Supplement (i)

Australian Guidelines for the Performance of the Sweat Test for the Diagnosis of Cystic Fibrosis

Report from the AACB Sweat Testing Working Party

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Introduction

Cystic Fibrosis (CF) is an autosomal recessive disease resulting from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7.[1] Many different mutations have been identified, but in Australia, over 90% of CF patients have at least one copy of the Delta F508 mutation. CF has an incidence here of about 1:2100 live births[2] and is usually diagnosed by the finding of an elevated sweat chloride concentration.

Because CF is a serious, life-threatening disease, false positive and false negative sweat test results can have very detrimental consequences. Sweat test methods must be standardised to ensure uniform quality. Guidelines for sweat testing have already been published by the National Committee for Clinical Laboratory Standards in the USA (Approved guideline C34-A2)[3] and by a multi-disciplinary working group in the UK. The UK guidelines which are both detailed and evidence based have been employed as a major resource document by this working party, and are published on the internet.[4]

The aim of these Australian guidelines is to emphasise important details that must be followed in order to obtain accurate results, and to expand on details not clearly described elsewhere. The aim is not to give a detailed account of how to perform a sweat test which can be found in the NCCLS and UK guidelines. These guidelines should be consulted if further details are required.

Indications for Sweat Testing

a) Positive newborn screening test for CF (elevated immunoreactive trypsin, followed by detection of one or two mutations in the CFTR gene)

b) Patient presenting with clinical signs suggestive of CF
   - Babies with CF may present with meconium ileus in the newborn period. They may have frequent chest infections, and often fail to thrive and have bulky, pale stools.
   - In older children, the major manifestations include chronic sino-pulmonary disease with infection and malabsorption due to pancreatic insufficiency. Finger clubbing is often present and *Staphylococcus aureus* or *Pseudomonas aeruginosa* is frequently cultured from the sputum. Other features may include chronic liver disease.[5]
   - Milder forms of CF may present in adults, and may even be found in male patients presenting with isolated obstructive azoospermia due to absence of the vas deferens.[1]

c) Family history of CF or family history of CF carrier status

Acceptable and Non-Acceptable Tests

Measurement of sweat chloride concentration is the definitive test for the diagnosis of CF.

Sweat sodium concentration may also be measured as a check on the chloride result, since there is usually little difference between sodium and chloride concentrations in sweat. Studies on 4771 patients from the Women's and Children's Hospital, Adelaide, showed a mean difference of 2.6 mmol/L, with an SD of 4.4 mmol/L. Therefore, if the difference in concentrations is >20 mmol/L, the test should be repeated. Sodium concentration must not be measured alone.

Sweat conductivity may be used as a screening test only. See page S5 for further discussion of sweat conductivity.

Measurement of sweat osmolality is NOT an acceptable test for the diagnosis of CF.

Who is Suitable to Test?

Generally, sweat tests are not performed until the subject is greater than two weeks of age and weighs more than three kg. Occasionally, the test may be attempted in younger, smaller babies, provided there are good clinical reasons for doing so. However, there are often technical problems doing the test in very small infants, and there may be a greater risk of complications (see below) or obtaining insufficient sweat. The test is contra-indicated in babies less than 48 hours of age because high concentrations of sweat electrolytes (sodium >70 mmol/L) can be found on the first day of life.[6] In a recent study in pre-term and full-term infants less than six weeks of age, it was concluded that sweat collection can be reliably performed in infants greater than or equal to 36 weeks postmenstrual age, greater than 2000 g and greater than three days postnatal age.[7]

If the patient is acutely unwell, dehydrated, oedematous or receiving corticosteroids, the test should be delayed.

Patient Preparation and Possible Complications

The purpose of the sweat test and how it will be carried out must be explained carefully to the parents of the child. They should be made aware that there is a small risk of complications. The most common one is some mild reddening of the skin. Damage to the skin, such as blistering or burns, occurs very infrequently and this risk can be minimised by careful attention to technique (see next section). It is a good idea to prepare a sweat test information sheet for parents. (See Appendix, page S7, for an example of a sweat test information sheet.)

Sweat Stimulation and Collection

(Based on the NCCLS and UK Guidelines)

- Sweat for measurement of chloride +/- sodium must be collected either by pilocarpine iontophoresis, using the Gibson Cooke method,[8] or by use of the Wescor Macroduct system.
- The power supply must be battery powered and should include a safety cutout. All electrical equipment must be checked on a 12 monthly basis and maintained in good working order. The current should be increased gradually to maximum and monitored throughout the procedure (<4mA).[9] It should not be necessary to maintain the current for more than 5 minutes.
- Electrodes are usually made of copper, stainless steel or carbon, and must be of proper size to fit the patient's limb. They must be regularly cleaned and inspected and kept free of surface oxidation. When using the Wescor Macroduct system, follow the manufacturer's instructions.
- The flexor surface of either forearm is the preferred site for sweat collection. Other sites, e.g. upper arm, thigh or calf, may rarely be used if the arms are too small. The site must be free of any skin disorders, such as eczema.
- The electrolyte solutions to be used include United States Pharmacopoeia grade pilocarpine nitrate (0.2 – 0.5%).[10] This can be used at both electrodes, or alternatively, a solution of magnesium sulphate (0.05 – 2.0 mol/L) or potassium sulphate 1% can be used at the cathode. Solutions containing sodium or chloride should be avoided because of the risk of contamination. Pilocarpine may be used in the form of a gel, as with the Wescor system.
- Pads placed under the electrodes and soaked with the electrolyte solutions must be thick (3 – 8 thicknesses of hospital lint) and maintained in full contact with the skin. They must be about 1 cm larger than the electrodes in all directions to avoid burning or blistering the skin.
- The following can be used for sweat collection:

  (i) Gauze pads which have been repeatedly washed in distilled water to remove any traces of NaCl.
  (ii) Filter paper, e.g. Whatman No 42/44
  Both of these are covered with a sheet of impervious material that is sealed on all sides with tape to prevent evaporation.
  (iii) Wescor disposable collectors.

- Sweat should be collected for no more than 30 minutes and no less than 20 minutes. Great care should be taken to prevent contamination and evaporation.
- For the non-Wescor system, a balance sensitive to 0.0001g must be used to weigh the sweat, with the same balance used throughout. It is preferable to use a balance that prints out its results.
- The minimum sweat secretion rate should not be less than 1g/m^2/min over the collection period. Insufficient volumes of sweat should not be pooled but rather, the sweat test should be repeated. If this is necessary, use a different site on the limb. Generally, a sweat test cannot be performed if <0.075 g of sweat is obtained using the Gibson Cooke method, or <15µL when using the Wescor Macroduct system over 30 minutes.[3] The weight or volume of sweat collected from each patient must be recorded, and the laboratory should aim to keep inadequate collections under 5%.[4]
- If insufficient sweat is collected, only one repeat stimulation (on the same day) may be performed, using an alternative site, usually the opposite arm. Do not re-stimulate the same site. If the sweat volume is still insufficient, the test should be re-scheduled for another date.

Methods of Analysis

The following measurement techniques are acceptable:

Chloride: colourimetry (titrimetric or spectrophotometric), coulometry (chloridometer) or ion-selective electrode.

Sodium: flame photometry, atomic absorption spectrophotometry or ion-selective electrode.

Reference Intervals, Interpretation and Reporting

A sweat chloride concentration >60 mmol/L strongly supports the diagnosis of CF. There are a number of other conditions, including adrenal insufficiency, which can cause an elevated sweat chloride concentration, but in practice these disorders rarely cause diagnostic confusion.[3]

A sweat chloride concentration between 40 and 60 mmol/L is suggestive of the diagnosis of CF.[11] The lower end of this range is not well defined and some patients with sweat chloride concentrations below 40 mmol/L may have CF.[12]

Where interpretive remarks are incorporated into the report, they must be overseen by a senior scientist experienced in sweat testing, in consultation with an appropriate pathologist or clinician.

Those patients in whom the sweat chloride result is equivocal should be referred to a physician specialising in the diagnosis and care of patients with CF. The diagnosis may be clarified by repeat sweat testing, genetic mutational analysis, measurement of nasal potential difference[13] and in some instances, by tests of exocrine pancreatic function.

Reference intervals have been developed from CF and non-CF children. Caution should be exercised when interpreting results in the adult population, as the concentration of sweat electrolytes may alter with age in both the CF and non-CF groups.[14,15]

Sweat Conductivity

It is the recommendation of this working party that sweat conductivity, which is related to the concentration of all ions, not just chloride, only be used as a screening test, and that for all borderline or positive sweat conductivity results, a formal measurement of sweat chloride be performed. This is in line with the NCCLS guidelines. Conductivity can be measured in an acceptable manner using the Wescor equipment.

Conductivity >80 mmol/L is very likely to be due to CF. Higher values are obtained for conductivity, because of the presence of ions other than Cl and Na in sweat. It has been reported that, on average, sweat conductivity is 15 mmol/L higher than sweat chloride concentration.[16]

Conductivity between 50 and 80 mmol/L may be due to CF, so all patients with conductivities >50 mmol/L should have measurement of sweat chloride concentration.

Several recent studies have demonstrated that measurement of sweat conductivity can reliably differentiate patients with CF from those without the condition.[17,18] Therefore, with further evidence, the recommendation that conductivity be used solely as a screening test may be changed.

Quality

Evidence shows that poor performance of sweat test analysis can lead to misdiagnosis.[19,20] The area of sweat testing most susceptible to incorrect performance is operator competency. Internal quality control and external quality assurance programs are valuable for the evaluation of methods of analysis, analytical competency and interpretation of results but do not test the ability of the operator to collect the sweat accurately.

False negative and false positive results can occur from any of the following:

- Inadequate sweat collection
- Improper method selection and performance
- Poor technical competency

One way of testing all components of the sweat test is to perform the test on a staff volunteer at regular intervals. This exercise is also good for training purposes.

Internal Quality Control (QC)

Aqueous electrolyte solutions of known sodium and/or potassium chloride concentrations should be used as internal controls.[3] When using gauze pads or filter paper, the internal QC material should be added to the paper or gauze and then analysed with the patient samples. For the Wescor system, it is acceptable to analyse the internal QC material directly. It is recommended that two levels of QC be analysed with each batch of patient samples. It is also recommended that one of these controls is close to the decision level for chloride concentration (40 mmol/L) and the other is in the abnormal range.

Between batch coefficients of variation (CV) for chloride measurement should be 5% or less at a concentration of 40–50 mmol/L.[4]

External Quality Assurance

Laboratories undertaking sweat testing in Australia must participate in a suitable external quality assurance (EQA) scheme. EQA can only assess the analytical component of sweat testing and not the stimulation and collection components. Therefore, errors arising from poor stimulation and/or collection cannot be identified. However, EQA can identify weighing errors, poorly performing methods, discrepancies in standardisation, calculation errors and errors in interpretation.

- The College of American Pathologists commenced a proficiency testing program in 1994.
- The UK external QA scheme started in 1999.
- The Royal College of Pathologists of Australasia (RCPA) Chemical Pathology Quality Assurance Programs Group have a quality assurance program for Sweat Electrolytes which began as a pilot in 1999. Following this, there have been two cycles per year. Each cycle consists of 6 linearly related samples distributed in duplicate. Currently, the samples are aqueous salt solutions of NaCl and KCl. The range of chloride concentrations is from 10 mmol/L to 120 mmol/L. Part of the program also looks at interpretation of the results. This is the qualitative aspect of the report, and currently there

is a choice of "negative", "equivocal" or "positive". Using these data, a cumulative summary report is produced graphically, representing the number of results returned and the interpretations selected by the laboratories.

Experience and Training

The laboratory must be performing sweat tests on a regular basis to maintain expertise. As the proper performance of sweat testing is operator dependent, thorough training of staff is particularly important. It is recommended that each person trained to carry out sweat tests perform at least 10 tests each year, ideally spaced throughout the year.

If those involved in collecting the sweat are not laboratory based, e.g. nurses, their training must be supervised by the laboratory and certified by a senior member of laboratory staff responsible for sweat testing.

Details regarding training must be fully documented, and training records kept up to date in accordance with National Association of Testing Authorities (NATA) requirements. Accreditation of laboratories for sweat testing by NATA should include an assessor with competence in this area.

Competing Interests: None declared.

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Appendix

Here is an example of a laboratory's information sheet regarding CF and the sweat test.

Fact Sheet
Sweat test

What is a sweat test?

Your doctor has asked us to give your child a sweat test. In this test, we collect a small amount of sweat from the skin and test it to see if your child has a condition called cystic fibrosis. Cystic fibrosis is a genetic disease that affects the lungs and digestive system.

How is it done?

The sweat is collected by applying a very small electrical current to your child's skin along with a chemical called pilocarpine. Pilocarpine helps to cause sweating. The test is usually done on your child's arm or leg.

How long does it take?

The sweat test usually takes about a half to one hour.

Does it hurt? Is it safe?

Sweat testing is a very safe procedure and thousands of children have the test without any problem. It usually causes only minor discomfort. Because the child's skin heats up over a small area during the test, there is a very slight risk of causing a minor skin irritation. Young babies less than six weeks old are most affected.

On rare occasions, we cannot collect enough sweat for the test, for instance, in very small babies. If this happens, we will have to repeat the test some weeks later.

Remember

- **Sweat testing is a safe procedure.**
- **The test may have to be repeated if not enough sweat is collected.**
- **Should only be performed at a recognised centre for sweat testing.**

This fact sheet is for education purposes only.
Please consult with your doctor or other health professional to make sure this information is right for your child.

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