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# A European consensus for the evaluation and management of infants with an equivocal diagnosis following newborn screening for cystic fibrosis

S.J. Mayell [a], A. Munck [b], J.V. Craig [a], I. Sermet [c], K.G. Brownlee [d], M.J. Schwarz [e], C. Castellani [f], K.W. Southern [a,*]
On behalf of the European Cystic Fibrosis Society Neonatal Screening Working Group [1]

* Corresponding author. Institute of Child Health, University of Liverpool, Royal Liverpool Children's Hospital, Eaton Road, Alder Hey, Liverpool, L12 2AP, United Kingdom. Tel.: +44 151 2525054; fax: +44 151 2525456.
*E-mail address:* kwsouth@liv.ac.uk (K.W. Southern).

[1] Cystic Fibrosis Specialists who contributed to the consensus:

| | |
|---|---|
| Balascakova Miroslava | Institute of Biology and Medical Genetics, Charles University Prague-2, Czech Republic |
| Barben Juerg | Children's Hospital, St Gallen, Switzerland |
| Gabriel Bellon | Hopital Debrousse, Lyon, France |
| Brownlee Keith | Regional Paediatric CF Unit, St James University Hospital, Leeds, United Kingdom |
| Burrows Elinor | Royal Liverpool Children's Hospital, Liverpool, United Kingdom |
| Bush Andrew | Royal Brompton Hospital, London, United Kingdom |
| Castellani Carlo | Cystic Fibrosis Center, Azienda Ospedaliera Verona, Piazzale Stefani 1, 37126 Verona, Italy |
| Corbetta Carlo | Laboratorio di Riferimento regionale, Ospedale dei Bambini "V. Buzzi", Milano, Italy |
| Dankert-Roelse Jeannette | Atrium Medical Centre, Department of Pediatrics, Heerlen, The Netherlands |
| De Boeck Kristiane | Department of Pediatric Pulmonology, University Hospital, Leuven, Belgium |
| Desai Maya | Birmingham Children's Hospital, Birmingham, United Kingdom |
| Dodge John | University of Wales, Swansea, United Kingdom |
| Doull Iolo | Children's Hospital for Wales, Cardiff, United Kingdom |
| Eichler Irmgard | Cystic Fibrosis Center, University Children's Hospital, Vienna, Austria |
| Green Anne | Birmingham Children's Hospital, Birmingham, United Kingdom |
| Huet Frederic | Hôpital d'enfants, Dijon and AFDPHE, Paris, France |
| Holubova Andrea | Institute of Biology and Medical Genetics, Charles University Prague-2, Czech Republic |
| Iapichino Luciana | Centro Regionale Fibrosi Cistica, Palermo, Italy |
| Lebecque Patrick | Cliniques St Luc, Université Catholique de Louvain, Brussels, Belgium |
| Macek Milan | Institute of Biology and Medical Genetics, Charles University Prague-2, Czech Republic |
| Melotti Paola | Cystic Fibrosis Center, Azienda Ospedaliera di Verona, Verona, Italy |
| Munck Anne | Hôpital Robert Debré & AFDPHE, Paris, France |
| Padoan Rita | Cystic Fibrosis Service, Spedali Civili Brescia, Italy |
| Quattrucci Serena | Department of Paediatrics, University of Rome, Rome, Italy |
| Reid Alastair | Royal Belfast Hospital for Sick Children, Belfast, UK |
| Renner Sabine | Cystic Fibrosis Center, University Children's Hospital, Vienna, Austria |
| Roussey Michel | Hopital Sud, Rennes and AFDPHE, Paris, France |
| Satish Rao | Birmingham Children's Hospital, Birmingham, United Kingdom |
| Sands Dorota | Institute of Mother and Child, Warsaw, Poland |
| Seia Manuela | Laboratorio Genetica Molecolare, Fondazione Policlinico, Mangiagalli, Regina Elena, Milano, Italy |
| Sermet Isabelle | Hopital Necker Enfants Malades, Paris, France |
| Skalicka Veronika | Cystic Fibrosis Centre Prague, University Hospital Motol, Prague, Czech Republic |
| Southern Kevin | University of Liverpool, Royal Liverpool Children's Hospital, Liverpool, United Kingdom |
| Schwarz Martin | Regional Molecular Genetics Laboratory, St Mary's Hospital, Manchester, United Kingdom |
| Taylor Christopher | Sheffield Children's Hospital, United Kingdom |
| Taccetti Giovanni | Cystic Fibrosis Centre, Meyer Hospital, Florence, Italy |
| Tiddens Harm | Dept. Pediatric Pulmonology , Erasmus MC/Sophia children's Hospital, Rotterdam, Netherlands |
| Tümmler Burkhard | Klinische Forschergruppe, Medizinische Hochschule, Hannover, Germany |
| Vavrova Vera | Department of Pediatrics, Charles Univ. Prague-2, School of Medicine, Czech Republic |
| Votava Felix | Department of Pediatrics, Charles Univ. Prague-3, School of Medicine, Czech Republic |
| Weller Peter | Birmingham Children's Hospital, Birmingham, United Kingdom |
| Wilschanski Michael | Hadassah University Hospital, Jerusalem, Israel |


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[a] University of Liverpool, United Kingdom
[b] Hopital Robert Debre, AP-HP and AFDPHE, Paris, France
[c] Hopital Necker Enfants Malades, Paris, France
[d] Regional CF Unit, St James's University Hospital, Leeds, United Kingdom
[e] Regional Molecular Genetics Service, St Mary's Hospital, Manchester, United Kingdom
[f] Cystic Fibrosis Center, Azienda Ospedaliera, Verona, Italy

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## Abstract

Screening newborns for cystic fibrosis (CF) is considered to be an ethical undertaking in regions with a significant incidence of the condition. Current screening protocols result in recognition of infants with an equivocal diagnosis. A survey of European practice suggested inconsistencies in the evaluation and management of these infants.

We have undertaken a consensus process using a modified Delphi method. This has enabled input of CF specialists from a wide geographical area to a rigorous process that has provided a clear pathway to a consensus statement. A core group produced 21 statements, which were modified over a series of three rounds (including a meeting arranged at the European CF Conference). A final document of 19 statements was produced, all of which achieved a satisfactory level of consensus. The statements cover four themes; sweat testing, further assessments and investigations, review arrangements and database.

This consensus document will provide guidance to CF specialists with established screening programmes and those who are in the process of implementing newborn screening in their region.


*Keywords:* Cystic fibrosis; Neonatal screening; Management; Sweat test; Gene analysis

## 1. Introduction

There is a good agreement that screening newborns for cystic fibrosis (CF) is a valid and ethical undertaking in regions such as Europe with a significant incidence of the condition [1]. Protocols for screening rely on the recognition that infants with CF have a high level of immuno-reactive trypsinogen (IRT) in their blood in the first week of life [2]. This test is sensitive but has poor specificity and therefore a second tier of investigations is necessary to identify those infants most at risk of CF [3]. In most newborn screening (NBS) protocols, this involves examining for common mutations of the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene which are associated with CF [4]. Such protocols will result in the recognition of carrier infants and data from well-established NBS programmes suggest that more carriers are recognised by NBS than might be expected for the incidence of CF in that population [5]. It has been concluded that this reflects the fact that carriers have a higher IRT than the non-carrier population and this is supported by data from population studies [6]. In most NBS protocols infants recognised as putative carriers have further assessment including a sweat test to exclude CF [4].

Another significant challenge of NBS for CF is the recognition of infants with an equivocal diagnosis [4,7]. This reflects the heterogeneous nature of the condition and poses a challenge to CF teams. Infants with one recognised *CFTR* mutation or persistent hypertrypsinaemia may have an intermediate sweat test result or an infant may be recognised with two *CFTR* mutations, one or both of which have unclear phenotypic consequences [7]. There is significant variability in the evaluation and management of these infants with an equivocal diagnosis [3,8]. We have used a modified Delphi method to form a consensus on the evaluation and management of these infants [9]. The Delphi method is a recognised technique that provides a formal strategy to gather expert opinion and form a consensus when there is a lack of high quality evidence on which to base practice. The method facilitates the inclusion of experts from a geographically disperse region and establishes a framework which makes it possible to clearly trace back how the group came to a decision.

## 2. Methods

Twenty-one preliminary statements were composed by a core group of experts in the field (CC, AM and KWS), taking into account the results of a survey of European practice [4]. The statements covered four thematic areas; sweat testing, further assessments and investigations, review arrangements and database. Two European Cystic Fibrosis Society (ECFS) working groups (the Diagnostic Network (ECFDN) and the Neonatal Screening Working Group) were approached and their members, which include clinicians, biochemists and geneticists, contacted by email. Additional invitations were made to increase multidisciplinary input. Consensus was determined *a priori* to be 80% of ratings providing agreement with the statement (considered sufficient for this type of study) [9].

For round one, specialists were asked to rate their opinion of each statement by choosing one of three options; 1) agree, 2) could agree if reworded or 3) disagree. Specialists choosing options 2) or 3) were asked for comments and also suggestions for alternative or modified statements.

After round one, statements not achieving consensus (or achieving consensus with provisos) were modified by the core

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group, taking into account the comments and suggestions made by respondents. These modified statements were then circulated in round two together with the original statements, the group ratings from round one and a summary of comments. Individuals who replied to round one were included in round two.

Following round two, statements not achieving consensus were presented and discussed in the European Cystic Fibrosis Neonatal Screening Working Group meeting at the 30th European Cystic Fibrosis Society Conference, Belek, Turkey in 2007. This meeting involved members of the consensus group (although not all) and other CF Specialists. The entire consensus document was presented, but the focus of the meeting was on statements that had not achieved consensus. This facilitated an open discussion that enabled deeper reflection on the issues around these statements. Statements were subsequently revised by the core group taking into account the discussions at this meeting and comments already received from round two. The revised statements were again circulated in round three to all respondents together with the original statements, the level of agreement from round two and respondents' comments (Fig. 1). Four appendices were constructed to provide further information and background to the statements (Table 1) and cover a) Sweat Testing, b) Gene Testing, c) Clinical Features and d) Further Physiological Testing.

## 3. Results

### 3.1. Round one

Forty-one responses from specialists in 11 European countries were received for round one. A consensus of over 80% was achieved on twelve of 21 statements. A further five statements were approaching consensus with greater than 60% agreement. Four statements had poor level of agreement (<60%).

### 3.2. Round two

Nine statements not achieving consensus in round one and three that did with provisos were revised by the core group, following analysis of respondents' comments. Respondents were asked to rate their agreement with both the revised and modified statements. Thirty-eight responses were obtained following round two. A consensus of greater than 80% was achieved on a further ten statements. Consensus was not achieved in two.

### 3.3. Round three

The two statements not achieving consensus were discussed at the ECFS Screening Working Group Meeting. Taking into account this meeting and respondents comments, it was clear that modification and combination of four statements were required to obtain consensus, reducing the number of statements from twenty one to nineteen. Thirty-four responses were obtained to round three and consensus was achieved on all nineteen statements (Table 1). An algorithm was developed from the

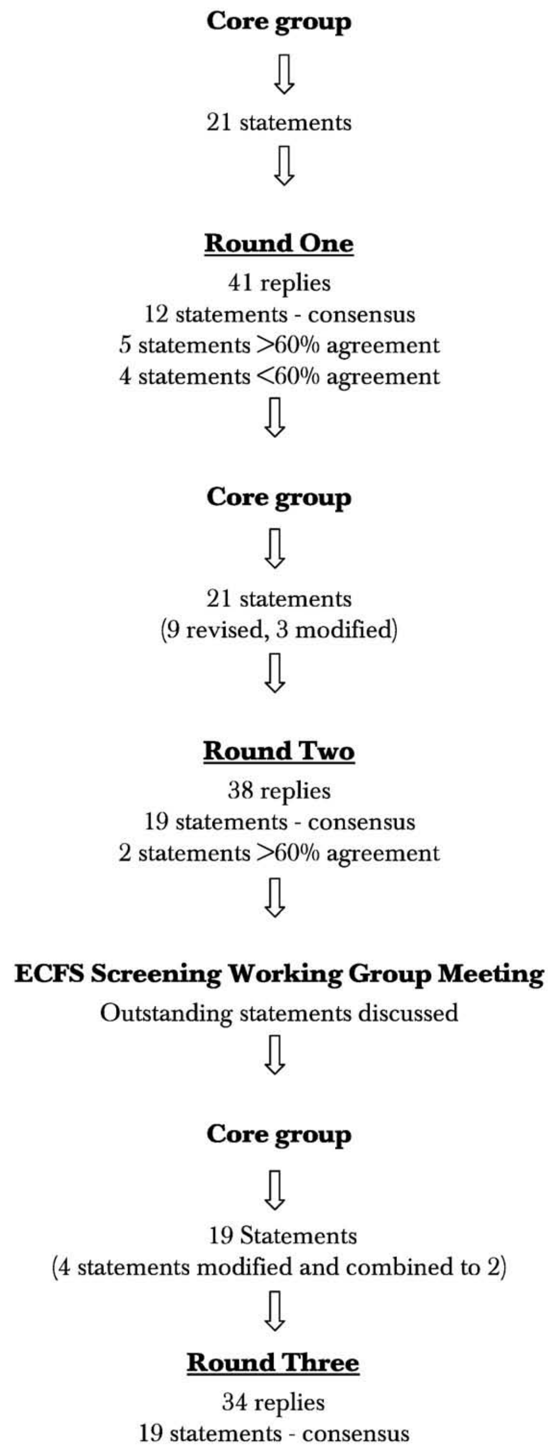


Fig. 1. The stages and outcomes of the modified Delphi method.

consensus statements (Fig. 2). Seven specialists who responded to round one did not respond to round three (17% attrition rate).

### 3.4. Specific issues and comments

#### 3.4.1. Clinic size

Original statements for round one defined specialist CF clinic size greater than 100 patients and achieved consensus. Despite this, several respondents discussed the size of clinics

Table 1
Nineteen statements at the end of the consensus process

| | |
|---|---|
| 1 | An infant with one or more raised IRT measurements and an equivocal sweat test (sweat $Cl^- \geq 30$ and $<60$ mmol $L^{-1}$) requires assessment and review in a specialist CF clinic (with >50 patients). |
| 2 | An infant with two *CFTR* gene changes (one of which has unclear clinical significance) and a normal sweat test requires assessment and review in a specialist CF clinic (>50 patients). |
| 3 | In these cases (infants from statements 1 and 2) a repeat sweat test should be undertaken in a centre with suitable experience (>150 sweat tests pa) of a validated technique for measuring sweat chloride ( Appendix A). |
| 4 | Infants from statement 1, who have a normal repeat sweat test in an accredited centre (sweat $Cl^- <30$ mmol $l^{-1}$), do not require further clinical review (negative CF screening test). |
| 5 | Extended gene analysis must be undertaken in infants with two equivocal sweat tests and one or no *CFTR* mutations recognised ( Appendix B). |
| 6 | Infants with one or more raised IRT measurements, one *CFTR* mutation and a normal sweat test ($Cl^- <30$) do not require extended gene analysis. |
| 7 | Infants with one or more raised IRT measurements, one *CFTR* mutation and a normal sweat test ($Cl^- <30$) do not require review in a CF clinic (negative CF screening test). Appropriate advice regarding carrier status should be given. |
| 8 | Infants with two equivocal sweat tests require detailed baseline assessment for respiratory disease (airways culture and chest radiograph). Further investigations may be indicated as determined by the clinical situation (for example, chest CT scan, and bronchoscopy). |
| 9 | Infants with two equivocal sweat tests require detailed baseline assessment for non-respiratory disease (fecal elastase). Other investigations as clinically indicated. |
| 10 | Infants with two equivocal sweat tests and any clinical evidence supportive of a CF diagnosis should have regular follow up in a CF specialist clinic ( Appendix C). |
| 11 | Infants with two equivocal sweat tests, one or no CF causing mutations and no clinical evidence of CF should be *considered* for further investigation of a physiological defect in a centre with appropriate experience ( Appendix D). |
| 12 | Infants with two equivocal sweat tests, one or no CF causing mutation, no clinical evidence of CF and evidence of ion transport defect on further testing should be followed up in a specialist CF clinic. |
| 13 | All infants with two equivocal sweat tests, one or no CF causing mutation, no clinical evidence of CF should be reviewed in a specialist CF clinic with a repeat sweat test at 6–12 months of age. |
| 14 | Infants with two *CFTR* gene changes (as statement 2) but a normal sweat test (at least one performed in a centre with adequate experience, as per statement 3) should have detailed clinical assessment (as 8 and 9). |
| 15 | Infants with two *CFTR* gene changes (as statement 2), a normal sweat test and any clinical evidence of CF should have regular follow up in a CF specialist clinic. |
| 16 | Infants with two *CFTR* gene changes (as statement 2), a normal sweat test and no clinical evidence of CF should be *considered* for further investigation of a physiological defect in a centre with appropriate experience ( Appendix D). |
| 17 | Infants with two *CFTR* gene changes (as statement 2), a normal sweat test, no clinical evidence of CF but evidence of abnormal ion transport should have regular follow up in a specialist CF clinic. |
| 18 | Infants with two *CFTR* gene changes (as statement 2), a normal sweat test and no clinical evidence of CF should be reviewed in a specialist CF clinic with a repeat sweat test at 6–12 months. |
| 19 | Clinical and demographic information on all infants with an equivocal diagnosis should be entered onto a database or registry (pending consent from legal guardian). |

(>50 or >100 patients) citing concerns that in some areas of Europe specialist CF clinics are traditionally smaller. Overall however it was felt beneficial to include a proposed number and the statements were modified to “specialist CF clinic (with >50 patients)”, which again achieved consensus.

### *3.4.2. Sweat test*

The sweat testing experience of the centre was suggested at greater than 150 sweat tests performed annually and this achieved consensus in round one (Statement 3). See also Appendix A.

### *3.4.3. Equivocal sweat test result*

All infants considered by the guideline will have one or more raised IRT. When these infants subsequently have an equivocal sweat test result they require assessment and review in a specialist CF clinic with a repeat sweat test (Statement 1). If the sweat test in an accredited centre remains equivocal (chloride, 30–60 mmol $L^{-1}$) further investigation is required. This should include extended gene analysis if one or no *CFTR* mutations have been identified (Statement 5) and baseline assessment for respiratory and non-respiratory disease (Statements 8 and 9). If these infants show any clinical evidence supportive of the diagnosis of CF they should have regular follow up in a specialist CF clinic (Statement 10). If there is no clinical evidence of CF they should be reviewed in a specialist CF clinic with sweat test repeated between 6 and 12 months of age (Statement 13).

### *3.4.4. What constitutes a negative screening result?*

Infants who have equivocal sweat test (chloride 30–60 mmol/l) that on repeat test is found to be normal (<30mmol/l) do not require further clinical review (Statement 4). This was agreed to be a negative screening result i.e. CF not suspected (85%, round one).

Infants who have one or more raised IRT measurements, one *CFTR* mutation and a normal sweat test have a negative screening test (Statement 7). This statement was approaching consensus in round one (71%). Following respondents comments, the statement was modified to advise discussion of carrier status and this achieved 92% consensus in round two.

### *3.4.5. Extended gene analysis*

Extended gene analysis is indicated when an infant has had two equivocal sweat test results and only one or no *CFTR* mutation has been identified on the local common mutation panel (Statement 5, 85% consensus round one) (Appendix B). Extended gene analysis should not be performed in infants with one or more raised IRT, one *CFTR* mutation and a normal sweat test. These infants have a negative screening result (Statement 6, 80% consensus round one).

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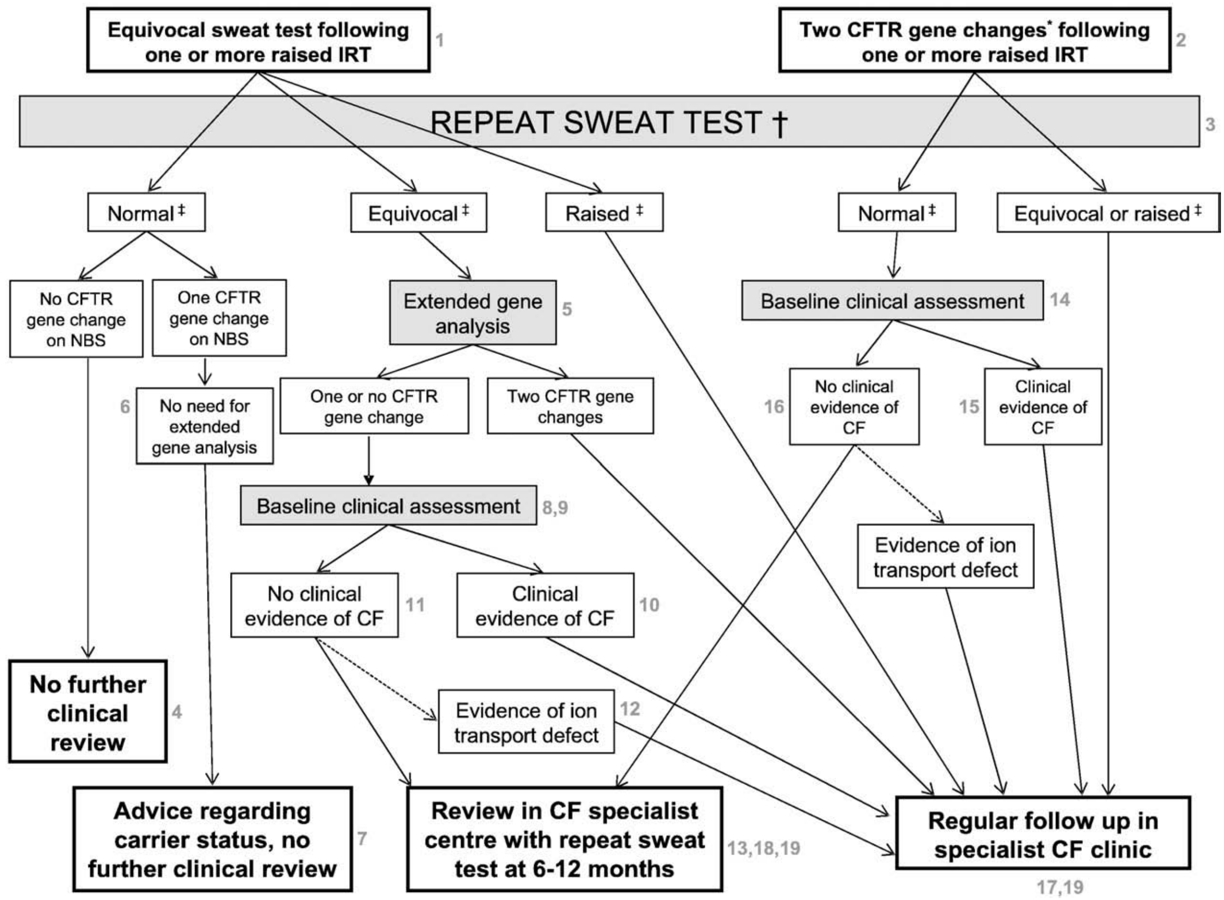


Fig. 2. The pathway of interventions that infants with an equivocal diagnosis may follow according to the results of this consensus process. The two distinct presentations of these infants (equivocal sweat test or two *CFTR* gene mutations of unclear clinical significance) represent the starting points at the top of the figure. The progress of the infant is then tracked following the repeat sweat test in an experienced centre. Subsequent interventions depend to some degree on the result of the repeat sweat test. The numbers indicate the consensus statement (Table 1) that corresponds to that part of the pathway. An important point to note is that infants who enter the pathway with an equivocal sweat test and then have a normal repeat sweat test do not require extended gene analysis or further clinical review (although some may require advice regarding carrier status). Clinical assessment for evidence of CF is considered important with respect to determining subsequent review arrangements (infants with any clinical evidence supporting a CF diagnosis should be seen in a specialist CF clinic). * One of which has unclear clinical significance. † In a centre with suitable experience (Appendix A). ‡ Normal = sweat Cl < 30 mmol $l^{-1}$, equivocal = sweat Cl ≥ 30 and < 60 mmol $l^{-1}$, raised = sweat Cl ≥ 60 mmol $l^{-1}$.

### 3.4.6. Baseline assessment for respiratory and non-respiratory disease

It was agreed that infants who had two equivocal sweat test results or who have two *CFTR* gene changes and a normal sweat test should have baseline clinical assessment (Statements 8, 9 and 14). In round one, baseline assessment for respiratory disease included CT scan and bronchoscopy and this statement achieved a poor level of agreement (41%). The statement was modified to include these investigations only when indicated by the clinical situation and 89% agreement was achieved in the second round (Statement 8). Similarly a significant number felt that some of the proposed non-respiratory investigations were unnecessary and this statement was changed accordingly (to faecal elastase with other investigations as clinically indicated). The statement achieved 92% agreement in round two (Statement 9).

### 3.4.7. Further investigation of physiological defect

A number of measurements of transepithelial salt transport exist that may help in investigating an equivocal diagnosis of CF. However, none have the face validity of sweat electrolyte measurement and are essentially extensions of research methodology. This was reflected in the variability of responses to statements concerning further electrophysiological investigation ranging from enthusiastic advocates to confirmed sceptics. It was agreed with 83% consensus in round two that infants who had two equivocal sweat tests and no clinical evidence of CF should be considered for further investigation of a physiological defect (Statement 11). If there is evidence of ion transport defect even in the absence of clinical evidence of CF with only one *CFTR* mutation these infants should be followed up in a specialist CF clinic (Statement 12).

It proved difficult to obtain a consensus regarding the subsequent management of infants who had no evidence of ion transport defect. In the absence of consensus, these infants are covered by Statement 13. This reflects a general anxiety that it would be inappropriate to exclude a diagnosis of CF on the basis of these measures alone, even when there is no clinical suspicion following baseline assessment.

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#### 3.4.8. Two CFTR gene mutations one or both of which have unclear clinical significance

The term *CFTR* gene change was used in the statements to highlight that these mutations have unclear phenotypic consequences. Subsequently, an ECFS consensus has been achieved on the use and interpretation of CF mutation analysis in clinical practice and has concluded that the term "mutation" should be used to define any molecular alteration in the DNA sequence of the *CFTR* gene [10]. Therefore for the purposes of this process the terms *CFTR* gene change and mutation are interchangeable. The dilemma remains that a number of frequently recognised *CFTR* gene mutations have unclear phenotypic consequence. The situation in CF NBS is further exacerbated by the fact that some *CFTR* mutations that are clearly "CF causing" can have little if any phenotypic consequence in the first years of life, related to the confounding impact of environment and other non-*CFTR* genes.

There was clear agreement that if these infants have any clinical evidence of CF they should have regular follow up in a specialist CF clinic, even with a normal sweat test result (Statement 15). If there is no clinical evidence of CF they should be considered for further investigation of ion transport defect (Statement 16) but regardless should be reviewed in a specialist CF clinic with sweat test repeated between 6 and 12 months of age (Statement 18). If there is evidence of abnormal ion transport these infants should have regular review in a specialist CF clinic (Statement 17).

## 4. Discussion

There is a good agreement that screening infants for CF is an ethical undertaking in regions with a significant incidence of the condition [1]. Unfortunately, current NBS protocols result in recognition of infants with an equivocal diagnosis of CF. To some degree this reflects the heterogeneity of the condition and the sensitivity of the IRT measurement. Infants with an equivocal diagnosis fall into two groups; those with intermediate sweat electrolytes (above the level expected for this age group), but no or one recognised *CFTR* gene mutation and those with two *CFTR* gene mutations, one or both of which have unclear long term phenotypic consequences.

Using a modified Delphi method, we have produced 19 statements that will act as a guide for CF teams in the evaluation and management of infants with an equivocal diagnosis following newborn screening. Strengths of this process have been the inclusive nature across a wide geographical area and a robust framework that enables clear identification of decision making pathways. From the consensus guideline, we have produced an algorithm to aid clinicians involved in CF NBS programmes. It was not the aim of this group to provide a diagnostic framework; rather to provide a pragmatic guideline for the management and evaluation of these infants. The end-points in the algorithm are therefore functional rather than categorical (Fig. 2).

Although the majority of statements attained consensus in the early phases of the process, some areas were more challenging. The group meeting was an essential part of the Delphi process to identify issues around these statements and achieve a final consensus. These guidelines can be used in established screening regions and in those with emerging programmes to guide the evaluation and management of this challenging clinical dilemma.

## Acknowledgement

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## Appendix A

Notes on sweat testing

1) Sweat collection in infants is challenging [11].
2) Sweat collection and analysis should be undertaken in a centre with adequate experience. CF physicians should be guided by national standards. If these are not available, consensus documents are available from the United Kingdom (http://acb.org.uk/docs/sweat.pdf) and North America [11]. These suggest that a laboratory should be undertaking at least 50 tests per year; however for infants with an initial equivocal result, the repeat sweat test should be done in a centre with more experience (>150 sweat tests per year, Statement 3).
3) Two equivocal sweat test results may be available on the same day that were undertaken in a suitably experienced centre, in which case the infant should proceed along the algorithm as described (Fig. 2).
4) Sweat chloride concentration remains the gold standard analytical measure to confirm a diagnosis of CF [11].
5) Sweat sodium should not be used [11].
6) Sweat conductivity may have a role in excluding a diagnosis of CF but does not have sufficient face validity in cases with an initial equivocal result [11].
7) Sweat electrolyte values fall over the first 4 weeks of life [12,13]. A sweat chloride value over 30 mmol $L^{-1}$ should prompt clinical review and a repeat sweat test [11].

## Appendix B

Notes on extended DNA analysis

1. Further investigation of these infants should be undertaken with close liaison with the local molecular genetics service. The extent of DNA analysis should reflect the clinical suspicion. Care should be taken in avoiding the situation where gene changes (mutations) are recognised with unclear phenotypic characterisation, although in most circumstances this will be unavoidable, particularly as laboratories move more quickly to comprehensive *CFTR* gene sequencing.
2. *CFTR* gene change is equivalent to *CFTR* mutation.
3. Further DNA analysis should be guided by the screening protocol (i.e., protocols that initially only examine for a limited panel of *CFTR* mutations would prompt further DNA analysis).
4. Infants recognised to be compound heterozygotes for R117H should have further characterisation of the poly T variant region (and TG repeats if found to be on a 5T background) [14,15]. Infants with R117H on a 7T background may have a normal or equivocal sweat test. Long term clinical outcome is

variable and the management of these infants requires specialist input (as per statements 2, 14, 15, 16, 17 and 18) [7,16].

5. Some *CFTR* mutations that are clearly "CF causing" (in particular, 3849+10 kb C>T) are associated with normal or equivocal sweat electrolyte values. Close liaison with the local molecular genetics service is needed to determine these infants.
6. Infants with persistent intermediate sweat electrolytes and clinical features (Appendix C) should have extensive DNA analysis after discussion with the local molecular genetics service.

## Appendix C

Clinical features consistent with a diagnosis of CF following newborn screening

### *C.1. Respiratory*

1. Symptoms
   a. Cough
   b. Wheeze
2. Clinical findings
   a. Over-expanded chest
   b. Crackles
   c. Wheeze
   d. Tachypnoeic
   e. Abnormal chest shape
3. Chest radiograph changes
   a. Overinflation
   b. Increased markings
   c. Areas of collapse or consolidation
4. Chest high resolution computerised tomogram (HRCT) changes
   a. Air trapping
   b. Early evidence of airway inflammation/bronchiectasis
5. Positive respiratory culture for characteristic CF pathogens
   a. Cough swab/plate
   b. Broncho-alveolar lavage

### *C.2. Non-respiratory*

1. Clinical evidence of malabsorption
   a. Meconium ileus
   b. Poor weight gain
   c. Distended abdomen
   d. Loose offensive stool
   e. Poor head growth
   f. Rectal prolapse
2. Laboratory evidence of malabsorption
   a. Low fecal elastase (or chymotrypsin)
   b. Positive fat microscopy
   c. Low fat soluble vitamin levels
3. Radiological evidence of pancreatic disease
   a. Pancreatic calcification on Abdominal radiograph
   b. Pancreatic fibrosis on abdominal ultrasound scan
4. Liver disease
   a. Prolonged cholestatic jaundice
   b. Elevated liver enzymes (ALT/AST)
   c. Abnormal liver appearance on ultrasound scan
5. Salt loss
6. Absence of the vas deferens

## Appendix D

Further physiological testing.

A number of electrophysiological techniques are available to demonstrate the salt transport defect that characterises CF. These are undertaken in specialist centres. None have the face validity of sweat testing or genotype analysis, but may provide useful additional information in equivocal cases. Some of these tests are particularly challenging in infants.

| Test | Technical details | What it involves for the infant | Availability[a] |
|---|---|---|---|
| Nasal Potential Difference (PD) | Ion transport across airway epithelium can be assessed by measuring the baseline PD. The impact on the PD of perfusing different solutions and drugs provides further information to differentiate CF from non-CF recordings. | The exploring electrode is placed in the nose. A reference electrode is placed either subcutaneously or over abraded skin on the forearm. Solutions are perfused through the exploring electrode into the nose and can be swallowed. | Very few centres are able to undertake this measurement in infants although it is more widely available in older children and adults. |
| Intestinal Current Measurements (ICM) | A biopsy is mounted in the laboratory in a device (Ussing chamber) that enables measurement of transepithelial ion transport. Various aspects of ion transport can be examined. | Biopsy of rectal mucosa. This procedure is painless and well tolerated by young infants. Does not require general anaesthesia or sedation. | This technique requires a dedicated laboratory service with highly skilled technicians. Available in limited number of centres in Europe. |
| Small bowel biopsy | Similar measures of transepithelial transport processes can be undertaken in the laboratory on upper gastro-intestinal (GI) mucosal biopsies. | Upper GI biopsy; requires general anaesthesia in most cases. | Limited (only currently available in Sheffield, UK; contact Prof Chris Taylor). |

[a]For details of centres in Europe that undertake appropriate electrophysiological investigations on infants, contact Dr Michael Wilschanski, chair of the European CF Society Diagnostic Network (michaelwil@hadassah.org.il).

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