A Laboratory Guide to Newborn Screening in the UK for

CYSTIC FIBROSIS

Handbook for laboratories incorporating:

• Background to the CF Screening Programme
• General organisation
• Screening protocol
• Pre-analytical aspects
• Analysis of immunoreactive trypsinogen (IRT)
• Mutation analysis
• Clinical follow-up and referral
• Reporting and communication of results
• Laboratory standards and guidelines
• Quality and performance monitoring
• Data collection and audit
• References

Major contributions from:
Professor Anne Green, Dr David Isherwood and Professor Rodney Pollitt

And for fourth revised edition:
Mr Paul Griffiths, Mr Wyn Griffiths, Dr Philippa Goddard and Miss Emma Scott
About the NHS Newborn Blood Spot Screening Programme

The NHS Newborn Blood Spot Screening Programme has responsibility for developing, implementing and maintaining a high quality, uniform screening programme for all newborn babies and their parents. The UK National Screening Committee (UK NSC) recommends that all babies in the UK are offered screening for phenylketonuria, congenital hypothyroidism (CHT), sickle cell disease (SCD), cystic fibrosis (CF) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD). There is a service specification for the NHS Newborn Blood Spot Screening Programme (No.19) available as part of the public health functions exercised by NHS England (www.gov.uk/government/publications/public-health-commissioning-in-the-nhs-from-2013).

The UK NSC and NHS Screening Programmes are operated by Public Health England (PHE). PHE’s mission is to protect and improve the nation’s health and to address inequalities through working with national and local government, the NHS, industry and the voluntary and community sector. PHE is an operationally autonomous executive agency of the Department of Health.

This is the fourth edition of the guide

For queries relating to this publication, please contact: phe.screeninghelpdesk@nhs.net

© Crown copyright 2014

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence v2.0. To view this licence, visit OGL or email psi@nationalarchives.gsi.gov.uk. Where we have identified any third party copyright information you will need to obtain permission from the copyright holders concerned.

A Laboratory Guide to Newborn Screening in the UK for Cystic Fibrosis
(OMIM #219700 www.omim.org/entry/219700)
A UK National Screening Committee publication
Published February 2014
Review date February 2017
PHE publications gateway number: 2013493
1.0 Introduction

1.1 Background
In April 2001 the Minister for Public Health (England) announced that all parents will be offered the choice of whether to have their baby screened for cystic fibrosis (CF). Screening was already taking place in Wales, Northern Ireland and some parts of England. Screening started in Scotland in February 2003 and became universal across the UK in October 2007.

The UK National Screening Committee (UK NSC) endorsed the protocols recommended by the CF Screening Advisory Board. This handbook is provided for newborn screening laboratories as a guide to support service provision in the UK and is available together with other relevant documents on the NHS Newborn Blood Spot Screening Programme website (www.newbornbloodspot.screening.nhs.uk).

The programme is a service to babies and their parents and seeks to balance the interests of parents whose children have CF with the majority, whose children are unaffected. The programme was also designed to minimise the number of carriers unavoidably detected.

At the time of going to print, every attempt has been made to provide the correct, up-to-date information. If there are any errata or comments, please send them to the NHS Newborn Blood Spot Screening Programme at phe.screeninghelpdesk@nhs.net for incorporation into the next edition.

1.2 Scope and purpose
This document provides guidance for those laboratories that provide a newborn blood spot screening service for CF in the UK. It is intended to define 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 guidance on achieving good quality by application of standards and audit.

1.3 Scientific background to the screening protocol
Cystic fibrosis (CF) is a heterogeneous disorder with a large number of different gene mutations and a range of different clinical phenotypes. For further information see the CF Trust website (www.cysticfibrosis.org.uk) and Wallis (1997).

Blood spot screening for CF is founded on the work of Crossley et al. (1979) who showed that immunoreactive trypsinogen (IRT) in blood is significantly increased in affected newborns. Screening programmes based on IRT were introduced in East Anglia in 1980 and subsequently elsewhere in the UK. IRT is not a particularly good marker for CF and nearly all the early programmes used a second ‘tier’ test, usually IRT in a second blood sample collected at 2-4 weeks of age. A two-stage IRT procedure has the disadvantage of requiring a relatively high number of second samples, which increase the anxiety generated by screening, and the presumptive diagnosis is made relatively late.

Once the cystic fibrosis transmembrane conductance regulator (CFTR) gene affected in cystic fibrosis had been identified mutation analysis began to replace the second-tier IRT assay. Initially, in 1990, only the most common mutation, delta-F508 (p.Phe508del - now known as c.1521_1523delCTT), was used. Homozygosity for this mutation was regarded as diagnostic. Babies showing a single copy of the mutation were investigated by the sweat test. As more disease-causing CFTR mutations were discovered and applied retrospectively it was found that some of the cases that had been classified as unaffected carriers on the basis of a sweat test chloride <60 mmol/L were in fact compound heterozygotes (heteroallelic homozygotes) and a number were showing clear clinical signs of CF. Consequently ‘equivocal’ sweat chloride results, together with a raised IRT in the initial screening sample, are now regarded as suggestive
of cystic fibrosis. Some mutations, R117H (now known as c.350G>A) being the most common, may be associated with equivocal or even normal sweat test results in the newborn period though later some results become clearly abnormal.

The availability of mutation analysis has greatly extended the range of clinical phenotypes known to be associated with abnormal CFTR function (Wallis, 1997) and the IRT-IRT and IRT-DNA newborn screening protocols differ significantly in the disease spectrum detected. A retrospective study (Boyne et al., 2000) of babies screened by the IRT-IRT protocol in the Trent region found that approximately 1% of babies with raised IRT in the initial sample but normal levels in the second had c.1521_1523delCTT (p.Phe508del) and a second CFTR mutation, the majority being “mild” mutations such as c.350G>A (R117H). In a 20-year period the East Anglia programme reported sensitivity of nearly 98% using the IRT-IRT protocol (Heeley et al., 1999) so that it appears that only a minority of the “missed” cases present in childhood with the classical signs of cystic fibrosis. The c.350G>A (R117H) mutation is much more common in the general population than would be expected from its occurrence in clinically diagnosed CF (Brock et al., 1998).

There is controversy as to whether it is appropriate to identify in the newborn period individuals with non-classical late presentations, for example milder forms of pulmonary disease or male infertility with congenital bilateral absence of the vas deferens. Wilfond and Rothenberg (2002) argue that the newborn screening panel should include only mutations clearly associated with pancreatic-insufficient CF. However, the CFTR genotype is a rather poor predictor of disease severity and some patients with an apparently “mild” genotype develop symptoms in the first few years and would benefit from earlier diagnosis. Thus, it seems reasonable to include such mutations in the screening panel, even though clinical management is problematical when the sweat test is normal or equivocal and there are no overt clinical signs.

For a general overview of the issues surrounding CF screening see the papers presented at the Centers for Disease Control and Prevention (CDC) meeting (Atlanta, November 2003) on newborn screening for CF: CDC (2005) and Grosse et al. (2004). More recently, Southern et al. (2009) reviewed two trials on the effects of screening on clinical outcome and Balfour-Lynne (2008) has discussed screening in the UK context.

1.4 General organisation

CF screening is fully integrated within the existing blood spot screening programme and based on the same screening laboratory populations. The initial screening test, the assay of IRT, uses blood collected on the standard newborn screening blood sample collection card. Quality assurance and performance management arrangements follow the same general principles as those for other newborn screening programmes.

With CF, as for other blood spot screening programmes, the screening laboratory is a major communication hub. Screening results are fed back to child health records departments (CHRDs), with onward transmission of negative results to the parents via health visitors. While over 99% of results are ‘CF not suspected’ and generated promptly for some of the remaining cases tests may not be completed until the baby is over a month old. Where the screening result report is used by the CHRD to check for completeness of coverage the effect of the CF screen on the timeliness of this process needs to be taken into account. There should be a system for acknowledging the receipt of specimens in the laboratory (using status code 01) in addition to reporting test results – see section 9.1.
2.0 The screening protocol

There is no universally-agreed approach to screening for cystic fibrosis. Wilcken (2007) identified seven basic strategies and a number of minor variations which are being used in newborn screening programmes around the world.

The UK protocol is intended to:

- **Maximise** diagnosis of CFTR defects producing preventable or treatable disease (respiratory, digestive) in infancy or childhood

- **Minimise**
  - Second heel pricks
  - Diagnostic delay
  - Detection of unaffected heterozygotes
  - Diagnosis of very mild forms of CFTR defect producing late-onset, essentially unpreventable, disease (e.g. congenital bilateral absence of the vas deferens)

- **Allow** for the fact that a clear diagnosis is not always possible

2.1 The screening protocol

This protocol is summarised diagrammatically in Figure 1. No alternative is to be offered.

The first step is the IRT assay followed by a one or two-stage mutation analysis of the CFTR gene on all samples with IRT values at or above the 99.5th centile. Subsequent action depends on the results of mutation analysis.

- Babies with **CFTR mutations detected in both genes** (either a homozygote or compound heterozygote) have a presumptive positive diagnosis of CF and are reported as: **CF suspected**. They are referred to a CF paediatric service for evaluation (clinical assessment, sweat test, confirmatory mutation analysis) - see section 8.1

- Most babies with **only one mutation detected** will be unaffected carriers but there is a risk that they carry a second abnormal allele not detected by the mutation panel used. They are therefore tested again (second IRT) on a repeat dried blood spot specimen taken ideally on day 21 (between day 21 and 28) (see section 7). Laboratories should request that this second IRT sample is collected on day 21:
  1. If IRT is ≥ cut-off 2 in the second sample the baby has a presumptive positive diagnosis of CF, is reported as **CF suspected** and is referred to the CF paediatric service for evaluation (clinical assessment, sweat test, further mutation analysis) - see section 8.1
  2. A baby whose second sample IRT result is < cut-off 2 is a **Probable carrier** with a ‘low likelihood’ of CF. Management of this group is described in section 8.2

The main aim of this approach is to minimise emotional trauma for parents of unaffected carrier babies (Parsons et al., 2003) and to reduce the number of negative sweat tests.
performed. With this approach to families there is less pressure on professionals dealing with ‘low likelihood’ cases to provide a definitive ‘answer’, and indeed an implicit admission that in the short-term it may not be possible to provide such an answer.

- Babies with **no detected mutation (by the first 4 panel DNA test)** are divided into two groups depending on the initial IRT result:

1. Those with 1st IRT below the 99.9th centile: report as ‘**CF not suspected**’

2. Those with 1st IRT equal to or above the 99.9th centile: a second IRT test is undertaken on a repeat dried blood spot specimen taken ideally on day 21 (between day 21 and 28) (see section 7):
   a) Babies with a second sample IRT < cut-off 2 are reported as **CF not suspected**
   b) If the second sample IRT is \( \geq \) cut-off 2 the baby has a **presumptive positive** diagnosis of CF, is reported as **CF suspected** and is referred to the CF paediatric service for evaluation (clinical assessment, sweat test, further mutation analysis) - see section 8.1

This procedure is adopted because CF has a significant incidence in non-Europeans, particularly of Asian origin. Many such cases have mutations not covered by the panels currently used in screening, a tendency seen also in cases originating in southern Europe. Additionally, in sub-populations with a tradition of intermarriage, a high proportion of CF affected babies are homozygous for rare “private” mutations (McCormick et al., 2002). The second IRT test will detect many of these cases.

For a summary of the rationale and definition of the cut-offs used in the screening protocol - see section 4.5.

**Figure 1. CF screening algorithm**

![CF screening algorithm diagram](image-url)

Note: for further definition of cut-offs see section 4.5
2.2 Sibling testing
Older siblings (of a confirmed case diagnosed from newborn screening) may be at risk of CF. Any testing will be at the discretion of the clinician.

For any subsequent siblings newborn screening should be undertaken as normal.

2.3 Late sampling
The routine (first) newborn screening blood spot sample should be taken on day 5 and in exceptional circumstances between day 5 and day 8 (date of birth to be counted as day 0).

If a second blood spot sample is required for IRT assay (one CFTR mutation detected or initial IRT \( \geq 99.9\)th centile) it should be collected ideally on day 21 (between day 21 and 28). If collection of the second sample IRT is delayed it can still be taken up to and including day 56.

The blood spot IRT is increased in most babies with CF in the first few weeks of life. However an initially raised IRT declines with age and becomes unreliable as an indicator of CF around 8 weeks (Crossley et al., 1979; Rock et al., 1990). This decline in IRT has implications for the reliability of the CF screening if samples are taken outside the specified time windows and it is important that appropriate cut-off values be applied to the times at which samples are taken. It also means that some babies cannot be reliably tested for CF (or testing cannot be completed) and includes babies in the following situations:

1. Babies who have arrived in the UK after 8 weeks (56 days) of age
2. Babies who have failed to have their first screening sample collected by 8 weeks of age
3. Babies who require a second blood spot sample for IRT assay but fail to have it taken at the correct time (sampling delayed or sample lost in transit)

The following guidance applies in these situations:

2.3.1 Late first samples (Figure 2)

a. For samples before day 21 cut-offs 0 and 1 apply as per screening protocol

b. For samples taken after baby is \( \geq 3\) weeks (\( \geq 21\) days) and up to 8 weeks (\( \leq 56\) days) old then a lower IRT cut-off (cut-off 2) should be applied as the decision point for mutation analysis - see section 4.5 for definition of cut-offs

c. Any samples taken after 8 weeks (>56 days) of age should not be tested for IRT. Procedures should be used in laboratories to select out these samples

d. If a first (routine screening) sample taken after 8 weeks is unavoidably tested then proceed as follows:
   i) If the IRT is <cut-off 2 it should be reported: CF – not screened (baby too old >8 weeks age)
   ii) If the IRT is \( \geq \)cut-off 2 (average result) the baby should be referred to a CF clinician and a blood spot sent at the same time for mutation analysis (according to screening protocol with the four mutation analysis first). Advice needs to be given to the family health visitor/midwife about the reason for referral so that this can be explained to parents. The screening report should state: CF suspected
2.3.2 Late or absent second samples (Figure 3)

e. For second samples taken by 8 weeks cut-off 2 is applied as in the screening protocol

f. If there is a delay and a second sample is taken after 8 weeks it is too old for screening to be carried out reliably and it should **not be analysed for IRT**. The screening laboratory staff should report: **CF suspected**. These babies should be referred to the CF clinical team for follow-up. The same action is required if no second screening sample is collected or received (because baby is too old) i.e. baby should be referred. Laboratory staff should provide information about the reason for referral in such a situation so that an explanation can be given to the parents by the midwife/health visitor/screening nurse specialist

g. If the second sample is taken after 8 weeks and unavoidably analysed the baby should be referred to a CF clinical team regardless of the IRT concentration, i.e. same action as for above (f)

Babies that have transferred out of the UK and are still awaiting the collection of a repeat first or second IRT sample should be reported as: **Screening incomplete**. See Appendix 1 for a template.
2.4 Unscreened babies

These include babies who were never part of the CF screening process, i.e. born abroad or not tested (e.g. too late, screening declined) and no screening specimens have been collected. For any subsequent requests for CF screening from the family it should be explained to the family (usually by the family health visitor) why their baby/infant has not been screened for CF. The GP, if not aware of the request, should also be informed (see Appendix 1 for an example template). Such babies should not routinely be offered any testing and should only be referred on a clinical basis if required.

If the family (or GP) has any concerns then a referral for assessment (which may include sweat testing) by the local designated CF team would be appropriate.

2.5 Notification form

A form has been developed by the CF Screening Advisory Board Diagnostic Outcome Sub-group to assist the identification, investigation and reporting of babies diagnosed with cystic fibrosis and not identified through the newborn screening programme. This may involve CF centres, newborn screening and molecular laboratories.

The form will assist communication between the relevant organisations and provides a record of critical points in the investigation. The details also enable the NHS Newborn Blood Spot Screening Programme to evaluate the CF Screening Programme and share any lessons learned from an investigation.
It is available from the NHS Newborn Blood Spot Screening Programme website (www.newbornbloodspot.screening.nhs.uk/cf).

The form should be completed as much as possible prior to sending to colleagues. All forms containing identifiers should be sent and received via secure NHS.net email addresses or via secure fax lines.

All fields shaded in grey should be removed prior to sending to the NHS Newborn Blood Spot Screening Programme. There should be no identifiable data on the form. Once completed, please forward to phe.screeninghelpdesk@nhs.net as an attachment.

2.6 Previously screened babies with subsequent discrepant results

2.6.1 Raised IRT ≥ cut-off 2 on sample taken after day 21 where there is a previous CF not suspected result

- Discrepant IRT result

Suspect contamination and request a further repeat if there is sufficient time to obtain a specimen up to and including day 56

- Non-discrepant IRT result ≥ cut-off 2

a. With previous IRT < cut-off 1 and CF not suspected reported
   - Send for mutation analysis
   - If one or more mutations detected – refer as: **CF suspected** or
   - If no mutations detected - ≤56 days – request repeat sample or
   - If no mutations detected - >56 days – refer as: **CF suspected**

b. With previous IRT ≥ cut-off 1, no mutations detected and CF not suspected reported
   - Persistently raised IRT
   - Refer and report as: **CF suspected**
3.3 Non-analytical factors affecting the screening result

3.3.1 Potential for false negative results

Several factors (in addition to late testing – see section 2.3) are known to lower blood IRT concentration in babies with CF, leading to falsely negative screening results.

- Many babies with CF and meconium ileus are likely to give negative screening results when tested during the first week of life, though IRT concentrations may become clearly abnormal a week or two later. It is unclear whether surgery itself is responsible or whether it is the...
concomitant lack of enteral feeding. In any case, CF should be strongly suspected in any baby with meconium ileus irrespective of the screening result. Neonatal units should have appropriate investigation protocols in place.

- The effects of blood transfusion on IRT are unclear but could result in a false negative IRT result. A repeat sample should therefore be taken after a minimum of 72 hours has elapsed. As white cells are removed prior to blood transfusion, misleading results with mutation analysis are unlikely (Brauner et al., 1997).

- Viral infection leading to acute gastroenteritis or respiratory illness may also be associated with a falsely negative screening result.

- Falsely negative results have also been reported in some premature or small for dates babies. It is not known whether such babies with CF will go through a period with high blood IRT concentrations as they mature but it seems possible that any such tendency may be overtaken by the processes leading to the later decline (as observed in term babies with CF).

It is not practicable to adopt alternative diagnostic approaches routinely in these last two groups of babies. It must always be borne in mind that not all cases of CF will be detected on newborn screening and that any child showing appropriate symptoms should be investigated accordingly.

3.3.2 Potential for false positive results

The reference range for IRT increases markedly with increasing prematurity, particularly so in babies born <29 weeks gestation. Elevated IRT levels have also been reported in association with congenital infections, renal failure, bowel atresias and nephrogenic diabetes insipidus. False positives also occur in children who are very sick on PICU who do not specifically have any bowel or renal problems. High IRT levels can occur in babies with a variety of chromosomal abnormalities particularly trisomies 13 and 18.

The standard screening protocol should be followed in all of these situations and if mutations are not found, laboratory staff should explain to the clinician looking after the baby that the raised IRT may be a secondary phenomenon and consider whether or not further investigation is required (e.g. repeat IRT, sweat test).
4.0 The IRT assay

Immunoreactive trypsinogen in the routine newborn screening blood spot is to be assayed using a methodology that has been demonstrated to be fit-for-purpose and approved by the CF Screening Advisory Board. Ideally the reagents and instrumentation should be CE marked. The laboratory should follow the procedures detailed in the manufacturer’s instructions. Procedures for specimen identification and disc punching are similar to those for the thyroid stimulating hormone (TSH) screening assay.

4.1 Quality monitoring and buddy groups

The sensitivity and specificity of the CF screen are crucially dependent on the performance of the IRT assay. In particular, if cut-off 1 is set too low, too many samples will be sent for mutation analysis and there will be a disproportionate increase in the number of unaffected carriers detected.

Unfortunately, this is a particularly difficult assay to control as human blood contains a variety of trypsinogen species which react differently with the various antibodies used for immunoassay. Additionally, the IRT species increased in neonates with CF has different properties from that present normally in neonatal blood (Dhondt & Farriaux, 1994). The use of different technologies and hence antibody configurations used to measure IRT may add a further level of complexity to the establishment of robust cut-off values.

For these reasons it is not possible to deliver the quality assurance required for newborn screening solely by means of an EQAS with circulated blood spots, particularly as there are very marked matrix effects.

Comparing data from several UK laboratories revealed significant variation in the population distribution of IRT values with different kit lots as well as systematic inter-laboratory variations due to differences in software set-up (Pollitt & Matthews, 2007). Some lot-to-lot variation seems unavoidable and a scheme of procurement of kit lot batches from the suppliers is essential to minimise lot-to-lot variability. Where possible laboratories that employ similar equipment should form buddy group arrangements. From previous experience of the buddy group system established within the UK in 2007, reduced variation and greater consistency in setting cut-off values has been demonstrated. Also, for the purpose of consistency, the kit shelf life should be as long as possible and should certainly exceed 6 months.

Although buddy grouping is considered favourable, in order to further reduce the variation in IRT population statistics between laboratories, each laboratory should adopt procedures to monitor statistics of the IRT values (e.g. means, medians and percentiles) from the samples they receive. Lot-to-lot variation between kits should not exceed +/-8%, although the aim should be to keep this lower.

When introducing a new kit lot for IRT, until sufficient data from the new kit lot has been collected, each screening laboratory should continue to use the existing cut-offs (i.e. from the previous kit batch). The suppliers of the kit must be able to provide additional quality control (QC) material for use with kit lot change over.

4.2 Population means and medians

The use of population means and medians can give helpful information about the performance of a particular IRT kit lot.
The population distribution of IRT results is skewed with the mean being typically about 4 ng/mL greater than the median. Laboratories should use median values to assess for any assay drift. For 5000 newborn results the 95% confidence interval of a typical median IRT concentration of 17 ng/mL is 16.8–17.2 ng/mL. The IRT level (cut-off 0) on which a decision is made to undertake repeat assays from a single IRT assay is set at approximately the 98.5th percentile and this will have a wider 95% confidence interval. The 99th percentile is typically 64.3 ng/mL with a 95% confidence range of 58–71 ng/mL. At this IRT level the lot-to-lot variation may also be as much as 20%. Variations of this magnitude can have a significant effect on the number of samples sent for CFTR gene mutation analysis leading to a change in the number of unaffected carriers detected from the programme.

4.3 Quality and performance monitoring

Internal quality control samples at three IRT levels should be included with each analysis batch. Each laboratory should assign acceptable ranges for these samples.

Dried blood spots available from the Centres for Disease Control and Prevention (CDC) can also be used for internal quality control purposes and achieve levels of precision (coefficient of variation - CV) of approximately 5–9% at IRT concentrations of approximately 50–140 ng/mL (Table 1). Each laboratory should assess and regularly monitor their own precision profiles.

For guidance examples of typical precision profiles using QC materials:

<table>
<thead>
<tr>
<th>Mean IRT ng/ml</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.4 *</td>
<td>2.30</td>
<td>9.0</td>
</tr>
<tr>
<td>64.9 *</td>
<td>5.30</td>
<td>8.2</td>
</tr>
<tr>
<td>90.0 *</td>
<td>6.56</td>
<td>7.2</td>
</tr>
<tr>
<td>125.8 **</td>
<td>7.45</td>
<td>5.9</td>
</tr>
<tr>
<td>224.4 **</td>
<td>14.36</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* PE control materials n=36 ** CDC control materials n=10

Samples obtained from babies show less precision than artificial control samples prepared in standardised conditions. In an example from one laboratory: 68 pairs of IRT results with a mean of 103.3 ng/mL had a standard deviation (SD) of differences 12.87 with a CV of 12.5%; 20 pairs of IRT data from babies with CF had a mean of 216.8 ng/mL with a SD of differences of 24.38 and CV of 11.2%.

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.

4.4 Stability of IRT in blood spots

It has been reported that there is only a gradual decline of trypsinogen in dried blood spots over several years (Crossley et al., 1981). When the blood spot IRT assay was initially being assessed dried blood spot samples from CF patients stored for 1.5–3 years demonstrated elevated trypsinogen in comparison to similarly stored controls (King et al., 1979). When specific work was undertaken to determine the deterioration of trypsinogen in dried blood spot samples stored at room temperature they lost half their immunoreactivity when measured by radioimmunoassay over a period of
three months (Heeley, 1980; Heeley et al., 1982). When stored for ten weeks in the dark at room temperature in a dry location in a cardboard box, dried blood samples from normal babies lost approximately two-thirds of their IRT as measured by Sorin reagents which measure mainly trypsinogen (Kirby et al., 1981).

The Lille group (Dhondt & Farriaux, 1994) studied samples stored at +4 °C using the Behring RIA-gnost® neonatal trypsin kit which detects both trypsin and trypsinogen and inhibited forms of the enzyme. After 4 months storage samples from babies with non-CF hypertrypsinaemia had lost approximately 25% of their activity and after 8 months, 45%. Unlike samples from normal babies, samples from CF babies showed a bimodal decay curve suggesting a different mix of IRT species.

CDC showed that IRT added to a filter paper matrix was stable when stored for one year at either -20 °C or 4 °C with desiccant. Only 75% of IRT remained in dried blood spots stored at ambient temperature for up to one year (Li et al., 2006).

It is inadvisable to rely on a screening result from a sample that has been significantly delayed in transit – empirically the reliability of results from samples received 14 days after collection should be regarded as suspect and a repeat specimen requested. However, if a high result is obtained from a sample analysed >14 days after collection it should be processed according to the national protocol.

4.5 Rationale and definition of IRT cut-offs

**Cut-off 0:**

The initial screening samples are normally assayed in singlicate. Those with IRT results above a preliminary threshold (cut-off 0) are then re-assayed in duplicate to give a more definitive result. This is to minimise effects of volumetric variability of the punched discs, day-to-day variation in IRT assay calibration, and to detect contamination of the sample with faeces, or possible sample misidentification. The value used for cut-off 0 is based on selecting an average of 1.5% of all results for repeat IRT analysis. It is approximately 10 ng/mL less than cut-off 1, typically a value of 60 ng/mL, and unless there is significant variation in the calibration of a particular kit lot, should not need to be changed.

**Cut-off 1 (99.5th centile):**

Experience in East Anglia using a two-stage IRT-IRT screen over a 17 year period showed that a 99.5th centile cut-off for the initial sample gave an overall sensitivity of 97% (Heeley et al., 1999). Therefore this centile cut-off (cut-off 1) was retained to determine the proportion of samples sent for mutation analysis in the current protocol. The long-term average IRT value for the 99.5th centile approximates to 70 ng/mL when assaying each sample in triplicate. However, there may be significant variations in calibration between kit lots and the cut-off value may sometimes require adjustment. If the cut-off is set too high the risk of false negatives is slightly increased. Setting the cut-off too low greatly increases the number of unaffected carriers detected.

**Cut-off 2:**

The rate at which blood IRT concentration declines with age in babies with cystic fibrosis is very variable. It is relatively slow in pancreatic-insufficient cystic fibrosis and cut-off 2 is set at 10 ng/mL below cut-off 1 to allow for this. However, a much more rapid decline has been observed in some milder cases (Boyne et al., 2000) and such babies may, as a result be classified as ‘Probable carriers’.
The 99.9\textsuperscript{th} centile:

The number of samples above the 99.9\textsuperscript{th} centile is too small to allow statistically significant numerical cut-offs to be determined in ‘real time’ for individual kit lots. Long-term data indicate that the number of samples with IRT values >120 ng/mL approximates to the required 0.1\%.
5.0 The first screening specimen

Samples are initially assayed in singlicate.

For babies with IRT value <cut-off 0 a negative result: **CF not suspected** is issued.

Samples with results ≥cut-off 0 should be re-assayed in duplicate with the next batch. Where possible the samples for re-assay should be taken from two separate spots on the card. The average of the three results should be taken.

The set of triplicate results should be reviewed for consistency as poor analytical performance can produce different results; spuriously high results can occur with faecal contamination or may be low if there is a missing spot or poor sample.

Further action therefore requires an assessment of agreement between results. The following sections provide **guidance only** and laboratories should use discretion for each individual set of results.

5.1 Results from duplicate re-assay (Figure 4)

If the average IRT is below the 99.5\(^{th}\) centile (cut-off 1) and provided there is no suspicion of a ‘missing spot’, a negative result: **CF not suspected**, is issued.

If the average of the three results is equal to or above the 99.5\(^{th}\) centile (≥cut-off 1) they should be assessed for consistency before proceeding further.

N.B. if the sample was taken on day 21 or later then cut-off 2 is applied (≥cut-off 2).

**A set of three results should be regarded as discrepant** if they meet any one of the following criteria, whichever is most convenient:

- A coefficient of variation (standard deviation/mean) ≥0.25 or
- One or more individual results being ≥30% above or below the average of all three or
- The ratio of the highest result to the average of the other two ≥1.5

**For sets of three results which are considered to be discrepant:**

a. If there is a single high result (≥99.5\(^{th}\) cut-off) with the other two below the 99.5\(^{th}\) centile

b. cut-off, and provided that there is no uncertainty about sample identification, the single high result can be ignored and a **CF not suspected** report issued

c. If two results are ≥99.5\(^{th}\) centile (≥cut-off 1) and the average of all three results at or greater than the 99.5\(^{th}\) centile but less than the 99.9\(^{th}\) centile, proceed to mutation analysis as per screening protocol

d. If the average of all three results is ≥99.9\(^{th}\) centile (≥cut-off 1), request a repeat blood specimen on the grounds that this is likely to be sample contamination

Assaying further samples from a card with discrepant results is unlikely to completely resolve the issue.
5.2 Insufficient sample: no re-assay possible
If it is not possible to punch any further discs from the card (i.e. for a single result ≥ cut-off 0) then a repeat specimen, to be taken as soon as possible, should be requested due to insufficient sample.

5.3 Only one re-assay possible
If only two IRT results can be obtained (i.e. after initial analysis only one further spot from the card is possible) proceed as follows:

a. If the average of the two results is below cut-off 1 report: **CF not suspected**

b. The average of both results are ≥99.5th centile, then proceed to mutation analysis. Widely discrepant results may require a repeat specimen, taken as soon as possible, to be requested – laboratory staff should use judgement on this

---

**Figure 4. Handling IRT results from the first screening specimen**

- **Three IRT results Average ≥ cut-off 1***
  - Discrepant? No → Accept the average result
  - Yes → **Two results ≥ cut-off 1?***
    - No → Report CF not suspected
    - Yes → **Average ≥ 99.9th centile?***
      - No → Send for mutation analysis
      - Yes → Request repeat sample
6.0 Mutation analysis

Mutation analysis must be performed in an accredited molecular genetics laboratory. The organisational arrangements for this are outlined in section 10.3.

6.1 Sample requirements, identity and transport

There is no requirement for a measured amount of blood so that the residual blood from a spot that has already had a disc punched out is likely to be sufficient.

There must be a tracking system to ensure that dried blood spot samples sent for mutation analysis are identified unequivocally. It is recommended that the card should not normally leave the screening laboratory.

Samples must be securely identified. The simplest method is to cut an irregularly shaped strip from the blood spot card, with the blood at one end, and on the blank section of the strip, write patient identifiers including at least two out of the baby's date of birth, surname and NHS Number. The irregularly shaped strip can then be matched with the card if subsequently required. With increasing automation and an electronic IT system a bar-code sample identifier is desirable.

Appropriate timely transport arrangements to the molecular genetics laboratory must be organised and the laboratory made aware of the imminent arrival of a screening specimen(s). Sample receipt by the molecular laboratory should be acknowledged back to the screening laboratory.

6.2 Two-stage analysis

Mutation analysis is carried out in two stages to minimise the detection of homozygotes for the 'milder' alleles and number of carriers detected.

The first stage will establish whether the infant has any of the four commonest alleles in the English population associated with severe disease. Nomenclature has been revised to comply with the Human Genome Variation Society Guidelines to ensure consistent and precise nomenclature. The four mutations are as detailed below.

<table>
<thead>
<tr>
<th>cDNA</th>
<th>Protein</th>
<th>Traditional</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1521_1523delCTT</td>
<td>p.Phe508del</td>
<td>(ΔF508)</td>
</tr>
<tr>
<td>c.1652G&gt;A</td>
<td>p.Gly551Asp</td>
<td>(G551D)</td>
</tr>
<tr>
<td>c.1624G&gt;T</td>
<td>p.Gly542*</td>
<td>(G542X)</td>
</tr>
<tr>
<td>c.489+1G&gt;T</td>
<td></td>
<td>(621+1G&gt;T)</td>
</tr>
</tbody>
</table>

Note 1: Depending on the technology used, the c.1519_1521delATC, p.Ile507del, (ΔI507) mutation may also be detected.

Note 2: Depending on the technology used, the c.1657C>T, p.Arg553*, (R553X) mutation may also be detected.

∞ The first 3 mutations are also described according to the predicted protein change so p.Gly551Asp indicates that the glycine at amino acid 551 is predicted to change to aspartic acid. c.489+1G>T is a mutation in the intronic part of the splice site and is thus has no directly associated amino acid change. The numbering indicates that by saying the 1st nucleotide (+1) after nucleotide 489 (which is at the last nucleotide of the exon) which is mutated.
This first stage will detect >80% of disease-causing mutations in the UK population.

There several technologies including a range of commercial kits available for detection of these four mutations. It is essential that in the first stage of molecular analysis no mutations other than the ones specified above are co-incidentally detected.

The second stage covers a wider range of mutations and is applied, using the original sample, in all cases where a single pathological mutation is detected in the heterogenous state at the first stage.

The mutation panels used will be specified by the CF Screening Advisory Board and reviewed periodically in the light of technical developments and on-going evaluation of the programme.

6.3 Reporting results
Samples with no detected CFTR mutation may be reported to the screening laboratory by list with appropriate specimen identifiers.

For any sample showing a CFTR mutation the molecular genetics laboratory must supply the screening laboratory with a formal report, largely limited to a factual description of the findings (mutations tested for and results). Standard advice on family studies, referring the baby to a CF centre for follow-up etc., is inappropriate in the screening context as these options are incorporated in the protocol. Copies (not transcriptions) of this mutation analysis report are to be sent with the screening laboratory’s report or referral letter to the baby’s consultant or GP as appropriate.

6.4 Action following mutation analysis
The screening laboratory will undertake the following action according to the protocol:

Two CFTR mutations detected – CF suspected - immediate referral to a CF specialist (see section 8.1).

One CFTR mutation detected – request a second blood spot sample for IRT analysis, to be taken ideally on day 21 (between day 21 and 28) (see section 7).

No CFTR mutation detected – further action depends on the level of IRT in the initial blood sample. If it is below the 99.9\textsuperscript{th} centile a, CF not suspected, result is issued. For results ≥99.9\textsuperscript{th} centile, a second blood sample is to be taken ideally on day 21 (between day 21 and 28) (see section 7).
7.0 The second specimen (i.e. repeat IRT test)

Requests for second blood samples for IRT testing will be via locally agreed pathways defined as part of the newborn screening responsibilities.

A second dried blood spot specimen for CF screening is requested in the following situations:

- Initial high IRT and one CFTR mutation detected
- Initial IRT ≥99.9th percentile and no mutations

The second dried blood spot specimen is for IRT testing only – it is recommended practice that other screening tests will not be repeated on this specimen.

Second samples should be taken ideally on day 21 (between day 21 and 28)
The explanation to be given to parents is that “further tests need to be done for cystic fibrosis”. The results of mutation analysis should NOT be given out at this stage as this may result in premature disclosure to the parents before the definitive screening result is known. The repeat request should be confirmed in writing to the appropriate health professional(s); a template is available on the NHS Newborn Blood Spot Screening Programme website (www.newbornbloodspot.screening.nhs.uk/cf).

If there is a delay and a second sample has not been taken by 8 weeks of age it is too old for IRT testing to be carried out reliably - see guidance in section 2.3.

The following are undertaken on the second sample depending on the reason for its request:

- One CFTR mutation detected
  This second sample is assayed for IRT in duplicate. Babies with an average of results ≥cut-off 2 are referred to a CF specialist as CF suspected (see section 8.1). Babies with an average of results below cut-off 2 are reported as CF carrier but are not referred. They are contacted by a health professional to discuss the screening results (see section 8.2)

- No mutation detected and initial IRT ≥99.9th percentile
  This second sample is assayed for IRT in duplicate. Babies with an average result ≥cut-off 2 are referred to a CF specialist as CF suspected (see section 8.1). Otherwise a CF not suspected result is issued
8.0 Clinical follow-up and referral

Each screening laboratory should have an agreed arrangement via a clinical liaison service (CLS) for the follow-up and referral of all presumptive positive cases (i.e. CF suspected) (see section 8.1). This should be part of a comprehensive newborn screening service specification agreed with commissioners and local clinical services together with other newborn screening programmes. Responsibility for undertaking the CLS must be documented and must include arrangements for backup.

The CLS role may be undertaken by person(s) based in the screening laboratory (i.e. screening clinical nurse specialist or duty biochemist), by the CF regional centre or by other designated health professionals in the community based on local arrangements.

These arrangements should be regularly updated to reflect personnel changes and the evolution of clinical services. For further details see the CF Screening Programme: National Standard Protocol Guidelines for Clinical Referral on the NHS Newborn Blood Spot Screening Programme website (www.newbornbloodspot.screening.nhs.uk/cf).

For information on regional CF centres please refer to the Cystic Fibrosis Trust’s Standards for the Clinical Care of Children and Adults with Cystic Fibrosis in the UK 2011 available at: www.cysticfibrosis.org.uk/media/82070/CD_Standards_of_Care_Dec_11.pdf and also the National Specialised Services Definition Set: 10 Cystic Fibrosis available at: www.specialisedservices.nhs.uk/document/cystic-fibrosis-services-all-ages.

8.1 Follow-up of CF suspected cases

These are babies in any of the following situations:

- two detected CFTR mutations or
- one mutation and a second sample with high IRT or
- high IRT concentration in two blood samples taken approximately 3 weeks apart or
- late first sample (unavoidably analysed), raised IRT (≥ cut-off 2) or
- late second sample (not analysed or unavoidably analysed) with first sample raised IRT

or

- previously reported ‘CF not suspected’ based on normal IRT, second sample unavoidably analysed with raised IRT (≥ cut-off 2) and no, one or more mutations or
- previously reported ‘CF not suspected’ based on raised IRT (≥ cut-off 1) with no mutations detected, second sample unavoidably analysed with raised IRT (≥ cut-off 2)

They should be referred to a designated clinician for CF or their deputy according to locally agreed and documented procedures within one working day of the definitive screening result becoming available (see Appendix 2 for a suggested template). The regional CF centre will usually be responsible for liaising with a more local CF clinic (as required by local arrangements).

Parents should not be informed of a positive screening result on a Friday, Saturday or Sunday. Ideally, Thursday should be avoided as well. Parents should be offered an appointment on the following day.
8.2 Follow-up of cases reported as CF carriers

Babies with one detected CFTR mutation and low IRT (normal) in the second blood sample are reported as CF carrier. Reporting of carrier status to parents is supported by the Human Genetics Commission (see Appendix 3).

Many of these families will already have raised anxieties because a second screening sample has been collected. The family should therefore be contacted within one working day of the second sample IRT result becoming available and arrangements made for a domiciliary visit to discuss the screening result. Some screening laboratories have associated nurse specialists who will initiate (and possibly participate in) this follow-up visit. In other areas a health visitor or other professional, with appropriate training, may carry out this responsibility.

The parents are to be told:

- The screening result has indicated CF carrier status but although CF is not suspected, the possibility that their child has cystic fibrosis cannot be ruled out completely

- Because of their child’s genetic status there is a small but significant risk (approximately 1 in 200) that any future children born to this couple will have ‘classical’ cystic fibrosis. Further tests can show whether they are at a higher or lower risk than this

- Parents are offered the option of having their baby seen straight away by a CF specialist if they wish and told to ask for such a consultation should they become anxious about their baby’s health

It is suggested that the parents consider further genetic investigations and appropriate counselling. However, partly because such investigations have the potential to uncover non-paternity, it is important that there is no undue pressure and that couples are given plenty of time to consider whether or not to pursue this option.

Parents should be given a copy of the NHS Newborn Blood Spot Screening Programme leaflet “Results of Newborn Blood Spot Screening - Carrier of cystic fibrosis gene” (available at: www.newbornbloodspot.screening.nhs.uk/cf).

Irrespective of who conveys this information to the family, the baby’s GP must be formally notified of the findings from screening and be sent a copy of the molecular genetics report and of the carrier leaflet for parents. See Appendix 4 for a template.
9.0 Reporting and communication of results

Screening laboratories shall use the newborn screening results status codes for acknowledging the receipt of specimens in the laboratory and when reporting results to the child health records departments (CHRDs) - see status codes section 9.1.

‘CF not suspected’ results should normally be communicated to the parents via health visitors. However, ‘CF not suspected’ screening results following a second sample (but not CF carrier result- see section 8.2) should be communicated as soon as possible because anxieties will have been raised. A reassuring second sample result requiring no further action can be communicated by telephone to the family by an appropriately trained health professional (e.g. screening clinical nurse specialist).

Results requiring follow-up/clinical referral of the baby (‘CF suspected’, ‘CF carrier’) are communicated directly to the parents by an appropriate health professional – see section 8.

Reports of all screening results should have a generic disclaimer attached: ‘These tests are screening tests; no screening test is 100% reliable’. Such a disclaimer is particularly relevant to CF because of falsely high IRTs from other non-CF causes and falsely low IRTs (see section 3.3).

The screening protocol is designed to pick up as few carrier babies as possible and therefore should not be regarded as a reliable means of detecting CF carrier infants.

9.1 Status codes

The outcome from all newborn screening tests is described in the form of a status code for each blood spot card that is received for the baby. The status codes are used to report results to child health records departments, particularly when reporting electronically. Status codes for each blood sample must be maintained within the child health system clearly linked to the baby’s record and the date of sampling. A baby may have blood taken for CF screening as a first sample, a repeat for the first sample, a second sample taken because the first IRT result was high and a repeat second sample if the initial ‘second’ sample is inadequate.

The status codes can be found on the NHS Newborn Blood Spot Screening Programme website and are summarised below for CF (www.newbornbloodspot.screening.nhs.uk/statuscodes).
### Table 2. Status codes for CF

<table>
<thead>
<tr>
<th>Code</th>
<th>Suggested term to be displayed in the child health system</th>
<th>Comments with reference to CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Specimen received in laboratory</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>CF screening declined</td>
<td></td>
</tr>
</tbody>
</table>
| 03   | CF – repeat/further sample required                       | Reasons for repeat sample will include the following pick list:  
  - Baby too young for reliable screening  
  - Too soon after transfusion (<72 hours)  
  - Unsuitable sample e.g. sample more than 14 days in transit, card out of date, contamination  
  - Insufficient sample  
  - Unsatisfactory analysis  
  - Inconclusive i.e. initial sample has “high IRT” and further 2nd IRT sample needed |
| 04   | CF not suspected                                          | **First sample**: Use for results on first sample  
  IRT < cut-off 0 (singlicate analysis)  
  IRT < cut-off 1 (after repeat duplicate analysis)  
  IRT < 99.9<sup>th</sup> percentile and no CFTR mutations have been detected  
  **Second sample**: Use for 2nd IRT < cut-off 2 if collected because first IRT ≥ 99.9<sup>th</sup> percentile |
| 05   | CF carrier                                                | One CF mutation and second sample IRT < cut-off 2 |
| 06   | Not applicable to CF                                      |                               |
| 07   | Not applicable to CF                                      |                               |
| 08   | CF suspected                                              | **These will include the following categories:**  
  - Two mutations detected  
  - One mutation and second IRT ≥ cut-off 2  
  - No mutations, first IRT ≥ 99.9<sup>th</sup> percentile and 2<sup>nd</sup> IRT ≥ cut-off 2 (also includes babies with a high IRT in a sample taken after 8 weeks and babies who had a revised screening result following unavoidable analyses)  
  - Second sample not taken after raised IRT on first sample |
| 09   | CF not screened/screening incomplete                      | Use with additional qualifying terms for:  
  - Baby who has died i.e. before first sample/repeat sample  
  - The baby is >8 weeks old (no sample taken) at first sample  
  - Baby has been transferred out of screening laboratory area and is still awaiting the collection of a repeat first or second IRT sample |
| 10   | Not applicable to CF                                      |                               |
10.0 Laboratory standards and guidelines

10.1 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; these standards include CF screening.


10.2 Screening laboratory

Organisation

• 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

• Laboratories undertaking newborn blood spot screening shall be accredited by Clinical Pathology Accreditation (UK) Ltd (CPA) now formally part of the United Kingdom Accreditation Service (UKAS). This shall include the newborn blood spot screening specialist assessment. There must be a senior member of laboratory staff at medical consultant or consultant clinical scientist level responsible for newborn blood spot screening with defined lines of accountability for all laboratory aspects of the service

• There shall be written agreed procedures describing the working arrangements between the screening laboratory and their referral laboratory

• 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. Processes shall be provided in line with relevant national standards and guidance and screening specifications. Processes shall be reviewed periodically taking into account audit data, accumulating results, technical developments and local changes in healthcare provision

• The laboratory must release reports on screening performance, including external quality assurance and CPA assessments to any agency with a legitimate interest in the quality and safety of the programme on behalf of the public

Analytical processes

• Assay for immunoreactive trypsinogen must be performed by an approved method. Any proposal to introduce new analytical methods needs careful collective consideration by the Cystic Fibrosis Scientific Advisory Group and the Cystic Fibrosis Screening Advisory Board and meet any recommended specification
• Samples for CFTR mutation analysis should be sent to an accredited molecular genetics laboratory satisfying the standards listed in section 10.3. The referring screening laboratory is responsible for determining further action on the basis of the molecular genetics result. For samples where a mutation has been detected the molecular genetics laboratory will issue a written report, a full copy of which is to be forwarded by the screening laboratory with the clinical referral letter to either the baby’s GP, or paediatrician, depending on the final screening result (see section 8).

• Laboratories shall participate in audit at local, regional and national levels, to assess the effectiveness of the national screening programme.

• Laboratories should publish the results and performance of their newborn blood spot screening programme within an annual report.

• There shall be a documented risk management policy for the laboratory aspects of the CF Screening Programme. These 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.

• Screening incidents shall be managed in accordance with the UK National Screening Committee’s Managing Incidents in National NHS Screening Programmes – Interim Guidance (Sep 2013) (UK National Screening Committee, 2013).

10.3 Mutation analysis

Organisation

• In general each screening laboratory should send samples to a single molecular genetics laboratory. Molecular genetics laboratories may receive samples from more than one screening laboratory.

• Screening laboratories may contract for this service with any molecular genetics laboratory which is capable of meeting the performance standards specified below, is accredited (Clinical Pathology Accreditation (UK) or ISO 15189) and has been accepted as a member of the UK Genetic Testing Network (www.ukgtn.nhs.uk).

Analytical processes

• The mutation panels used will be specified by the Cystic Fibrosis Board and reviewed periodically in the light of technical developments and ongoing evaluation of the programme.

• There must be a tracking system to ensure that dried blood spot samples sent for mutation analysis are identified unequivocally. It is recommended that the card should not normally leave the screening laboratory.

• Samples are to be processed with sufficient frequency so that mutation results are received by the screening laboratory in a timely manner to meet the overall performance standard required by the programme.

• Results will be returned to the screening laboratory only. At this stage no other party is to be informed. For samples where a mutation has been detected the molecular genetics laboratory will issue a written report detailing the genetic findings but without detailed advice. The screening laboratory will forward a full copy of this report with the clinical referral letter to either the GP or paediatrician depending on the final screening result.
10.4 Overall performance

Timeliness

- Laboratory services should be configured to enable CF newborn screening to be completed in time for all babies with positive screening results to have their first clinic appointment by day 28, for babies in whom 2 mutations have been detected, and by day 35 for babies who have required a second sample IRT measurement.

- Analysis for IRT must be performed frequently enough to generate a screening test result (including any retest results where required to be confirmed in duplicate) no later than 4 working days from receipt of an adequate sample.

- Definitive results from mutation analysis must be available on or before the 4th working day from receipt of the sample from the screening laboratory.

- CF suspected results should be reported to the appropriate clinical team within 1 working day of becoming available. Intermediate reports (i.e. increased IRT in the initial sample without follow-up results) should not be issued.

- Parents should not be informed of a positive screening result on a Friday, Saturday or Sunday. Ideally, Thursday should be avoided as well. Parents should be offered an appointment on the following day.

Quality assurance

- 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.
It is essential that data be collected to monitor the performance of the national CF screening protocol, thus allowing us to assure parents that the screening works effectively in detecting clinically relevant CF cases in infancy but without unnecessary carrier detection and also enable us to compare the national programme with that of other newborn screening programmes throughout the world.

The laboratory based data required ([www.newbornbloodspot.screening.nhs.uk/datacollection](http://www.newbornbloodspot.screening.nhs.uk/datacollection)) should be collected by the laboratory in each area and submitted on a retrospective basis to the NHS Newborn Blood Spot Screening Programme by the 31st July for the previous financial year (1st April - 31st March).

Clinical information ([www.newbornbloodspot.screening.nhs.uk/datacollection](http://www.newbornbloodspot.screening.nhs.uk/datacollection)) 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 Screening Programme. It is the responsibility of the clinical services to ensure that these forms are completed and returned to their respective laboratory directors.

Follow-up information is also required on all cases reported as CF carriers ([www.newbornbloodspot.screening.nhs.uk/datacollection](http://www.newbornbloodspot.screening.nhs.uk/datacollection)). A form for each case should be sent to the relevant health professional (specialist health visitor/counsellor) for completion. The anonymised data from the form should comprise part of the annual data submission by the screening laboratory to the NHS Newborn Blood Spot Screening Programme.

If a screening laboratory director, clinical team or molecular laboratory director is made aware of a CF case (born after April 1st 2007) that has not been detected via the CF Screening Programme, it is very important that information on the case be reported to the NHS Newborn Blood Spot Screening Programme. The details should be gathered by the clinical team in conjunction with the laboratory director using the ‘CF diagnosis not identified through screening’ form ([www.newbornbloodspot.screening.nhs.uk/datacollection](http://www.newbornbloodspot.screening.nhs.uk/datacollection)). The screening laboratory should issue the relevant clinical team with this form (see section 2.5). The data should be collated and anonymised by the screening laboratory director and returned as soon as possible to NHS Newborn Blood Spot Screening Programme. Data on these cases will be collated on an annual basis as an important part of the audit of the programme.
References


References


We would like to thank Professor Anne Green, Dr. David Isherwood and Professor Rodney Pollitt for their major contribution to this document in the previous editions; Dr. Sarah Ball, Ms. Cathy Coppinger and Dr. Paul Newland for their contributions to this edition and to many contributors from the UK screening laboratories who provided valuable information.

Any comments on the content of this handbook should be sent for the attention of the CF Scientific Advisory Group c/o the NHS Newborn Blood Spot Screening Programme:

phe.screeninghelpdesk@nhs.net.

Appendix 1 Fax template for notification of CF screening not completed (by screening laboratory to GP and/or CHRD)

**For patient’s notes**

<table>
<thead>
<tr>
<th>Item</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby’s name</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>Gender</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>D.O.B</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>Birth weight</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>Gestation</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>NHS number</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>Address</td>
<td>_______________________________________________</td>
</tr>
</tbody>
</table>

**To the GP**

Please be aware that the above baby has not received screening for cystic fibrosis (CF). This is because [delete as appropriate the baby has moved into the country or was tested late (after 8 weeks of age), the parents declined screening] and no screening specimens have been collected.

Screening specimen date(s) ______________________________________________________________

The screening tests for phenylketonuria, medium-chain acyl-CoA dehydrogenase deficiency (MCADD), sickle cell disease and congenital hypothyroidism are ______________________________
______________________________________________________________________________________

It should be explained to the family (usually by the family health visitor) why their baby/infant has not been screened for CF. Such babies should not routinely be offered any testing and only be referred on a clinical basis if required.

To the GP - If the family (or GP) has been worried about the baby before today (for example, persistent cough or poor weight gain) then you should discuss referral to the local CF centre with the family for further assessment. Similarly, if you or the family have concerns in the future about the child (for example persistent cough, poor weight gain, loose stools, rectal prolapse) then referral to the CF team should be considered.

**CF Centre [insert name]**

<table>
<thead>
<tr>
<th>Item</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>Telephone</td>
<td>_______________________________________________</td>
</tr>
<tr>
<td>Email</td>
<td>_______________________________________________</td>
</tr>
</tbody>
</table>

If you have any questions about this fax, do not hesitate to contact the screening laboratory.

Signed: ________________________________   Date: _________________________

**Screening laboratory contact details:** ___________________________________________
Appendix 2 Template for notification of presumptive positive for designated clinician (by screening laboratory to clinician)

For patient’s notes

Baby’s name ____________________________________________
Gender ________________________________________________
D.O.B _________________________________________________
Birth weight __________________________ Gestation ____________
NHS number ___________________________________________
Address _______________________________________________
______________________________________________________________________
GP ______________________________________________________
To the clinician __________________________________________

Screening specimen date(s) __________________________________

The above baby was found to have a positive (abnormal) newborn screening test for cystic fibrosis (CF). The results from the mean blood spot immunoreactive trypsinogen (IRT) concentration were __________________________ ng/mL. The screening tests for phenylketonuria, medium-chain acyl-CoA dehydrogenase deficiency (MCADD), sickle cell disease and congenital hypothyroidism are __________________________________________________________________________________________
____________________________________________________________________________________

The mutation analysis shows ______________________________________
____________________________________________________________________________________

Recommended action as per CF Screening Programme Guidelines for Clinical Referral (available from www.newbornbloodspot.screening.nhs.uk/cf):

• Parents should not be informed of a positive screening result on a Friday, Saturday or Sunday. Ideally, Thursday should be avoided as well. Parents should be offered an appointment on the following day.
• The baby must be seen within five working days of the specialist centre being informed of a positive result.
• A sweat test should be performed at the first visit according to the ACB sweat test standards.
• The baby will be reviewed by the paediatrician and the CF nurse specialist and a full assessment undertaken.
• If a sweat test proves normal (sweat chloride concentration less than 30 mmol/L, mutation analysis must be performed on a repeat blood sample to verify the screening result.

Signed: ________________________________   Date: _________________________

Screening laboratory contact details: __________________________________________
Resources available from [www.newbornbloodspot.screening.nhs.uk/cf](http://www.newbornbloodspot.screening.nhs.uk/cf)

‘Cystic fibrosis is suspected’ leaflet

‘When Cystic Fibrosis is Suspected: Communication Guidelines’
Appendix 3 Extract from Human Genetics Commission (reporting of carriers)

Human Genetics Commission

Making babies: reproductive decisions and genetic technologies

January 2006

Chapter 3. Page 43.

3.48 The extension of the neonatal screening programme to cystic fibrosis (CF) highlights the issue of detecting carriers for these recessive conditions. Screening for this condition with the current testing technique identifies only a very small proportion of carriers, and they require clinical assessment to ensure that they are not affected by the disease. The numbers of CF carrier infants identified in this way is similar to the number of affected infants likely to benefit from early diagnosis. Whilst some would say this information ought not to be divulged, others, including patient representatives, argue strongly that the result of the tests, once generated, should be given to the parents. We support this latter position and this information should not be withheld from parents who indicate that they wish to have it when agreeing to have their child tested.

(see also 3.49 and 3.50)
Appendix 4 Fax template for notification of probable CF carrier (by screening laboratory to GP)

For patient’s notes

<table>
<thead>
<tr>
<th>Field</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby’s name</td>
<td>____________________</td>
</tr>
<tr>
<td>Gender</td>
<td>____________________</td>
</tr>
<tr>
<td>D.O.B</td>
<td>____________________</td>
</tr>
<tr>
<td>Birth weight</td>
<td>____________________</td>
</tr>
<tr>
<td>NHS number</td>
<td>____________________</td>
</tr>
<tr>
<td>Address</td>
<td>____________________</td>
</tr>
<tr>
<td></td>
<td>____________________</td>
</tr>
<tr>
<td>To the GP</td>
<td>____________________</td>
</tr>
</tbody>
</table>

**Screening specimen date(s)** ____________________

The above baby was found to have an abnormal newborn screening test for cystic fibrosis (CF), suggesting that he/she is a **carrier** for CF. The result suggests that a diagnosis of cystic fibrosis is unlikely. The results from the mean blood spot immunoreactive trypsinogen (IRT) concentration were ____________________ ng/mL. The screening tests for phenylketonuria, medium-chain acyl-CoA dehydrogenase deficiency (MCADD), sickle cell disease and congenital hypothyroidism are ____________________

The mutation analysis shows ____________________

What happens next?
The nurse from the screening laboratory has made arrangements to visit the family at home with the family health visitor on **[insert date and time of appointment]** to explain the result. The baby does not require any further clinical follow-up. Below is a link to the information sheet that will be given to the family.

Do I need to do anything?
The family may wish to be referred for genetic counselling to discuss options for further testing for the family. They will be advised to contact you should this be their request. If the family wishes to be referred for genetic counselling for further testing, they should be referred to **[insert name and address of regional genetics centre]**.

What if I am concerned about the baby, now or in the future?
If you have been worried about the baby before today (for example, persistent cough or poor weight gain) then you should discuss referral to the local CF Centre with the family for further assessment. Similarly, if you or the family have concerns in the future about the child (for example persistent cough, poor weight gain, loose stools, rectal prolapse) then referral to the CF team should be considered.
CF Centre [insert name]
Address
Telephone
Email

If you have any questions about this fax, do not hesitate to contact either the screening laboratory [inset telephone number and email address] or the CF Centre above.

Signed: ________________________________   Date: _________________________________________

Screening laboratory contact details: ____________________________________________________

Resources available from [www.newbornbloodspot.screening.nhs.uk/cf](http://www.newbornbloodspot.screening.nhs.uk/cf)

‘Carrier of cystic fibrosis gene’ leaflet

‘Communication Guidelines: Carrier of Cystic Fibrosis Gene’