Newborn Bloodspot Sampling Guidelines

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Introduction

The aim of the Newborn Blood Spot Screening Programme is to identify specific conditions, as soon after birth as possible and before the onset of recognisable clinical symptoms. By detecting these conditions early it is possible to treat and reduce their severity. The current policy in Scotland is that all newborn babies are offered screening for phenylketonuria (PKU), congenital hypothyroidism (CHT), sickle cell disease (SCD), cystic fibrosis (CF) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD). Additionally all babies moving into Scotland from elsewhere who have not been screened/have a verifiable screening result should also be offered screening up to 6 months of age. These guidelines aim to:

- Provide a consistent and clear approach to offering newborn blood spot screening and taking the sample
- Provide a framework for Health Professionals to support parents in making informed choice about newborn bloodspot screening for their baby
- Support Health professionals in obtaining good quality samples to prevent the need for avoidable repeats

Why is the quality of the bloodspot so important?

Good quality blood spot samples are vital to ensure that babies with rare but serious conditions are identified and treated early. Poor quality samples can cause inaccurate newborn screening results and therefore these samples cannot be accepted by the laboratory. The most significant effects of poor quality samples are:

1) Falsely low analyte concentrations (false-negative results), which can be caused by:
   - Small volume spots (i.e. under-filled circles)
   - Compression of the sample

2) Falsely high analyte concentrations (false-positive results), which can be caused by:
   - Layering the blood
   - Applying the blood to the front and the back of the card

Poor quality blood spots could therefore lead to false-negative and false-positive screening results – this means that babies with a condition might be missed or referred for further tests unnecessarily. If poor quality blood spots are received, the newborn screening laboratory will have to request a repeat sample. Avoidable repeat samples can cause anxiety for parents, distress to babies and delays in the screening process (for example a baby might miss CF screening because it can only be screened for accurately up to eight weeks of age). They are also a waste of healthcare resources. In some cases, parents may refuse to consent to a repeat – this means that the baby will have incomplete screening.

The Scottish Newborn Screening Laboratory (SNSL) have adopted the UK, evidence based consensus on blood spot quality with standardised acceptance and rejection criteria. To ensure that an avoidable repeat sample is not requested, sample takers are advised to obtain four good quality blood spots and complete all the fields on the blood spot card accurately. If you are using one of the older bloodspot cards (always check the expiry date on the card before use) if possible fill the fifth spot but it is more important to have four good quality spots than five inadequate ones. Good quality blood spots are those where the circle is filled and evenly saturated by a single drop of blood that soaks through to the back of the blood spot card.
1. **Offer of screening**

It is important that parents can make an informed choice about screening for their baby and are prepared for the blood sampling procedure. Ideally parents should be advised of the screening options offered for their baby and provided with a copy of the national screening leaflet to support them to make an informed decision at least 48 hours prior to taking the blood sample. The information leaflet will provide information on the conditions screened for, how the bloodspot will be taken and how the results will be received. Where applicable provide the leaflet in the appropriate language, some translated versions are available for download from Health Scotland. If the required language is not available, alternative arrangements should be made with local interpreter services.

It is the responsibility of the NHS Board through the Screening Coordinator and the Director of Public Health, to ensure that robust systems are in place locally to confirm that every newborn baby or child up to the age of 6 months, resident in their NHS Board, is invited to participate in screening. Midwives working in hospital or in the community are responsible for ensuring that testing is offered and national protocols are followed which includes, in Scotland, the legal requirement for written consent. If the baby is still in hospital on the fifth day of life, it is the responsibility of the ward, Neonatal Intensive Care Unit (NICU), Special Care Baby Unit (SCBU), or the Surgical Unit staff to obtain the blood spot sample.

2. **Parental consent and refusal**

Parents should have a pre-test discussion with the healthcare professional taking care of them and their baby regarding the newborn blood spot test. Discussions should include the purpose, processes and benefits of the test. Parents will be asked to complete a consent form stating that they have received sufficient information to understand the reasons for testing, the significance of the results and the possible consequences of not having tests performed.

The form should be completed accepting or declining each individual test, storage of the card and the use of samples for anonymised research. This information should also be recorded on the bloodspot card in the comments section. The form should be countersigned by the healthcare professional taking the blood sample and filed in the maternity records. The midwife will also ascertain family contact details for the first month and establish the preferred method of communication, should it be necessary to repeat testing.

Occasionally parents decline testing of some or all of the conditions. In these circumstances the midwife must complete the blood spot card, in the usual manner, stating that the parents have declined the offer of screening. Parents are given the option to take up screening at a later date and are provided with a copy of the information sheet “**When the blood spot screening test has not been undertaken**”. The information sheet lists the possible signs and symptoms of the conditions that are screened for. This information is also given to the family’s GP, so that the GP can also look out for these signs and symptoms.

3. **The newborn bloodspot card**

It is vital that every section of the blood spot card is completed accurately. The information supplied is entered onto the Scottish Newborn Screening Laboratory’s (SNSL) Laboratory Information Management System (LIMS) and is used for:

- the identification of the infant – to ensure that the correct result is issued to the correct baby
the determination of results – certain parameters have to be fulfilled before results are valid and issued
ensuring the correct protocol is followed – different protocols are applied in particular tests

4. Completing the bloodspot card

Check the expiry date before completing the blood spot card. Expired cards should not be used, they should be returned to the SNSL.

The middle section of the front of the card contains demographic details

It is best practice to use labels for this section if available. Always ensure that it is the baby’s label and not mother’s.

<table>
<thead>
<tr>
<th>CHI number</th>
<th>The use of the CHI number is a mandatory requirement and should always be noted in full. Ensure the baby’s CHI number is supplied and not the mothers.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother’s or Baby’s Alternative Surname</strong></td>
<td>If more than one name is known please document both</td>
</tr>
<tr>
<td><strong>Address</strong></td>
<td>This is the home address of the baby at the time of birth</td>
</tr>
<tr>
<td><strong>Post code</strong></td>
<td>This should be in full</td>
</tr>
<tr>
<td><strong>Parent contact telephone number</strong></td>
<td>This is important as immediate contact with parents may be required</td>
</tr>
<tr>
<td><strong>Hospital of birth</strong></td>
<td>Name of hospital or if born before arrival then the hospital that the baby is delivered in. Home should be stated if not associated with a hospital</td>
</tr>
<tr>
<td><strong>GP</strong></td>
<td>General Practitioner with whom the baby will be registered</td>
</tr>
<tr>
<td><strong>GP address</strong></td>
<td>Name of surgery and address</td>
</tr>
<tr>
<td><strong>Baby's DOB</strong></td>
<td>This must be accurate as the interpretation of results for some tests is dependent on the age of the child. If the sample is taken too early, i.e. before 4 days of age, this can invalidate the results</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Tick box; M-Male  F-Female  U-Unknown or Uncertain</td>
</tr>
<tr>
<td><strong>Rank</strong></td>
<td>This is important to indicate whether baby is a twin etc as shown on the back of the card eg 1/1, 1/2. This will avoid the specimen being treated as a duplicate specimen of another baby</td>
</tr>
<tr>
<td><strong>Gestation</strong></td>
<td>This is the gestation that the baby was born at and is important for some of the tests. Mark in weeks and days</td>
</tr>
<tr>
<td><strong>Baby's Ancestry</strong></td>
<td>Choose from one of the family origin codes, as detailed on the back of the card</td>
</tr>
<tr>
<td><strong>Mother born in the UK</strong></td>
<td>This is used in relation to some epidemiological studies</td>
</tr>
<tr>
<td><strong>Mothers DOB</strong></td>
<td>Used as another link to the baby if details differ from those held by Child Health Information System</td>
</tr>
</tbody>
</table>

The right hand side of the front of the card is information used by the laboratory:

| **Type of feed** | If more than one type applies tick both i.e. breast and bottle. ‘Other’ is for total parenteral nutrition (TPN) in cases of sick babies. |
| **Current Hospital and ward if applicable** | If the baby is still in hospital please supply the hospital and ward, this will allow child health to locate the baby if a repeat sample is needed. |
| **Has baby had a transfusion** | Date of transfusion must be included. Please also indicate if the transfusion was a blood transfusion or other product such as platelets or FFP. The interpretation of results will differ depending on whether blood or other products were given. |
| **Comments** | **This box should be used to record any condition(s) where screening has been declined. This box should also be used to record ‘No Storage’ or ‘No Research’ if parents have requested this.** This space should also be used to record any relevant antenatal screening results, parent(s) known carriers of haemoglobin variants/CF, Meconium ileus, any family history relevent to the conditions screened for and reason for sample if not taken on day 5-8 (e.g. pre transfusion, preterm CHT) |
| **Date Specimen Taken** | **This is essential.** The SNSL uses this to determine age of baby when sample was taken. Cards without this date will not be issued with a result until this has been established. |
The left hand side of the front of the card is for the specimen collection

- Baby's Surname and DOB must also be completed on this portion of the card as it shall be separated from the rest of the card and this is required for identification purposes in addition to the barcode included on both sections of the card.

The reverse side of the card describes the following:

- Family origins – This information is important for SCD testing
- Rank – This gives birth order in multiple pregnancies
- Blood Collection – Brief instructions

5. Timing of the sample
The blood spot sample should ideally be taken as soon as possible after the baby reaches 96 hours old. Babies who are premature, unwell, or have had blood transfusions should all have the newborn blood sample taken as usual and this information must be recorded on the blood spot card.

6. Preparation for taking the bloodspot sample
In order to take the newborn blood spot sample, the following is required:
- Blood spot card (check expiry date), glassine envelope and prepaid/addressed envelope
- Non-sterile protective gloves
- Age appropriate automated incision device designed for use on newborns
- Cotton wool/gauze
- Spot plaster (if required)
- Sharps box
- Maternity/baby record
- Water for cleansing

7. **Collecting the bloodspot sample**
Recommend comfort measures for the baby. Ensure the baby is cuddled and in a secure position for taking the sample, swaddling the baby may reduce pain/discomfort. Engaging the baby through face to face contact, voice and touch may be beneficial. Suggest that the baby is breast fed during the heel prick as an analgesic (or expressed breast milk). Whilst there is no evidence that formula feed has analgesic properties, parents may comfort formula-fed babies with a feed. Alternatively a pacifier may provide comfort.

- Explain the procedure to parents
- Complete all boxes on the card including the baby’s surname and date of birth on the blood spot sample section to the left of the card. When completing the card care must be taken to place the card on a clean surface to avoid contaminating the sample.
- Ensure the baby is comfortable and in a secure position for taking the sample
- Clean the heel by washing thoroughly with tepid, plain water. The water should not be heated and the baby’s foot should not be immersed. If faecal matter cannot be removed from the foot with water, use a mild, unperfumed soap to clean away the faecal matter and then rinse the foot thoroughly. This is because faecal matter contains very high concentrations of immunoreactive trypsinogen (IRT). IRT is measured during screening for CF therefore faecal contamination may lead to a false positive result. Do not use alcohol or alcohol wipes.
- Wash hands and apply gloves
- The heel should be warm (but in most cases additional prewarming of the foot is not required) and completely dry. Soft paraffin solutions should not be used as they can alter the result of the screen and can clog the equipment used.
- Obtain the sample using an automated incision device designed for use on newborns. An arch-shaped incision device is recommended.
- Skin puncture must be no deeper than 2.0 mm. The external and internal limits of the calcaneum are the preferred puncture sites (diagram A).
- The whole heel plantar surface may be used in term and pre-term infants who have repeated heel punctures to a penetrative depth of no more than 1.00mm (diagram B). Avoid the posterior curvature of the heel. Allow the heel to hang down to assist blood flow. This reduces the soft tissue damage and pan from repeated heel puncture in the same area.
- Before activation place the automated incision device against the heel in accordance with manufacturer's instruction. This is to ensure the correct depth of incision is achieved – not too deep to cause harm to the baby, and not too shallow to prevent adequate blood flow.

Diagram A
For full and preterm infants

Diagram B
For infants who have had repeated heel punctures

Adapted from Jain and Rutter
The aim is to fill each 4 circles on the blood spot card using a single drop of blood (if using the old cards and there is sufficient blood to fill the fifth then please complete the fifth spot but it is more important to get 4 good quality spots). Wait for the blood to flow and a hanging drop to form. Allow one spot of blood to drop onto each of the circles on the blood spot card. Do not allow the heel to make contact with the card. Do not squeeze the foot in an attempt to increase blood flow. The first drop of blood should be used. Allow the blood to fill the circle by natural flow, and seep through from front to back of the blood spot card. Fill each of the circles completely. Always ensure the sample is taken from the front of the card and not from the back. Spots that exceed the dotted lines on the filter paper are acceptable provided that a single drop of blood has been used. Do not compress or apply pressure to the blood spots (for example when sealing the postage envelope) as this reduces the density of blood on the sample and there is a significant risk that this could lead to a “suspected” result being missed.

If the blood flow ceases the congealed blood should be wiped away firmly with cotton wool or gauze, to disturb the clot and encourage blood flow. Gently massage the foot, avoid squeezing, and drop the blood onto the blood spot card. If the baby is not bleeding a second puncture is necessary. The second puncture should be performed on a different part of the foot, as marked by the shaded areas in diagrams A and B. When the sample collection is complete, wipe excess blood from the heel and apply gentle pressure to the wound with cotton wool or gauze. Apply a hypoallergenic spot plaster if required and remind the parent to remove the plaster in a few hours.

Ethylenediaminetetraacetic Acid (EDTA) is an anticoagulant used in some blood collection tubes. EDTA can interfere with the interpretation of testing and could lead to false negative results. Blood from EDTA tubes, citrate tubes or capillaries should never be used to fill blood spots as this will affect the measurement of Thyroid-Stimulating Hormone (TSH) for CHT and Immunoreactive Trypsinogen (IRT) for CF leading to false negative results.

8. Good quality samples

Initially the lab needs to take four 3.2mm ‘punches’ from the sample that is provided. If the result from one of the punches is above the cut-off for one of the conditions tested for, then the test has to be repeated to confirm the original result. To do this a two further punches for each test which gave a high result are taken. This would mean that another two to eight punches may be required. Additionally, sometimes a further sample for mutation analysis (e.g. as in the protocol for cystic fibrosis screening), will require a further two punches. This means that up to 14 punches may be required. This is another reason good quality bloodspots are required.

This is a very good quality sample: i.e. there is plenty of blood and it is soaked all the way through.
9. Insufficient samples

Research has shown that the levels of analytes tested for by newborn screening can be compromised when a small sample size is used. As a result new criteria have been agreed nationally in relation to the minimum requirements from the samples provided to ensure the screen result is robust. The minimum sample size the SNSL can accept is 8mm in diameter. This means that cards such as the following are insufficient and would be rejected resulting in an “avoidable repeat”.

Samples will also be rejected as insufficient if there is not enough blood to perform all the tests required or if the blood has not soaked all the way through to the back of the card (i.e. white paper can be seen where there should be blood) then there is a risk of a false negative result as the concentration of the sample will be diluted and an affected baby could be missed. This also applies to a multi spotted sample (e.g. several small spots of blood). The following samples are insufficient, in the first the blood has not soaked through to the back of the card properly and what has come through is patchy with white paper visible on the punched spots. In the second card no blood has soaked through to the back at all.
The sample also cannot be accepted if it is a layered sample. This is where one spot of blood is layered directly on top of another or the blood has been applied to one side of the card and then turned over and re-applied on the other side. Again this could leave a sandwich of white paper in the middle which will affect the results and an affected baby may be missed. The following is an example of layering where the pattern of the bloodspot is obviously different to that on the back.

Front  
Back

Finally, the sample will not be accepted if the blood has leached into the glassine cover in transit, as this means the sample was wet when it was put into the glassine and compression may have occurred. Always ensure that the sample is completely dry before placing it in the glassine cover.

For further information on sample quality, please contact the Scottish Newborn Screening Laboratory on 0141 354 9277 or email GG-UHB.NewbornScreeningLaboratory@nhs.net

10. After taking the bloodspot sample

Check the completed bloodspot card with the parent and make any necessary changes and record that the sample has been taken in the maternity record, in compliance with local protocols. The blood spot card should be air dried flat in a horizontal position and then placed in a glassine envelope provided with the card and sent directly to the Scottish Newborn Screening Laboratory in the pre-addressed, free post envelope as soon as possible after collection, ideally on the same day and no more than 24 hours after taking the sample. Only one blood spot card should be placed in each glassine envelope. No more than six blood spot cards should be placed in a single envelope as this will exceed the contracted postal weight and delay the delivery of specimens. Dispatch should not be delayed in order to batch cards together for postage.
In the event of a postal strike or severe weather which may disrupt deliveries, staff should refer to their local NHS Board protocols and contingency plans to ensure the timely delivery of blood spot cards to the SNSL.

Screening is offered routinely to all babies whose mothers are known or suspected to be infected with HIV or Hepatitis B. The blood spot card should be identified as a Biohazard. The envelope in which the card is placed must not be marked as 'Biohazard' to avoid breach of confidentiality.

11. **Repeat samples**

Occasionally it may be necessary to repeat a test and a second blood sample will need to be collected. If a repeat sample is required, the SNSL notifies the relevant Child Health/Screening Department to arrange for a repeat sample to be obtained by the midwife or health visitor.

There are a number of reasons why a repeat sample may be requested:

- There was insufficient blood available to perform all tests
- The blood spot card was damaged or did not reach the laboratory
- Layering or compression of the blood
- Equivocal or borderline test results – this means that the test result is not abnormal enough for the baby to be referred to a specialist but is not completely within normal ranges. There are several reasons why tests give an inconclusive result and often the repeat specimen provides a definitive result. If the repeat sample is also inconclusive then arrangements will be made for the baby to be assessed by a nominated clinical specialist
- There were insufficient details on the card to allow accurate analysis of the results
- The baby was too young when the specimen was collected (less than 72 hours old)
- The card was not dried properly prior to being posted
- The analysis was unsatisfactory due to specimen contamination or deterioration
- The specimen took more than 14 days to reach the laboratory and was therefore unsuitable for testing
- The baby was premature (less than 32 weeks gestation at birth)
- The baby had a blood or blood product transfusion less than 72 hours before the sample was taken

The 'repeat sample' box should be ticked on the blood spot card.

12. **Special circumstances**

12.1 **Babies born prematurely or cared for in neonatal units**

Babies admitted to neonatal units are likely to have multiple bloodspot samples taken. Blood spot screening should be coordinated with other tests where possible to minimise the number of invasive procedures performed. Venepuncture or venous / arterial sampling from an existing line is an alternative method to collect the blood spot sample. This is providing the sample is not contaminated with EDTA/heparin and the line is cleared of infusate. Do not use heparinised capillary tubes as lithium heparin is difficult to detect and can affect DNA testing.

Babies less than 96 hours of age should have a single circle bloodspot sample taken on admission/prior to blood transfusion for the routine screening test for SCD. This should be on a separate bloodspot card marked "Pre-transfusion" and should be completed fully as described previously. Tape or a sticky label can be placed over the unused circles to avoid the routine sample being added to the pre-transfusion bloodspot card.
The ‘Pre-transfusion’ blood spot card should be stored with the baby’s medical records in line with local protocols and despatched to the newborn screening laboratory together with the routine sample if the baby has received a blood transfusion in the interim to prevent the need for DNA analysis to complete SCD screening. The single circle blood spot sample taken and marked as ‘Pre-transfusion’ can be discarded appropriately if the baby does not receive a blood transfusion.

If the baby is transferred to another unit before the routine sample has been taken, ensure the pre-transfusion blood spot card accompanies the infant. Details of newborn sampling should be documented and included in transfer information.

The routine blood spot sample (four spots) should be taken as soon after the baby reaches 96 hours old as possible for all babies regardless of medical condition, medication, milk feeding and prematurity.

When a baby has had a blood transfusion or blood product, either intrauterine or in the newborn period, an interval of at least 3 days (72 hours) is required between the transfusion and the routine blood spot sample for CF, CHT, MCADD and PKU to enable metabolite concentrations to return to pre-transfusion levels (for intrauterine transfusion count day of birth as date of transfusion). In the event of multiple blood transfusions however, even if it has not been 3 days (72 hours) since the last transfusion, a routine blood spot sample should be sent by day 8 at the latest regardless to ensure all babies are screened by day 8 regardless of blood transfusion status.

If there has not been an interval of at least 3 days (72 hours) between the last transfusion and the routine sample, a repeat sample should be taken at least 3 days (72 hours) after the last transfusion to reduce the chance of missing a baby with one of the conditions. The date of the last blood transfusion before the blood spot must be recorded on the blood spot card and on discharge / transfer notifications to permit appropriate interpretation of results.

If a baby has not had a pre-transfusion sample taken, the laboratory may forward the routine sample to the DNA laboratory for analysis as a failsafe however an additional cost would be incurred and not all conditions screened for by the SCD screen will be detected.

Provider organisations should ensure failsafe arrangements for notifying screening status when the care of babies is transferred. This includes babies who are transferred in the neonatal period. The screening status of the baby is to be recorded on an auditable system and included in the discharge/transfer documentation.

Babies born at less than 32 weeks (equal to or less than 31 weeks + 6 days) require a second blood spot sample to be taken in addition to the routine sample taken 96 hours after birth to ensure a valid sample for congenital hypothyroidism screening as immaturity can mask this condition. These babies are to be tested when they reach 28 days of age (counting day of birth as day 0) or day of discharge home, whichever is the sooner.

Complete the details on the blood spot card as described previously, recording ‘CHT preterm’ on the blood spot card so the laboratory is aware of the reason for the second sample. Write the gestational age on the card. If the baby is being discharged home before 28 days of age, write ‘discharged home’ on the blood spot card, again this is so the laboratory knows why the repeat sample was taken before day 28. The responsibility for taking each sample lies with the healthcare professional that is responsible for clinical care at the time the blood spot sample is due to ensure babies who are transferred at less than 28 days of age have all newborn blood spot tests completed.
12.2 Babies transferring into area (up to the age of 6 months old)

For a child transferring into the NHS Board from within the UK, the Child Health/Screening Departments should check any available Health Visitor records received from the previous NHS Board of residence and document any results available for the child.

If there are no records or results available, the previous NHS Board or health provider, if known, should be contacted to ascertain whether screening took place and if any written results are available.

If a child has transferred in from abroad or no results of screening are available, screening for PKU, CHT, MCADD and SCD should be offered up until the child is 6 months of age through local NHS Board arrangements. CF screening is only reliable up until the child is 8 weeks of age, however abnormal results would be notified to a clinical specialist.

If screening is declined, this should be documented as per the normal procedure, including completing a newborn blood spot card and sending it to the laboratory stating that screening for the child has been declined. A copy of the information sheet “When the blood spot screening test has not been undertaken” should be given to parents.

12.3 Repeat samples

Informed consent must be taken for all repeat samples. Parents should be informed of the reason for the repeat. To enable parents to make an informed choice about screening for their baby.

Unavoidable repeat samples may be required from a few babies due to prematurity, borderline thyroid stimulating hormone (TSH) results, inconclusive CF screening or having received a blood transfusion. These samples should be taken as soon as possible or at the age directed by the screening laboratory.

A one week interval between samples is recommended for borderline TSH results to detect any meaningful change in TSH levels. Take a four blood spot sample and mark the blood spot card ‘CHT borderline’. Ensure that the ‘repeat sample’ box is ticked on the blood spot card.

Laboratories may also request a repeat sample due to any of the following:

- Too young for reliable screening which may give rise to a false positive result for CHT
- Too soon after transfusion (<72 hours) therefore metabolite concentrations may not have returned to pre-transfusion levels yet
- Insufficient sample therefore a risk of a false negative result
- Inappropriate application of blood therefore a risk of a false negative or false positive result
- Compressed, damaged or contaminated sample therefore a significant risk of a false negative or inaccurate result
- Day 0 and day 5 sample on same blood spot card therefore unable to determine baby’s age at time of sample
- Possible faecal contamination risk of inaccurate CF result
- Incomplete or inaccurate data on the blood spot card, e.g. no/inaccurate CHI number, no/inaccurate date of sample or no/inaccurate date of birth therefore unable to confirm identity of the baby
- Expired blood spot card used
- > 14 days in transit, too old for analysis therefore risk of inaccurate result
- Damaged in transit therefore risk of inaccurate result
- Sickle – too premature for testing therefore risk of inaccurate result

When a repeat sample is requested for any of the above reasons, the sample should be taken within 72 hours of the receipt of the request (unless ongoing transfusions).