total homocysteine measurements. Ideally, this should be done approximately 1 week and 2 weeks after starting pyridoxine.

A fall in homocysteine <20% or <20µmol/L is unlikely to be significant. If there is no response, stop pyridoxine, continue folate & start a methionine-restricted diet (unless the baseline total homocysteine was <100µmol/L).

If the plasma total homocysteine falls to $<100\mu\text{mol/L}$, continue pyridoxine and folic acid. The pyridoxine dose should be adjusted to the minimum that controls the homocysteine (ideally $<10\text{mg/kg/d}$).

If the total homocysteine falls by $>20\%$ but remains $>100\mu\text{mol/L}$, consider monitoring the total homocysteine for longer, as it may take up to 6 weeks to see the full response. If the total homocysteine still remains $>100\mu\text{mol/L}$, reduce the pyridoxine dose to 10mg/kg/d & start a methionine-restricted diet.

See HCU diagnostic protocol for details

- 12 Dietary management as per [dietary guidelines](#)
- 13 Post-diagnosis discussion should ensure parents have good understanding of the condition, support information and the correct telephone numbers for the HCU team.
- 14 A specialist nurse should be available to provide advice and support.
- 15 The specialist dietitian should make contact with the local dietitian if appropriate.
- 16 Older siblings to be tested as appropriate – or arrangements made for testing. Family to be made aware of opportunities for early post-natal testing of subsequent siblings.

See HCU sibling protocol for details

Contact with dietitians

- 17 The parents of children on dietary treatment should have on-going access to a specialist dietitian.
- 18 Discussion between parents and the dietitian should cover:
 - Age appropriate advice on feeding (including breastfeeding and weaning)
- 19 Post-confirmation of diagnosis appointments to be scheduled to meet needs of the family. For patients on dietary management, this should include:
 - Dietetic review approximately one week after starting dietary management, followed by regular telephone reviews

- Dietetic review in clinic approximately every 2 months between the age of 4 to 12 months
- Post-weaning clinical and dietetic review

See [dietary management pathway](#)

Appendix 8: Status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|---|----------|---|--|
| 01 | Specimen received in laboratory | N/A | <p>Same value applies to all screening tests (ie relates to the blood spot card)</p> <p>Additional data items to be provided with this status code and entered into Child Health systems. electronically or by manual means:</p> <ul style="list-style-type: none"> • Date sample taken • Date sample received in laboratory • Laboratory identifier | |
| 02 | (Condition screened for) declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | (Condition screened for) repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |

| | | |
|------|---|---|
| 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/contamination, discoloured spots) |
| 0306 | Unsuitable sample: day 0 and day 5 on same card | |
| 0307 | Unsuitable sample for CF: possible faecal contamination | |
| 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| 0310 | Unsuitable sample: Date of birth not accurately matched | |
| 0311 | Unsuitable sample: Expired card used | |
| 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| 0313 | Unsuitable sample: Damaged in transit | |
| 0314 | Sickle - Too premature for testing | |
| 0315 | CHT - Pre-term | |
| 0316 | CHT - Borderline result | |
| 0317 | CF - Inconclusive | |

| | | | |
|-----------|--|------|---|
| 04 | (Condition screened for) not suspected | N/A | |
| 05 | (Condition screened for) carrier | N/A | |
| 06 | Sickle Cell Disease not suspected, carrier of other haemoglobin | 0601 | Result consistent with haemoglobin C carrier |
| | | 0602 | Result consistent with haemoglobin D carrier |
| | | 0603 | Result consistent with haemoglobin E carrier |
| | | 0604 | Result consistent with haemoglobin O-Arab carrier |
| 07 | (Condition screened for) not suspected, other disorders follow up | N/A | |
| 08 | (Condition screened for) suspected | N/A | |
| 09 | (Condition screened for) not screened/screening incomplete | 0901 | CF: > 8 weeks, too old for screening |
| | | 0902 | All screens: >1 year, too old for screening |
| | | 0903 | Moved out of area |
| | | 0904 | Not contactable, reasonable efforts made |
| | | 0905 | Baby died |
| | | 0906 | Not required, previous valid result |
| 10 | Haemoglobin S not suspected (by DNA) No other haemoglobin /thalassemia excluded | N/A | Applies to sickle cell disease screening only |

Appendix 9: PKU status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|--|----------|--|--|
| 01 | Specimen received in laboratory | N/A | | |
| 02 | PKU declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | PKU repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |
| | | 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| | | 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/ contamination, discoloured spots) |
| | | 0306 | Unsuitable sample: day 0 and day 5 on same card | |

| | | | | |
|-----------|--|------|---|--|
| | | 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| | | 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| | | 0310 | Unsuitable sample: Date of birth not accurately matched | |
| | | 0311 | Unsuitable sample: Expired card used | |
| | | 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| | | 0313 | Unsuitable sample: Damaged in transit | |
| 04 | PKU not suspected | N/A | | <p>According to the following criteria:</p> <p>1. [Phe] <200 µmol/L (singlicate analysis)</p> <p>OR</p> <p>2. [Phe] <240 µmol/L (repeat analysis, mean of triplicate)</p> <p>AND</p> <p>[Tyr] <240 µmol/L (mean of duplicate)</p> |
| 05 | Not applicable to PKU | | | |
| 06 | Not applicable to PKU | | | |
| 07 | PKU not suspected, other disorders follow up | N/A | | <p>According to the following criteria:</p> <p>[Phe] ≥200 µmol/L (singlicate analysis)</p> <p>AND</p> <p>1. [Phe] <240 µmol/L</p> |

| | | | | |
|-----------|---|------|---|--|
| | | | | (repeat analysis, mean of triplicate) |
| | | | | AND |
| | | | | [Tyr] ≥ 240 $\mu\text{mol/L}$ (mean of duplicate) |
| | | | | OR |
| | | | | 2. [Phe] ≥ 240 $\mu\text{mol/L}$ (repeat analysis, mean of triplicate) |
| | | | | AND |
| | | | | [Tyr] ≥ 240 $\mu\text{mol/L}$ (mean of duplicate) |
| 08 | PKU suspected | N/A | | According to the following criteria: |
| | | | | 1. [Phe] ≥ 200 $\mu\text{mol/L}$ (singlicate analysis) |
| | | | | AND |
| | | | | 2. [Phe] ≥ 240 $\mu\text{mol/L}$ (repeat analysis, Mean of triplicate) |
| | | | | AND |
| | | | | [Tyr] < 240 $\mu\text{mol/L}$ (mean of duplicate) |
| 09 | PKU not screened/ screening incomplete | 0902 | All screens: >1 year, too old for screening | |
| | | 0903 | Moved out of area | |
| | | 0904 | Not contactable, reasonable efforts made | |
| | | 0905 | Baby died | |
| | | 0906 | Not required, previous valid result | |

10 Not applicable to
PKU

Appendix 10: MCADD status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|--|----------|--|---|
| 01 | Specimen received in laboratory | N/A | | |
| 02 | MCADD declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | MCADD repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |
| | | 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| | | 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/contamination, discoloured spots) |
| | | 0306 | Unsuitable sample: day 0 | |

| | | | | |
|-----------|---------------------|------|---|---|
| | | | and day 5 on same card | |
| | | 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| | | 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| | | 0310 | Unsuitable sample: Date of birth not accurately matched | |
| | | 0311 | Unsuitable sample: Expired card used | |
| | | 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| | | 0313 | Unsuitable sample: Damaged in transit | |
| 04 | MCADD not suspected | N/A | | <p>1. C8 <0.4 µmol/L (singlicate analysis)</p> <p>OR</p> <p>2. C8 <0.5 µmol/L (re-test in duplicate, mean of triplicate)</p> <p>OR</p> <p>3. C8 ≥0.5µmol/L (re-test in duplicate, mean of triplicate)</p> <p>AND</p> <p>C8:C10 ratio <1.0 (mean from triplicate results)</p> |

| | | | |
|----|--|---|---|
| 05 | Not applicable to MCADD | | |
| 06 | Not applicable to MCADD | | |
| 07 | Not applicable to MCADD | | |
| 08 | MCADD suspected | N/A | <p>According to the following criteria:</p> <ol style="list-style-type: none"> 1. C8 \geq 0.4 μmol/L (singlicate analysis) <p>AND</p> <ol style="list-style-type: none"> 2. C8 \geq 0.5 μmol/L (re-test in duplicate, mean of triplicate) <p>AND</p> <p>C8:C10 ratio \geq 1.0 (mean from triplicate results)</p> |
| 09 | MCADD not screened/ screening incomplete | <p>0902 All screens: >1 year, too old for screening</p> <p>0903 Moved out of area</p> <p>0904 Not contactable, reasonable efforts made</p> <p>0905 Baby died</p> <p>0906 Not required, previous valid result</p> | |
| 10 | Not applicable to MCADD | | |

Appendix 11: MSUD status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|--|----------|--|--|
| 01 | Specimen received in laboratory | N/A | | |
| 02 | MSUD declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | MSUD repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |
| | | 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| | | 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/ contamination, discoloured spots) |
| | | 0306 | Unsuitable sample: day 0 and day 5 on same card | |

| | | | | |
|-----------|------------------------|------|---|---|
| | | 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| | | 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| | | 0310 | Unsuitable sample: Date of birth not accurately matched | |
| | | 0311 | Unsuitable sample: Expired card used | |
| | | 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| | | 0313 | Unsuitable sample: Damaged in transit | |
| 04 | MSUD not suspected | N/A | | <p>1. 'Leucines' <500 $\mu\text{mol/L}$ (singlicate analysis)</p> <p>OR</p> <p>2. 'Leucines' <600 $\mu\text{mol/L}$ (re-test in duplicate, mean of triplicate)</p> |
| 05 | Not applicable to MSUD | | | |
| 06 | Not applicable to MSUD | | | |
| 07 | Not applicable to MSUD | | | |
| 08 | MSUD suspected | N/A | | <p>1. Leucines ≥ 500 $\mu\text{mol/L}$ (singlicate analysis)</p> <p>AND</p> <p>2. Leucines ≥ 600 $\mu\text{mol/L}$ (re-test in duplicate, mean of triplicate)</p> |

| | | | |
|-----------|--|------|---|
| 09 | MSUD not screened/ screening incomplete | 0902 | All screens: >1 year, too old for screening |
| | | 0903 | Moved out of area |
| | | 0904 | Not contactable, reasonable efforts made |
| | | 0905 | Baby died |
| | | 0906 | Not required, previous valid result |
| 10 | Not applicable to MSUD | | |

Appendix 12: IVA status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|--|----------|--|--|
| 01 | Specimen received in laboratory | N/A | | |
| 02 | IVA declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | IVA repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |
| | | 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| | | 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/ contamination, discoloured spots) |

| | | | | |
|-----------|-----------------------|------|---|--|
| | | 0306 | Unsuitable sample: day 0 and day 5 on same card | |
| | | 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| | | 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| | | 0310 | Unsuitable sample: Date of birth not accurately matched | |
| | | 0311 | Unsuitable sample: Expired card used | |
| | | 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| | | 0313 | Unsuitable sample: Damaged in transit | |
| 04 | IVA not suspected | N/A | | <p>According to the following criteria:</p> <ol style="list-style-type: none"> 1. C5 <1.6 µmol/L (singlicate analysis) <p>OR</p> <ol style="list-style-type: none"> 2. C5 <2.0 µmol/L (re-test in duplicate, mean of triplicate) |
| 05 | Not applicable to IVA | | | |
| 06 | Not applicable to IVA | | | |
| 07 | Not applicable to IVA | | | |
| 08 | IVA suspected | N/A | | <p>According to the following criteria:</p> <ol style="list-style-type: none"> 1. C5 ≥1.6 µmol/L (singlicate analysis) |

AND

2. C5 ≥ 2.0 $\mu\text{mol/L}$
(re-test in duplicate, mean of triplicate)

| | | | |
|-----------|--|------|--|
| 09 | IVA not screened/ screening incomplete | 0902 | All screens: >1 year, too old for screening |
| | | 0903 | Moved out of area |
| | | 0904 | Not contactable, reasonable efforts made |
| | | 0905 | Baby died |
| | | 0906 | Not required, previous valid result |
| 10 | Not applicable to IVA | | |

Appendix 13: GA1 status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|--|----------|--|--|
| 01 | Specimen received in laboratory | N/A | | |
| 02 | GA1 declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | GA1 repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |
| | | 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| | | 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/ contamination, discoloured spots) |
| | | 0306 | Unsuitable sample: day 0 and day 5 on same card | |

| | | | | |
|-----------|-----------------------|------|---|--|
| | | 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| | | 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| | | 0310 | Unsuitable sample: Date of birth not accurately matched | |
| | | 0311 | Unsuitable sample: Expired card used | |
| | | 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| | | 0313 | Unsuitable sample: Damaged in transit | |
| 04 | GA1 not suspected | N/A | | <p>1. C5-DC <0.56 $\mu\text{mol/L}$ (singlicate analysis)</p> <p>OR</p> <p>2. C5-DC <0.70 $\mu\text{mol/L}$ (re-test in duplicate, mean of triplicate)</p> |
| 05 | Not applicable to GA1 | | | |
| 06 | Not applicable to GA1 | | | |
| 07 | Not applicable to GA1 | | | |
| 08 | GA1 suspected | N/A | | <p>According to the following criteria:</p> <p>1. C5-DC \geq0.56 $\mu\text{mol/L}$ (singlicate analysis)</p> <p>AND</p> <p>2. C5-DC \geq0.70</p> |

µmol/L (re-test in duplicate, mean of triplicate)

| | | | |
|-----------|--|------|--|
| 09 | GA1 not screened/ screening incomplete | 0902 | All screens: >1 year, too old for screening |
| | | 0903 | Moved out of area |
| | | 0904 | Not contactable, reasonable efforts made |
| | | 0905 | Baby died |
| | | 0906 | Not required, previous valid result |
| 10 | Not applicable to GA1 | | |

Appendix 14: HCU status codes and sub codes

| Status code | Suggested term used in child health system | Sub code | Description | Comment |
|-------------|--|----------|--|--|
| 01 | Specimen received in laboratory | N/A | | |
| 02 | HCU declined | 0201 | Declined, no history of being screened | |
| | | 0202 | Declined, screened in UK (as reported by parents) with no evidence of result | |
| | | 0203 | Declined, screened outside UK with evidence of result | |
| | | 0204 | Declined, screened outside UK with no evidence of result | |
| 03 | HCU repeat/further sample required | 0301 | Too young for reliable screening | |
| | | 0302 | Too soon after transfusion (<72 hours) | |
| | | 0303 | Insufficient sample | (Includes not enough blood, not soaked through, small area re Card scan users) |
| | | 0304 | Unsuitable sample (blood quality): incorrect blood application | (Incorrect blood application technique – includes multi-spotted, spotted both sides) |
| | | 0305 | Unsuitable sample (blood quality): compressed/damaged | (Includes compressed, evidence incomplete drying, stained glassine, scratched/abraded/ridged, liquid/water damage/ contamination, discoloured spots) |
| | | 0306 | Unsuitable sample: day 0 and day 5 on same card | |

| | | | | |
|-----------|-----------------------|------|---|--|
| | | 0308 | Unsuitable sample: NHS number missing/not accurately recorded | |
| | | 0309 | Unsuitable sample: Date of sample missing/not accurately recorded | |
| | | 0310 | Unsuitable sample: Date of birth not accurately matched | |
| | | 0311 | Unsuitable sample: Expired card used | |
| | | 0312 | Unsuitable sample: >14 days in transit, too old for analysis | |
| | | 0313 | Unsuitable sample: Damaged in transit | |
| 04 | HCU not suspected | N/A | | <p>1. Methionine <45 $\mu\text{mol/L}$ (singlicate analysis)</p> <p>OR</p> <p>2. Methionine <50 $\mu\text{mol/L}$ (re-test in duplicate, mean of triplicate)</p> <p>OR</p> <p>3. Methionine \geq50 $\mu\text{mol/L}$ (re-test in duplicate, mean of triplicate)</p> <p>AND</p> <p>THcy <15 $\mu\text{mol/L}$</p> |
| 05 | Not applicable to HCU | | | |
| 06 | Not applicable to HCU | | | |
| 07 | Not applicable to HCU | | | |

| | | | |
|-----------|--|------|---|
| 08 | HCU suspected | N/A | According to the following criteria: |
| | | | 1. Methionine ≥ 45 $\mu\text{mol/L}$ (singlicate analysis) |
| | | | AND |
| | | | 2. Methionine ≥ 50 $\mu\text{mol/L}$ (re-test in duplicate, mean of three results) |
| | | | AND |
| | | | THcy ≥ 15 $\mu\text{mol/L}$ |
| 09 | HCU not screened/ screening incomplete | 0902 | All screens: >1 year, too old for screening |
| | | 0903 | Moved out of area |
| | | 0904 | Not contactable, reasonable efforts made |
| | | 0905 | Baby died |
| | | 0906 | Not required, previous valid result |
| 10 | Not applicable to HCU | | |

Appendix 15: Newborn screening laboratory standards

| Newborn screening laboratory standards | | ISO 15189:2012 Clause |
|---|---|--------------------------------------|
| NBS1 | Laboratories undertaking newborn blood spot screening shall be accredited by the United Kingdom Accreditation Service (UKAS). This shall include the NBS specialist assessment. DNA laboratories shall be a member of the UK Genetic Testing Network (UK GTN) and comply with the quality criteria laid down by the UK GTN Steering Group. | 4.4 |
| NBS2 | Newborn blood spot screening shall be provided within the organisational structure of the newborn blood spot screening programme and undertaken by specialist newborn screening laboratories already providing screening programmes. | N/A |
| NBS3 | There shall be documented local policies and standard operating procedures describing the whole screening process including pre-analytical, analytical and post-analytical processes. Where appropriate these shall include reporting results, referral and follow-up arrangements for presumptive positive cases and carriers, as specified in laboratory handbooks. | 4.2.1 4.2.2.1 |
| NBS4 | Newborn blood spot screening tests shall be performed by a recommended method as defined in the screening programme laboratory handbook, capable of performing to the required sensitivity/specificity. | 4.4 5.5.1.2 5.5.1.3 |
| NBS5 | Processes shall be provided in line with relevant national standards and guidance and screening specifications. | 4.4 |
| NBS6 | Processes shall be reviewed periodically taking into account audit data, accumulating results, technical developments and local changes in healthcare provision. | 4.14.1.a |
| NBS7 | There shall be written and agreed procedures describing the working arrangements between the screening laboratory and any referral laboratory that is used. | 4.5 |
| NBS8 | There must be a senior member of the laboratory staff at medical consultant or consultant clinical scientist level, evidenced by FRCPATH or equivalent responsible for the | 4.1.1.4 5.1 |

| | | |
|-------|---|--------------------|
| | newborn blood spot screening with defined lines of accountability for all laboratory aspects of the service. | 4.1.2.1 4.1.2.5 |
| NBS9 | Laboratories undertaking newborn blood spot screening shall undertake internal quality control procedures for the screening test and demonstrate satisfactory performance in an approved external quality assurance scheme. | 5.6.2 5.6.3 |
| NBS10 | There shall be a documented risk management policy for the laboratory aspects of the newborn screening programme. This should describe the steps in the testing protocol where failures could occur and the procedures that have been implemented to minimise the risk of their occurrence. | 4.14.6 (4.12) |
| NBS11 | Screening incidents shall be managed in accordance with the guidance on Managing Safety Incidents in NHS Screening Programmes. | 4.4 4.9 |
| NBS12 | Laboratories shall participate in audit at local, regional and national level, to assess the effectiveness of the national screening programme. | 4.14 |
| NBS13 | The laboratory must release reports on screening performance, including external quality assurance and UKAS assessments to any agency with a legitimate interest in the quality and safety of the programme on behalf of the public. | 4.4 |
| NBS14 | Laboratories should publish the results and performance of their newborn blood spot screening programme within an annual report. | 4.4 |
| NBS15 | Screening laboratories shall use the newborn screening results status codes and sub codes for acknowledging the receipt of specimens in the laboratory and when reporting results to the child health records departments. | 4.4 5.4.6 |
| NBS16 | Clinical information should be requested from clinical referral centres on each presumptive positive case. Data on each case notified should be collated and anonymised before submission to the NHS Newborn Blood Spot Screening Programme. Cases presenting clinically should also be anonymised and reported to the NHS Newborn Blood Spot Screening Programme | |