

## Screening Programmes

A Laboratory Guide to Newborn Screening in the UK for

# CYSTIC FIBROSIS



**UK Newborn Screening Programme Centre**

*[www.newbornbloodspot.screening.nhs.uk](http://www.newbornbloodspot.screening.nhs.uk)*





# 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 referral and follow-up
- Reporting to child health records departments (CHRDs)
- Laboratory standards and guidelines
- Quality and performance monitoring
- Data collection and audit
- References and further reading

## Major contributions from:

**Professor Anne Green, Dr David Isherwood and Professor Rodney Pollitt.**



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This is the 3rd edition of the guide

A Laboratory Guide to Newborn Screening in the UK for Cystic Fibrosis

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**UK Newborn Screening Programme Centre**

Level 5, Frontage Building  
Great Ormond Street Hospital for Children NHS Trust  
Great Ormond Street  
London WC1N 3JH

<http://www.newbornbloodspot.screening.nhs.uk>

Phone: 020 7829 7883/7884

Fax: 020 7829 7881





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# 1 Background

## 1.1 Introduction

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 National Screening Committee (NSC) endorsed the protocols recommended by the CF Screening Programme 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 UK Newborn Screening Programme Centre (UKNSPC) website (<http://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 turn out to have CF and 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 UK Newborn Screening Programme Centre, Level 5, Frontage Building, Great Ormond Street Hospital, Great Ormond Street, London, WC1N 3JH ([uknewbornscreen@gosh.nhs.uk](mailto:uknewbornscreen@gosh.nhs.uk)) for incorporation into the next edition.

## 1.2 Scope and purpose

This document provides guidance for the laboratories which 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 (<http://www.cftrust.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 (now known as p.Phe508del), 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 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 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, 1998) 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 Rosenberg (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 McKay (2007) has reviewed systematic studies 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 and the AutoDELFIATM instrumentation used in screening for congenital hypothyroidism. 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. In those areas 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 separately from reporting the test results. This functionality is incorporated into the status codes to be used when electronic reporting to CHRD systems is introduced – see section 9.1.



## 2 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 standard 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 two-stage mutation analysis of the CFTR gene on all samples with IRT values above the **99.5<sup>th</sup> 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 between day 21 and day 28 of life and assayed for IRT (section 7):-
  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.

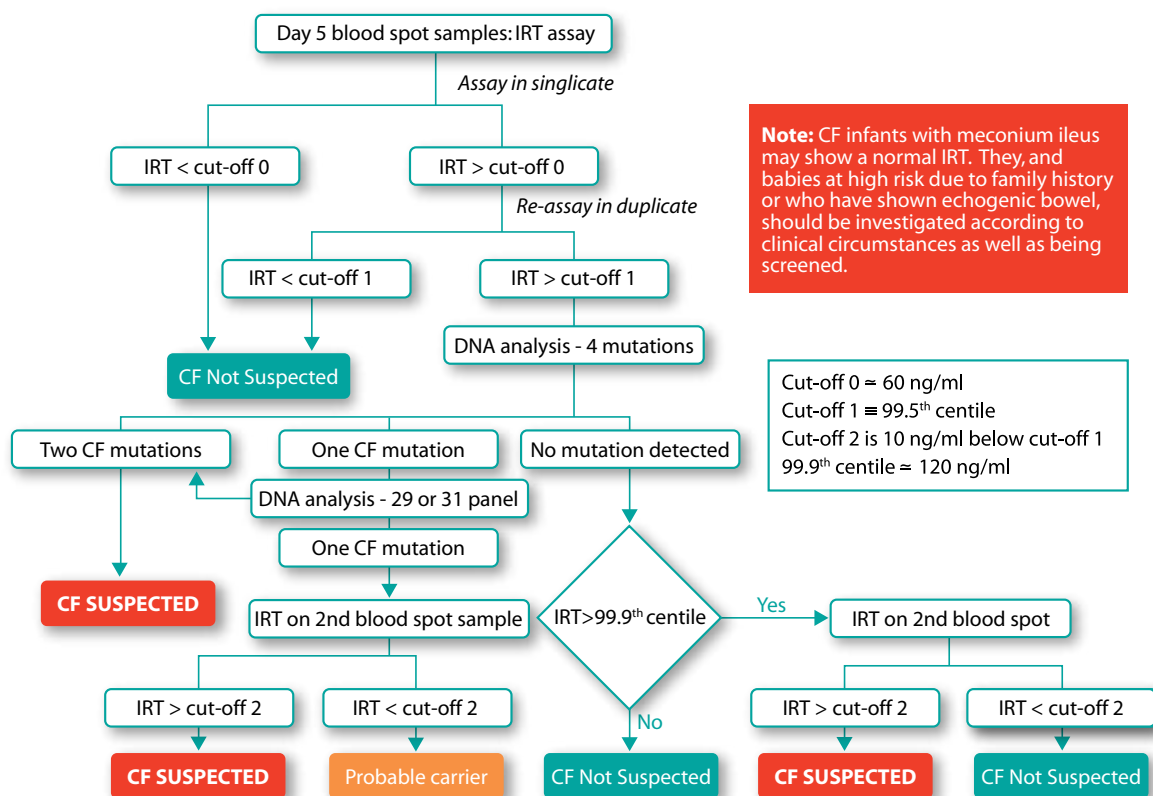
The main aim of this approach is to minimise emotional trauma for parents of unaffected carrier babies (Parsons *et al.*, 2003) and to reduce the number of negative sweat tests performed. With this approach to families there is less pressure on professionals dealing with 'low likelihood' cases to provide 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** are divided into two groups depending on the initial IRT result:
  1. Those with IRT below the 99.9<sup>th</sup> centile: report as **'CF Not Suspected'**.
  2. Those with IRT above the 99.9<sup>th</sup> centile: a second IRT test is undertaken on a repeat dried blood spot specimen taken between day 21 and day 28 of life (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 > 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 flow chart**



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 according to clinical procedures.

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 5-8 (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 >99.9<sup>th</sup> centile) it should be collected between day 21 and day 28.

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:

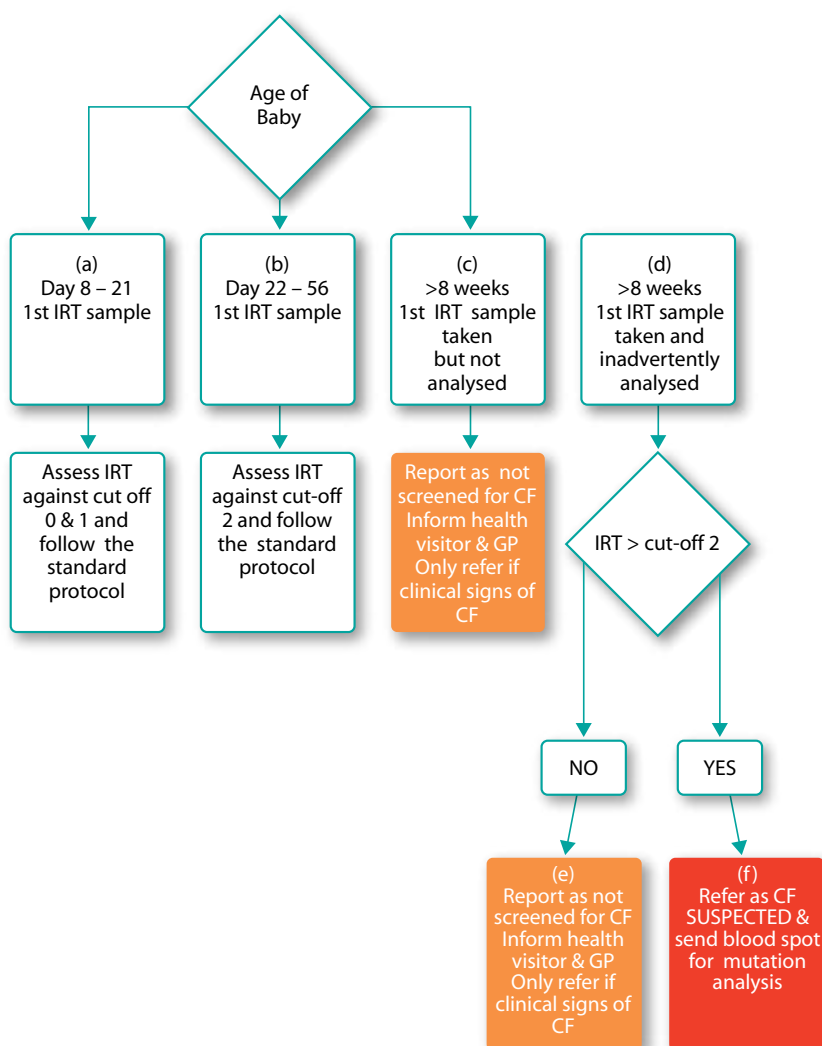
1. Babies who have arrived in the UK after 8 weeks (56 days) of age
2. Babies who have failed to have their first screening sample collected by 8 weeks of age
3. Babies who require a second blood spot sample for IRT assay but fail to have it taken at the correct time (sampling delayed or sample lost 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 standard protocol.
- b. For samples taken after baby is 3 weeks (21 days) but less than 8 weeks 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 of age **should not be IRT tested**. Procedures should be used in laboratories to select out these samples.
- d. If a first (routine screening) sample taken after 8 weeks is **inadvertently** tested then proceed as follows:
- e. If the IRT is < cut-off 2 it should be reported: **‘CF – Not Screened (baby too old >8 weeks age)’**.
- f. If the IRT is > 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’**.

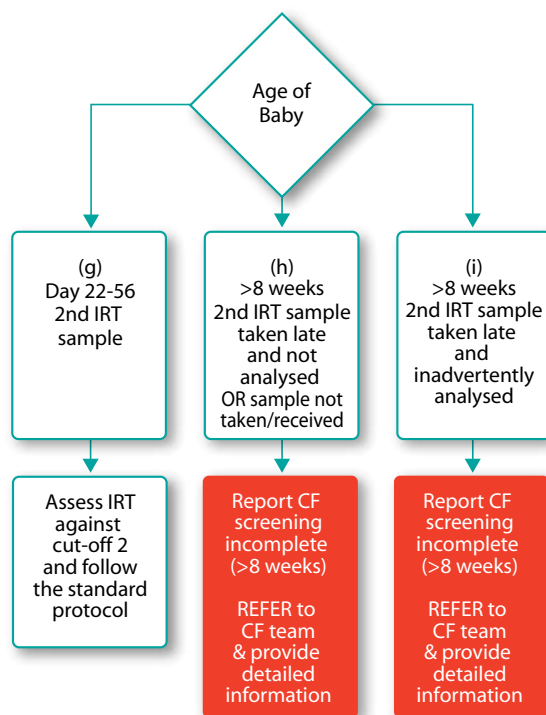
**Figure 2. Handling of late first IRT samples**



### 2.3.2 Late or absent second samples (Figure 3)

- g. For second samples taken by 8 weeks cut-off 2 is applied as in the standard protocol.
- h. 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 - Screening Incomplete (second sample taken when baby too old > than 8 weeks age)’**. 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.
- i. If the second sample is taken after 8 weeks and inadvertently analysed the baby should be referred to a CF clinical team regardless of the IRT concentration, i.e. same action as for above (h).

**Figure 3. Handling of late second IRT samples**



## 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 refused) 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. Such babies should not routinely be offered any testing and 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.



## 3 Pre-analytical aspects

### 3.1 Family history and other risk factors

Babies presenting with meconium ileus, those who have shown echogenic bowel *in-utero*, or are known to be at risk due to family history should be regarded as high risk and should be investigated independently according to clinical circumstances as well as being screened in the normal way.

In cases where mutation testing has been undertaken before routine screening it is advantageous for the results to be communicated to the screening laboratory. Where such results are communicated this may avoid unnecessary duplication of follow-up or diagnostic testing. It will also help avoid any confusion arising from a 'CF Not Suspected' screening result (from IRT analysis) which might cause concern for the family.

### 3.2 Specimen requirements

Faeces contain large amounts of trypsin/trypsinogen and even trace contamination of blood spots by faeces (not visible to the naked eye) can increase the IRT result. It is therefore extremely important that the heel should be carefully cleaned before the sample is taken (see UKNSPC Guidelines for Newborn Blood Spot Sampling, 2008 <http://newbornbloodspot.screening.nhs.uk/bloodspotsampling>).

The CF screen requires a good quality blood spot for the IRT assay. Specimens that are overlaid by multiple applications are likely to give falsely raised results and be genotyped unnecessarily. If the blood does not fully percolate to the reverse side of the sample paper the IRT could be measured falsely low, leading to a false negative result.

Anti-coagulated blood should not be used.

IRT is rather unstable – see section 4.4. Samples received in the laboratory more than 14 days after the date of collection are unsuitable for testing and a repeat sample must be requested as soon as possible. This is a general requirement for all dried blood spot screening tests.

For CF it is important to be aware that samples taken when the baby is more than 8 weeks old are not usually analysed (see section 2.3).

### 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. These babies should be investigated according to clinical circumstances; 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 transfusion, misleading results with mutation analysis are unlikely (Brauner *et al.*, 1997).
- **Viral infection** leading to acute gastroenteritis or respiratory illness is also sometimes 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 normal range of 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. High IRT levels can also 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 The IRT assay

Immunoreactive trypsinogen in the routine newborn screening blood spot is to be assayed using the PerkinElmer AutoDELFIA™ Neonatal IRT kit (B005-112) and the AutoDELFIA™ immunoassay system, following the procedures detailed in the manufacturer's instructions. Procedures for specimen identification and disk punching are similar to those for the thyroid-stimulating hormone (TSH) screening assay.

The AutoDELFIA™ assay measures the immunoreactive trypsinogen species as ng/ml (= µg/L as SI units).

### 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).

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 PerkinElmer for two laboratory buddy groups in the UK was implemented in 2007 in order to minimise variation and gain greater consistency in setting cut-off values. There is a kit shelf life of up to 9 months.

One buddy group is comprised of the laboratories at Belfast, Birmingham, Cambridge, Glasgow, Liverpool, Manchester, Newcastle, Sheffield and Portsmouth and the other group of Bristol, Cardiff, Carshalton, Leeds, London (Lewisham and Great Ormond Street), and Oxford. Each buddy group covers approximately 420 000 tests per annum (approx 50 % UK births); these workloads include assays of repeats, quality assurance samples and standards.

Although this grouping procedure has been implemented in the UK to 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. The suppliers (PerkinElmer) state that the variation is within +/-8 % from batch to batch of IRT kits, although aim 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 lab should continue to use the existing cut-offs (i.e. from the previous kit batch). PerkinElmer is 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 and laboratories should note the guidance about mean, median and percentile IRT values published by the manufacturer in the AutoDELFIA™ Neonatal IRT literature supplied with their assay kits.

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 98.5<sup>th</sup> percentile and this will have a wider 95 % confidence interval. The 99<sup>th</sup> percentile is typically 64.3 ng/ml with a 95 % confidence range 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 Internal quality control

The AutoDELFIA™ kits from PerkinElmer (PE) include internal quality control samples at three IRT levels; these should be included with each analysis batch. The manufacturers state that the established mean for each of the three controls should be within +/-20 % of the values stated on the QC certificate.

The actual inter-assay coefficient of variation (CV) in UK laboratories for the same control samples supplied with the AutoDELFIA™ Neonatal IRT kit typically ranges from 7-10 % (UKNSPC IRT Quality Working Group).

Dried blood spots available from the Centers for Disease Control and Prevention (CDC) can also be used for internal quality control purposes and achieve similar levels of precision with CV approx 5–7 % at IRT concentrations approx 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 1. Precision data from a UK laboratory**

Mean IRT ng/ml	SD	CV
25.4 *	2.30	9.0
64.9 *	5.30	8.2
90.0 *	6.56	7.2
125.8 **	7.45	5.9
224.4 **	14.36	6.4

\* PE control materials n=36

\*\* CDC control materials n=10

Samples obtained from babies have less precision than artificial control samples prepared in standardised conditions. 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 %.

## 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.

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 (99.5<sup>th</sup> centile):

Experience in East Anglia using a two-stage IRT-IRT screen over a 17 year period showed that a 99.5<sup>th</sup> 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 99.5<sup>th</sup> centile approximates to 70 ng/ml when using AutoDELFIA™ kits and 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 be classified as 'Probable carriers'.

### **The 99.9<sup>th</sup> centile:**

The number of samples above the 99.9<sup>th</sup> 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 The first screening specimen

Samples are initially assayed in singlicate.

For babies with IRT value below cut-off 0 a negative result '**CF Not Suspected**' is issued.

Samples with results greater than 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 result 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<sup>th</sup> 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 above the 99.5<sup>th</sup> centile (cut-off 1) they should be assessed for consistency before proceeding further.

**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
- One or more individual results being >30 % above or below the average of all three
- 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<sup>th</sup> cut-off) with the other two below the 99.5<sup>th</sup> centile 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.
- b) If two results are above the 99.5<sup>th</sup> centile and the average of all three results lies between the 99.5<sup>th</sup> centile and 99.9<sup>th</sup> centile, proceed to mutation analysis as per standard protocol.
- c) If the average of all three results is above the 99.9<sup>th</sup> centile, 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 disks from the card (i.e. for a single result above cut-off 0) then a repeat specimen, to be taken as soon as possible, should be requested due to insufficient sample.

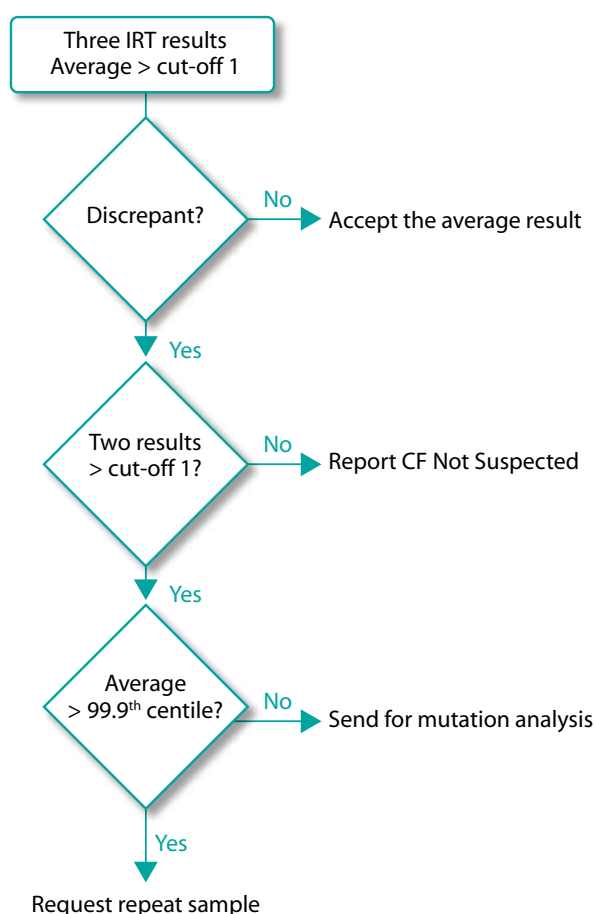
## 5.3 Only one re-assay possible

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:

- if the average of the two results is below cut-off 1 report '**CF Not Suspected**'.
- the average of both results are above the 99.5 % 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**





## 6 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 disk punched out is likely to be sufficient. It is recommended that the newborn screening blood sample collection card itself should not 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 write the identifiers, including the baby's date of birth, surname, and NHS Number (when available) on the blank section of the strip. 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 lab should be acknowledged back to the screening lab.

For the analysis careful check procedures are required to ensure that there is no transposition of identity.

## 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 UK population associated with severe disease. Nomenclature has been recently revised to comply with the Human Genome Variation Society Guidelines to ensure consistent and precise nomenclature\*. The four mutations are as detailed below with the terminology in brackets being the old versions:

p.Phe508del	c.1521_1523delCTT	(ΔF508)	See note 1
p.Gly551Asp	c.1652G>A	(G551D)	See note 2
p.Gly542X	c.1624G>T	(G542X)	
	c.489+1G>T	(621+1G>T)	

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

*Note 2: Depending on the technology used, p.Arg553X, c.1657C>T, (R553X) may also be detected.*

This first stage will detect >80 % of disease-causing mutations in the UK population.

There are a number of technologies in use including the Tepnel CF4 ARMs™ kit, pyrosequencing and a range of other commercial kits. When using the latter all but the alleles specified must be masked. It is highly desirable that regardless of the technology used for this first stage, no other mutations are inadvertently 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 at the first analysis. Commercially available panels covering approximately 30 mutations are suitable for this; the choice is left to molecular genetics laboratories depending on available technology.

*\* The first 3 mutations are 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 non-coding and has no directly associated amino acid change. The numbering describes that by saying the 1<sup>st</sup> nucleotide (+1) after nucleotide 489 (which is at the last nucleotide of the exon) will be mutated.*

### 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 (section 8.1).

*One CFTR mutation detected* – request a second blood spot sample for IRT analysis, to be taken between day 21 and day 28 of age (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<sup>th</sup> centile a, '**CF Not Suspected**', result is issued. For results >99.9<sup>th</sup> centile, a second blood sample is to be taken between day 21 and day 28 of age (section 7).



## 7 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 >99.9<sup>th</sup> 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 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 lest this should 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 UK Newborn Screening Programme Centre web site ([http://newbornbloodspot.screening.nhs.uk/hp\\_comms\\_guidelines](http://newbornbloodspot.screening.nhs.uk/hp_comms_guidelines)).

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 above 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 >99.9<sup>th</sup> percentile**

This second sample is assayed for IRT in duplicate. Babies with an average result above 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 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) and those cases where screening is incomplete (see sections 8.1 and 8.3). 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 UK Newborn Screening Programme Centre website ([http://newbornbloodspot.screening.nhs.uk/nat\\_std\\_cf\\_protocol](http://newbornbloodspot.screening.nhs.uk/nat_std_cf_protocol))

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 2001 available at: [http://www.cftrust.org.uk/aboutcf/publications/consensusdoc/C\\_3000Standards\\_of\\_Care.pdf](http://www.cftrust.org.uk/aboutcf/publications/consensusdoc/C_3000Standards_of_Care.pdf) and also the National Specialised Services Definition Set: 10 Cystic Fibrosis available at: [http://www.nscg.nhs.uk/documents/ssnds10\(v3\).pdf](http://www.nscg.nhs.uk/documents/ssnds10(v3).pdf)

### 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 high IRT *or*
- high IRT concentration in two blood samples taken approximately 3 weeks apart

They should be referred to a designated clinician for CF or their deputy according to locally agreed and documented procedures within 24 hours of the definitive screening result becoming available. The regional CF centre will usually be responsible for liaising with a more local CF clinic (as required by local arrangements).

### 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 1).

Many of these families will already have raised anxieties because a second screening sample has been collected. The family should therefore be contacted within 24 hours 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 UKNSPC leaflet "Results of Newborn Blood Spot Screening - Carrier of cystic fibrosis gene" ([http://newbornbloodspot.screening.nhs.uk/cf\\_info\\_leaflets](http://newbornbloodspot.screening.nhs.uk/cf_info_leaflets)).

Irrespective of who conveys this information to the family, the baby's GP must be formally notified of the findings from screening by the screening laboratory and be sent a copy of the molecular genetics report and of the carrier leaflet for parents ([http://newbornbloodspot.screening.nhs.uk/hp\\_comms\\_guidelines](http://newbornbloodspot.screening.nhs.uk/hp_comms_guidelines)).

### 8.3 Follow-up of cases with incomplete screening

These are babies who require a second sample for IRT analysis but which cannot be completed (see section 2.3.2). The referral documentation from the screening laboratory needs to make the reason clear.



## 9 Reporting and communication of results

Screening results should be reported by the screening laboratory to the child health records department (CHRD) - 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' or 'results incomplete') are communicated directly to the parents by an appropriate health professional – see section 8.

For babies who are referred the screening laboratory reports the results directly the clinician to whom the child is referred with copies to the health visitor and GP. Templates suitable for written communication from the laboratory e.g. in the form of letters or memos are available on the UKNSPC website ([http://newbornbloodspot.screening.nhs.uk/hp\\_comms\\_guidelines](http://newbornbloodspot.screening.nhs.uk/hp_comms_guidelines))

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 implementation of status codes are currently under development and will depend on the local IT in use. The status codes can be found on the Programme Centre's website and are summarised below for CF (<http://newbornbloodspot.screening.nhs.uk/statuscodes>).

**Table 2. Status codes for CF**

Code	Suggested term to be displayed in the child health system	Comments with reference to CF
01	Specimen received in laboratory	
02	CF screening declined	
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 2 <sup>nd</sup> IRT sample needed
04	CF not suspected	<b>First sample:</b> Use for results on first sample IRT < cut-off 0 ( <i>singlicate analysis</i> ) IRT < cut-off 1 ( <i>after repeat duplicate analysis</i> ) IRT <99.9 <sup>th</sup> percentile and no CFTR mutations have been detected. <b>Second sample:</b> Use for 2 <sup>nd</sup> 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	<i>These will include the following categories:</i> <ul style="list-style-type: none"> <li>• Two mutations detected</li> <li>• One mutation and second IRT &gt; cut-off 2</li> <li>• No mutations, first IRT &gt;99.9<sup>th</sup> percentile and 2<sup>nd</sup> IRT &gt; cut-off 2</li> </ul> (also includes babies with a high IRT in a sample taken after 8 weeks)
09	CF not screened/ screening incomplete	Use with additional qualifying terms for: - <ul style="list-style-type: none"> <li>• baby who has died i.e. before first sample/repeat sample</li> <li>• the baby is &gt;8 weeks old (no sample taken) at first sample or the baby is &gt;8 weeks old with an initial high IRT requiring follow-up *</li> </ul> * <i>It is critical that a process is in place to ensure this baby is recalled for follow up.</i> <ul style="list-style-type: none"> <li>• baby has been transferred out of screening laboratory area and is still awaiting the collection of a repeat first or second IRT sample.</li> </ul>



## 10 Laboratory standards and guidelines

### 10.1 Generic standards

The UKNSPC has generic standards (2008) for blood spot screening relating to completeness of offer, tracking, timely sample collection and despatch, quality of blood spot sample, timely sample receipt, processing of screen positives and completeness of uptake programme coverage; these standards include CF screening.

See the UK Newborn Screening Programme Centre Policies and Standards –

Standards and Guidelines for Newborn Blood Spot Screening 2008 available at:

<http://newbornbloodspot.screening.nhs.uk/standards#fileid10827>.

### 10.2 Screening laboratory

#### Organisation

- Newborn screening for CF should be provided within the organisational structure of the newborn blood spot screening programme. It should be undertaken by specialist newborn screening laboratories already providing screening programmes for phenylketonuria, congenital hypothyroidism, medium-chain acyl-CoA dehydrogenase deficiency and sickle cell disorders.
- Laboratories screening for CF must be accredited by Clinical Pathology Accreditation (UK). There must be a member of staff at consultant level responsible for CF screening with defined lines of accountability for all aspects of the service.
- There should be written agreed procedures describing the working arrangements between the screening laboratory and their partner molecular genetics laboratory.
- There should be documented local policies and standard operating procedures covering all aspects of newborn screening including pre-analytical, analytical and post-analytical procedures; these include reporting of normal and abnormal results, and follow-up procedures for presumptive positive and carriers results. Processes must be provided in line with relevant national standards and guidance and should be reviewed regularly taking into account audit data, accumulating results, technical developments and local changes in healthcare provision.

#### Analytical processes

- Assay for immunoreactive trypsinogen must be performed by an approved method (currently PerkinElmer AutoDELFIA™ Neonatal IRT kit (B005-112) and the AutoDELFIA™ immunoassay system). Any proposal to introduce new analytical methods needs careful collective consideration by the Cystic Fibrosis Scientific Advisory Group and approval by the Cystic Fibrosis Board.
- 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 should provide data on CF screening to the National CF Screening Programme at the UK Newborn Screening Programme Centre as well as regional and local audit/quality management groups as required.
- The results and performance of the CF Screening Programme should be included within an annual report produced by the screening laboratory for circulation to local directors of public health (and others as required). Normally this will be a combined report covering all blood spot screens, dealing also with common issues such as specimen quality, timeliness, etc. There should also be periodic multidisciplinary review of local policies for CF screening in the light of accumulated results, technical developments and any local changes in health care provision.
- There must be a documented risk management policy for the laboratory aspects of the CF Screening Programme.

## 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 (<http://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

### Timelines

- 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 4<sup>th</sup> working day from receipt of the sample from the screening laboratory.
- Presumptive positive results should be reported to the appropriate clinical team within 24 hours of becoming available. Intermediate reports (i.e. increased IRT in the initial sample without follow-up results) should not be issued.

*Note: Babies in whom CF screening is incomplete (see section 2.3.2) should be referred within 24 hours of knowing this is the situation*

## Quality assurance

- Laboratories must undertake appropriate internal quality control procedures for the screening test and demonstrate satisfactory performance in an approved external quality assurance scheme (Note; there is currently no formally accredited scheme) as part of the UKNSPC Quality Management arrangements; laboratories are expected to review, on a regular basis, their IRT population statistics.
- The molecular genetics laboratory must participate in the UK NEQAS for Molecular Genetics (<http://www.ukneqas-molgen.org.uk>) dried blood spot CFTR mutation scheme (currently organised by the Northern Genetics Service, Institute of Human Genetics, Newcastle upon Tyne, NE1 3BZ) and demonstrate satisfactory performance. All laboratories receiving samples from the CF newborn screening molecular QA programme must complete annual returns of full audit data to the UKGTN laboratory standards committee.
- The UK Newborn Screening Programme Centre and NHS Sickle Cell and Thalassaemia Screening Programme's National Newborn Blood Spot Laboratory Quality Assurance Development Group is currently reviewing quality assurance arrangements for dried blood spot screening. Screening laboratories and molecular genetics laboratories will be made aware of any changes in quality assurance arrangements and will need to follow any new policies when they are introduced.



## 11 Data Collection and Audit

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 (<http://newbornbloodspot.screening.nhs.uk/datacollection>) should be collected by the directors of newborn screening in each area and submitted on a retrospective basis to the UKNSPC by the 31<sup>st</sup> July for the previous financial year (1<sup>st</sup> April - 31<sup>st</sup> March).

The clinical data required (<http://newbornbloodspot.screening.nhs.uk/datacollection>) to accompany the screening data should be requested by the laboratory directors on a case by case basis; data on each case (presumptive positive and screening incomplete cases) notified to the clinical referral services should be collated and anonymised by the laboratory directors before submission to the UKNSPC. 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 (<http://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 completed form should be returned to the laboratory director and anonymised before transmission to the UKNSPC.

If a laboratory director is made aware of a CF case (born after April 1<sup>st</sup> 2007) that has been reported as 'CF Not Suspected' it is very important that information on the case be reported to the UKNSPC. The details should be gathered by the clinical team in conjunction with the laboratory director using the 'False Negative' form (<http://newbornbloodspot.screening.nhs.uk/datacollection>). The screening laboratory should issue the relevant clinical team with this form. The data should be collated and anonymised by the laboratory director and returned as soon as possible to UKNSPC. Data on these false negative cases will be collated on an annual basis as an important part of the audit of the programme.



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# Appendix 1

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**Making babies: reproductive decisions and genetic technologies**

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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)



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Any comments on the content of this handbook should be sent for the attention of the CF Scientific Advisory Group c/o the UK Newborn Screening Programme Centre: [uknewbornscreen@gosh.nhs.uk](mailto:uknewbornscreen@gosh.nhs.uk)

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# Screening Programmes



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