Newborn infants have been screened for cystic fibrosis (CF) as part of the Newborn Metabolic Screening Programme (NMSP) since 1986\(^1\). This is done by a two tier system from the newborn bloodspot.

**Tier 1:** Measurement of immunoreactive trypsin (IRT).

**Tier 2:** If the IRT level is in the highest 1%, this is followed by genetic analysis using a limited panel of the most common CF genetic mutations in NZ.

A positive CF screen is then notified if one or two of these CF mutations are identified in the bloodspot. A positive CF screen means the child is either a carrier (approximately 80%) or has CF (approximately 20%).

### Figure 1: Newborn screening protocol for cystic fibrosis in New Zealand

- **Newborn screening spot taken in first days of life**
- **Tier 1: Immunoreactive trypsin measurement**
  - Negative (below cut-off)
  - Positive (above cut-off)
- **Tier 2: CF Genetic screen (limited panel)**
  - Negative
  - Positive for 1 to 2 mutations
    - LMC notified that child has positive CF screen
    - Regional CF team notified child has positive screen
    - Regional CF team contacts LMC
    - LMC informs family & refers to CF team
    - CF team contacts family with an appointment
- **No further action CF not suspected**

**Time frame**
- 2-3 days
- 2-4 weeks
Figure 2: Newborn diagnostic protocol for cystic fibrosis in New Zealand

Paediatrician review: iv
- Clinical assessment
- Sweat test vi
- CF genetic testing (blood) vii
- Pancreatic function (stool)

CF unlikely Cl≤29mmol
- Discharge to community

CF probable Sweat Cl 30-59mmol/L viii
- Follow up in CF clinic for first year
- Repeat sweat test 6-9 months of age
- Request CFTR sequencing

CF Cl≥60mmol/L
- Paediatric counselling
- Referral of family to Genetic Health Services NZ xi,xii
- Return newborn screening audit sheet

CF ≥2 CFTR genetic mutations
- Transfer to CF regional clinic

i Infants that have meconium ileus often have a low IRT and are missed by newborn screening. All children with meconium ileus should have CF genetic mutation analysis and a sweat test organised regardless of the newborn screening result.

The exact nature of the second tier testing has changed over time. Prior to the genetic mutations being established it was a second IRT measurement. From 1997 to 2010 it was the detection of initially a four and then a three gene mutation panel when the clinical significance of one of the commonest genetic mutations (R117H or c.350G>A) was discovered to be variable4. In 2010 MELT technology was used which detects the temperature at which the two strands of the DNA sequence carrying the relevant part of the CFTR gene separate or ‘melt’5. This occurs at different temperatures if a CFTR mutation is carried on one or two strands compared to ‘normal’ DNA. More recently, from 2015, it has been done with genetic sequencing of the CFTR region exons 10 & 11 which cover the most common three genetic mutations seen in NZ (F508del or c.1521_c.1523delCTTT, G551D or c.1625G>A, G542X or c.1624G>T) and other rare mutations. This step may remain subject to change.

NOTE: The report to lead medical teams will remain the same regardless of exact Tier 2 technique – that is, a positive screen that includes one or two CF causing genetic mutations has been found in an infant.
iii The Newborn Metabolic Screening Programme testing centre notifies the lead maternity carer (LMC) and the nominated regional CF paediatrician or nurse. The nominated CF team member contacts the LMC and they plan to inform the family. The LMC informs the family, giving the family information sent by the newborn screening testing centre, and refers the child to the regional CF paediatrician. The nominated CF team member (usually the paediatric nurse) at each hospital will receive the referral, contact the family and organise review with the CF paediatrician. NOTE: The aim is to have the shortest time possible between the family being told their baby has a positive screen for CF, and the assessment by the regional CF paediatrician. Same day contact with the family – first by the LMC then the CF team – is ideal, with the first review taking place within two days.

iv Siblings of children diagnosed with CF: those with symptoms need prompt sweat testing with their own CF genetic mutational analysis if positive or intermediate sweat chloride levels determined; those without symptoms should have a sweat test when possible. This is regardless of their own newborn screening result.

v A sweat test can be done from 1 week of age onwards in a child with a weight >2kgs. The main issue in the very young infant is obtaining adequate sweat weight. Using the traditional Gibson-Cooke method a sweat weight of 75mg is needed, using the newer Wescor Macroduct® system a sweat of 15µL is needed.

vi Not all regional paediatricians have immediate access to sweat tests. The initial assessment may be clinical review, stool sample for pancreatic function (chymotrypsin +/- elastase) and blood genetic mutational analysis in the first instance while a sweat test is booked inter-regionally (see below).

**Sweat Test**

The access to this test is variable around the country. There are five DHBs that perform this locally, elsewhere the test is either conducted locally or sent to a second region for analysis, or the child needs to travel to another centre for testing. There are three potential delay time points. Firstly the availability of sweat tests – offered a number of times per week in the centres, offered sometimes only once every four weeks in the regions. Secondly is when the result becomes available once testing is done – usually within 48 hours if done locally, up to one week if sent elsewhere. Thirdly the age at testing – in some units it has been done when the child is 6 weeks of age and >5kgs to maximise the chance of getting adequate sweat weight and a test result. Also if the child has to attend another centre, this is dependent on family timing and ability to travel, often occurring at weeks to months of age. The sweat test should be considered a priority and the child remain under review until a result obtained.

vii The CFTR genetic analysis on the blood from the infant is a diagnostic test detecting the presence of 30-50 CFTR mutations including the 23 mutations recommended by the American College of Medical Genetics, with results usually available in one to two weeks. Because the genetic component of the screening test is currently sequencing of exons 10&11 of CFTR and diagnostic testing involves a limited panel of mutations (30-50 out of 1500+), it is possible that the diagnostic test will be mutation negative in babies with a positive screen. These results should be referred to the screening programme for confirmation of the initial result.

viii In infants up to six months of age those with an intermediate sweat test 30-59 mmol/L are still likely to have CF. Children in this range should be followed in clinic, and have a repeat sweat test and full CFTR mutation sequencing. The sweat test is most discriminatory between 9-12 months of age.
There is evidence of improved outcomes in children with early diagnosis of CF subsequent to newborn screening \(^{11-14}\) including before 2 months of age\(^{15}\).

Increasingly less common CFTR genetic mutations are being discovered, often without knowing the likely clinical outcome which can range from classical CF, CFTR Related Metabolic Syndrome (see below), or no clinical concern. The outcome may also depend on the second CFTR genetic mutation the ‘novel’ mutation is paired with. One genetic mutation in particular gives a variable outcome - R117H (legacy name) or c.350G>A (DNA sequence name) depending on its association with the number of adjacent polythymidine tracts. These have been determined as 5T, 7T, 9T and very rarely 11T. The less thymidine tracts the less efficient the DNA is in ultimately creating the correct protein. 5T is associated with classical CF phenotype or a more mild CF phenotype. 7T with possible CFTR-Related Metabolic Syndrome (see below). 9T and 11T are rarely cause for concern\(^{16,17}\).

Regardless of outcome - the family is offered counselling – initially this may be done by the local paediatrician but all families should be referred to Genetic Health Services New Zealand for counselling on future pregnancies and cascade testing to extended family members as appropriate. The family can choose whether or not to attend.

Letters regarding the clinical review and test results will be sent from the CF paediatrician to the LMC and the family’s general practitioner, usually with a copy to the family.

**Special Circumstances**

**Cystic fibrosis associated with sweat chloride <60mmol/L**

There is a group of children that do not have an immediately diagnostic sweat test (chloride ≥ 60mmol/L) or two known CF-causing genetic mutations, but results are not normal (normal chloride is ≤29mmol/L)\(^{17-19}\). Children with an intermediate result 30-59 mmol/L are likely to have CF (probable CF). Some will develop the classical clinical pattern for CF, some will develop ‘CFTR Related Metabolic Syndrome’ (e.g. nasal polyposis, pancreatitis, male infertility etc) and others may never have disease. The recommendation is to follow up all children with sweat chloride in this intermediate range (30-59 mmol/L) at least 3 monthly over the first year and to repeat the sweat test between 9 and 12 months of age when sweat chloride is lowest\(^{10}\). Those who remain with sweat chloride ≥40mmol/L at this stage should be considered to have CF and have continued follow-up.

**Children missed on newborn screening or born abroad**

About 8% of children with CF are missed on newborn screening (low IRT, no CFTR genetic mutations detected, other reason for no or incorrect testing, born abroad in country without CF newborn screening). This means some children will require investigations for CF at a later age.

The history and/or examination will usually raise suspicions of a possible CF diagnosis. Common features are recurrent respiratory infections and failure to thrive with steatorrhoea (but some have normal growth).

- In a baby, CF should be excluded with; meconium ileus, rectal prolapse, salty tasting skin, prolonged obstructive jaundice, and unexplained haemolytic anaemia with hypoalbuminaemia.
- In an older child or young adult, CF is possible with: recurrent respiratory infections, nasal polyps, severe sinusitis, presence of clubbing, failure to thrive, abnormal bowel habit or recurrent abdominal pain with no other explanation.
- The isolation of *Pseudomonas aeruginosa* in particular, and *Staphylococcal aureus* repeatedly from respiratory tract secretions is suspicious.
References


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