

AUSTRALIAN GUIDELINES FOR THE PERFORMANCE OF THE SWEAT TEST FOR THE DIAGNOSIS OF CYSTIC FIBROSIS

Report from the 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 7¹. 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 births² 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 can be found at the following website: <http://www.acb.org.uk/Guidelines/sweat.htm>.

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 Delta F508 mutation)
- b) Patient presenting with clinical signs suggestive of CF. Babies with CF may present with meconium ileus in the newborn period, and often fail to thrive and have bulky, pale stools. 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* are frequently cultured from the sputum. Other features may include chronic liver disease⁴.

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 deferens¹.

- c) Family history of CF
- d) Other DNA mutation studies indicating the possibility of CF

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. If the difference in concentrations is >20 mmol/L, the test should be repeated (see UK guidelines). Sodium concentration must not be measured alone.

Sweat conductivity may be used as a screening test only. See page 7 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?

As a general guideline, 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 contraindicated 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 life⁵.

If the patient is acutely unwell, dehydrated, oedematous or receiving corticosteroids, the test should be delayed. Patients receiving oxygen may be sweat tested but for safety reasons, the oxygen must be delivered in a closed system.

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. Burns to the skin occur very infrequently and this risk can be minimised by careful attention to technique. It is a good idea to prepare a sweat test information sheet for parents. (See Appendix, page 12, 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 “in-house equipment” based on the Gibson Cooke method⁶, 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 regularly 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)⁷. 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, eg, 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 USP grade pilocarpine nitrate (0.2 – 0.5%)⁸. This can be used at both electrodes, or alternatively, a solution of magnesium sulphate (0.05 – 2.0 moles/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 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, eg, Whatman No 42/44Both 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.
- 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 1 g/m²/min over the collection period. Insufficient volumes of sweat should not be pooled but rather, the full 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 < 15 uL when using the Wescor Macroduct system over 30 minutes³. The amount of sweat collected from each patient must be recorded, and the laboratory should aim to keep inadequate collections under 5% (UK Guidelines).
- 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.

A sweat chloride concentration between 40 and 60 mmol/L is suggestive of the diagnosis of CF⁹. The lower end of this range is not well defined and some patients with sweat chloride concentrations below 40 mmol/L may have CF¹⁰.

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

For those patients in whom the sweat chloride result is equivocal, genetic mutational analysis and/or measurement of nasal potential difference¹¹ may help to make the diagnosis.

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^{12,13}.

SWEAT CONDUCTIVITY

It is the recommendation of this working party that sweat conductivity 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 and the recommendation of the UK working party. 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 concentration¹⁴.

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.

It is interesting to note that in a recent study, conductivity was shown to be reliable in diagnosing CF¹⁵.

QUALITY

Evidence shows that the poor performance of sweat test analysis can lead to misdiagnosis^{16,17}. The area of sweat testing most susceptible to incorrect performance is operator competency. There is also a definite requirement for internal quality control and external quality assurance to evaluate methods of analysis, analyst competency and also interpretation of results.

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

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

INTERNAL QUALITY CONTROL (QC)

Aqueous electrolyte solutions of known sodium and/or potassium chloride concentrations should be used as internal controls². 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.

Based on the UK guidelines, between batch coefficient of variation (CV) for chloride measurement should be 5% or less at a concentration of 40 – 50 mmol/L.

One way of testing all components of the sweat test is to perform the test on a staff volunteer at regular intervals, e.g. every three months. Sweat chloride concentration should remain relatively constant in one normal subject. This exercise is also good for training purposes.

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. This scheme uses aqueous “salt” solutions of NaCl, KCl and KH₂PO₄ to mimic real sweat.

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 categories selected by the laboratories.

EXPERIENCE AND TRAINING

The laboratory must be performing sweat tests on a regular basis to maintain expertise. It is recommended that those 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.

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APPENDIX

Here is an example of a laboratory's information sheet regarding CF and the sweat test. This laboratory uses the Wescor Macroduct system.

SWEAT TEST FOR CYSTIC FIBROSIS

Authorised by the Director of Chemical Pathology

What is Cystic Fibrosis?

Cystic Fibrosis (CF) is a genetic disorder (passed from parents to child) that affects many functions of the body including digestion, breathing and reproduction. It can affect both males and females. This lifelong illness usually becomes worse with age.

The symptoms and severity of CF differ from person to person. In some patients, only lung function is affected while others have both lung and digestive problems. The common symptoms of CF include excessive mucus production, chronic coughing, recurrent pneumonia, wheezing, sinus infection, nasal polyps (bumps inside the nose), poor growth, frequent foul smelling stools, enlarged fingertips and salty tasting skin. CF does not affect intelligence.

In CF, the glands that produce mucus, saliva and intestinal fluids do not work properly. These glands produce secretions that are thick and sticky rather than thin and watery. Thick mucus in the lungs interferes with the removal of dust and germs and can cause breathing problems, infections and lung damage. In the intestine, this thick mucus stops food being digested properly which slows growth and development.

Treatment is improving the length and quality of life of people with CF. Antibiotics and physiotherapy can reduce the effects of thick mucus in the lungs. Enzymes and special diets can improve nutrition. The sooner this condition is diagnosed, the sooner treatment can begin.

Please see your own doctor for more information or further explanation about this condition.

How is a Sweat Test Done?

Patients with Cystic Fibrosis produce extremely salty sweat. Based on this observation, the sweat test was developed fifty years ago to diagnose CF. This test is still the standard for diagnosis. Two electrodes are covered with a special gel and strapped to the patients forearm. A small electrical current is passed through the electrodes for 5 minutes. This can produce a mild tingling feeling but no pain. The electrodes are then removed and a sweat collector (which is about the size and shape of a wristwatch) is strapped to the same spot. The sweat collector must stay on the arm for 30 minutes to collect

enough sweat to do the test. During this time, the patient can move around freely. It is important that the patient has plenty to drink on the day of the test, as this will help them to sweat.

The results should be with your doctor within 1-2 working days of our collection. Please contact the doctor, and not the laboratory, for the results.

Information for Parents – Sweat Testing poses a remote risk of minor skin burns

Information supplied by WESCOR, current at 12 January, 1998

“There is an element of risk inherent in all medical procedures, no matter how simple. The sweat test has been an important laboratory tool since the 1950's. It provides a quantitative test result to confirm or exclude a clinical diagnosis of Cystic Fibrosis. Unfortunately, the test has been accompanied by occasional minor burns.

The sweat test consists of three sequential procedures: (1) sweat stimulation, (2) sweat collection, and (3) sweat analysis. The first procedure is known as pilocarpine iontophoresis. It is the universally accepted by medical authorities as a safe and effective method of stimulating sweat glands. A sweat-inducing drug, pilocarpine, is delivered from the surface of the skin through the watery pathways of the sweat ducts into the sweat glands by a small electric current that is made to flow through the dermal layers. The electric current is supplied by a battery-powered device through a pair of electrodes fitted to the limb of the patient.

Minor skin burns have been an unwelcome, adverse side-effect of pilocarpine iontophoresis from the beginning. Some types of iontophoresis apparatus are prone to cause burns, particularly if there is procedural error. Fortunately, such burns are extremely rare with the Wescor iontophoretic system. It uses a sophisticated microprocessor current controller and a very low delivery current of only 1.5 milliamperes. Pilocarpine is contained in unique Pilogel drug reservoirs that are 96% water. These features substantially reduce, but do not eliminate, the possibility of skin burns.

Burn descriptions vary from “tiny black pinholes in the skin” to “crater-like, third-degree burns two to three millimetres in diameter.” In most of the incidents reported, the children have exhibited no sign of pain or discomfort during iontophoresis, and the burn was not discovered until the electrodes were removed.

Most individuals exhibit sensitivity to pilocarpine that is typically manifest as mild erythema (redness) of the skin at the electrode locations. In some cases, one or more blister-like welts may also form. These are often mistaken as burns, but they are simply the reaction of the skin to pilocarpine. Such “blisters” invariably disappear within 2 to 3 hours, leaving no after-effects.

Based on current data and reported events, the apparent burn rate is less than 1 in 50,000. Wescor prescribes proper test procedures which minimise the risk of burns from its equipment. It is highly unlikely that your child will suffer a burn during the sweat stimulation phase of the sweat test.

We realise these statistics will be of scant comfort to the parents of a child who has the misfortune of suffering from the “one burn in 50,000.” However, experience has shown that when burns do occur, the injuries are minor and there are no lasting effects. The burns usually heal completely within one to two weeks with little or no scarring.”

This method has been in use in our laboratory since 1992. In that time, no patient has had a permanent burn and only one patient has received a superficial burn.