

# **Guidelines for the Performance of the Sweat Test for the Investigation of Cystic Fibrosis in the UK**

**Report from the Multi-Disciplinary Working Group with  
representation from:**

*Association of Clinical Biochemists  
British Paediatric Respiratory Society  
British Thoracic Society  
Cystic Fibrosis Trust  
Royal College of Paediatrics & Child Health  
Royal College of Pathologists  
UK National External Quality Assessment Schemes*

**These guidelines have been submitted to the Royal College of  
Paediatrics and Child Health for their endorsement**

*Please note that the Royal College of Paediatrics and Child Health( RCPCH) is currently appraising these guidelines. We understand that the RCPCH is proceeding with appraisal of the Grade B recommendations only (there are no grade A recommendations and it is not practice to appraise Grade C recommendations). We understand that the appraisal will be completed by Spring 2003.*

**July 2002**

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**Key words: Sweat Test  
Cystic Fibrosis**

## **ACKNOWLEDGEMENTS**

The group is grateful to all the professional bodies for sponsorship of these guidelines, the peer reviewers for their helpful comments and suggestions, to the Specialist Advisory Group (Paediatrics) of UK National External Quality Assurance Schemes for stimulating the idea to undertake the guidelines, and also to many professionals for their interest and useful comments throughout the process.

## ABBREVIATIONS

ACB	Association of Clinical Biochemists
BTS	British Thoracic Society
CPA	Clinical Pathology Accreditation (UK) Ltd.
CF Trust	Cystic Fibrosis Trust
NCCLS	US National Committee for Clinical Laboratory Standards
NEQAS	National External Quality Assurance Schemes
RCPATH	Royal College of Pathologists
RCPCH	Royal College of Paediatrics and Child Health

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## GUIDELINES DEVELOPMENT GROUP

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## NOTES FOR USERS

It is intended that the recommendations contained in these guidelines will be adopted for local use wherever sweat testing for the investigation of cystic fibrosis is performed. It is important that discussion takes place between all relevant health professionals (clinical chemists, paediatricians, chest physicians, CF nurses), so that specific local guidelines can be derived and implemented.

The guidelines are owned by the professional bodies as listed on the title page, but they can be copied for local use.

Details of where to obtain copies can be obtained from:-

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## REVIEW AND UPDATING

This guideline was issued in 2002 and it is planned to review in 2004 or sooner if new evidence becomes available.

Comments are invited to assist the review process and all correspondence should be sent to :-

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## GRADING SCHEME FOR RECOMMENDATIONS

The criteria for the grading of recommendations in this document are based upon those used by the US Agency for Health Care Policy and Research (AHCPR) <sup>(1)</sup>, and published by the Scottish Intercollegiate Guidelines Network (SIGN) <sup>(2)</sup>.

### Levels of evidence:-

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Level	Classification of evidence (based on AHCPR 1992)
I a	Evidence obtained from meta-analysis of randomised controlled trials
I b	Evidence obtained from at least one randomised controlled trial
II a	Evidence obtained from at least one well designed controlled study without randomisation
II b	Evidence for at least one other type of quasi-experimental descriptive studies
III	Evidence obtained from well designed, non-experimental descriptive studies such as comparative studies, correlation studies and case studies
IV	Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities

### Grading of Recommendations

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Grade	Type of recommendation (based on AHCPR 1992)
A (levels I a and I b)	Requires at least one randomised controlled trial as part of the body of literature of overall good quality and consistency addressing the specific recommendation
B (levels II a, II b, III)	Requires availability of well conducted clinical studies but no randomised clinical trials on the topic of the recommendation
C (level IV)	Requires evidence from expert committee reports or opinions and/or clinical experience of respected authorities. Indicates absence of directly applicable studies of good quality

The process of developing the recommendations has been according to guidance from the RCPCH (3); further details are provided in the process of guidelines development section.

## References

1. Agency for Health Care Policy and Research. Acute pain management, operative or medical procedures and trauma 92-0032. Clinical practice guidelines. Rockville, Maryland, USA: Agency for Health Care Policy and Research Publications, 1992.
2. SIGN Guidelines: An introduction to SIGN methodology for the development of evidence-based clinical guidelines. Scottish Intercollegiate Network (SIGN). SIGN Publication Number 39, July 1999.
3. Standards for Development of Clinical Guidelines in Paediatrics and Child Health: Role of the Royal College of Paediatrics and Child Health. Report of the Quality of Practice Committee, December 1998. 2<sup>nd</sup> edition November 2001.

## SUMMARY OF RECOMMENDATIONS

<b>Patient Information</b>	<b>Grade</b>
<ul style="list-style-type: none"> <li>▪ It is good clinical practice to prepare the patient and, where appropriate, parent effectively before testing. Informed consent should be obtained in accordance with local policy. Pre-test information appropriate for the individual should include why the test is being done, how it will be performed, risks associated with the test, what the subject will experience, and contact details regarding the testing and final result. An example leaflet for patients/parents is provided (see Appendix document 1).</li> </ul>	<b>C</b>
<b>Subject Suitability</b>	
<ul style="list-style-type: none"> <li>▪ Sweat tests can be performed after 2 weeks of age in infants greater than 3 kg who are normally hydrated and without significant systemic illness</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat testing can be attempted in term infants after 7 days of age if clinically important, but will need repeating if insufficient quantity of sweat is collected</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests should be delayed in subjects who are dehydrated, systemically unwell or who have eczema affecting the potential stimulation sites</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests should be delayed in subjects who are oedematous and/or on systemic corticosteroids</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests should not be performed in subjects who are on oxygen by an open delivery system. This would not apply to an infant in headbox or on nasal prong oxygen.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests can be performed in subjects on flucloxacillin</li> </ul>	<b>B</b>
<b>Sweat Collection</b>	
<ul style="list-style-type: none"> <li>▪ The flexor surface of either forearm is the preferred site for sweat collection. Consideration may be given to other sites if both arms are eczematous, too small or otherwise unsuitable. Other sites used successfully include the upper arm, thigh and back.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Great care must be taken at all stages of the procedure to avoid contamination (see example SOP)</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ In response to a sweat test request it is sufficient to carry out one sweat collection only.</li> <li>▪ The power supply used must be battery powered and should include a safety cutout. <ul style="list-style-type: none"> <li>- monitoring of the current must be carried out throughout iontophoresis where possible. Wescor systems from model</li> </ul> </li> </ul>	<b>B</b>

<p>3600 onwards have no ammeter but have an appropriate safety cut out system</p> <ul style="list-style-type: none"> <li>- the power supply and electrodes must be regularly checked, maintained and records kept.</li> <li>- electrical safety of all power supplies must be checked annually</li> </ul> <ul style="list-style-type: none"> <li>▪ Electrodes should be of a suitable size and curvature to fit snugly on the patient's limb. <ul style="list-style-type: none"> <li>- they are most commonly made of copper or stainless steel.</li> <li>- electrodes should be firmly secured in position to the electrolyte support pads or gels using straps that are adjustable to fit the patient (e.g. Velcro or rubber).</li> <li>- electrodes must be regularly cleaned and inspected, and discarded if they show pitting or irregularities</li> </ul> </li> <li>▪ Selection of new equipment, and maintenance of existing equipment, must comply with CPA Accreditation (or equivalent standard).</li> </ul>	<p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p>
<p><b>Electrolyte Solutions</b></p> <ul style="list-style-type: none"> <li>▪ Aqueous solutions or Wescor gel discs containing Pilocarpine nitrate at 2-5g/l are recommended for use at both electrodes. Alternative solutions (e.g. magnesium sulphate) may be used at the cathode.</li> <li>▪ Solutions containing sodium and/or chloride should be avoided because of the risk of contamination of the collection.</li> <li>▪ Unbuffered acid solutions should not be used because of the increased risk of burns.</li> <li>▪ Electrolytes used for iontophoresis must either be obtained as part of a medical device (e.g. Wescor Pilogel Discs) or from a recognised manufacturer of unlicensed medical products. Solutions must not be produced in-house by hospital laboratories.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>C</b></p>
<p><b>Electrolyte Supports</b></p> <ul style="list-style-type: none"> <li>▪ Suitably thick pads must be used for the electrolyte solutions to minimise the risk of acid burns. Pads of Hospital Lint BPC Plain 500 gram folded to provide 4-8 thicknesses (greater than 1cm thick) are recommended as an electrolyte reservoir with filter paper collection systems. The pad should be at least 1cm larger than the electrode in all directions to prevent electrode-skin contact. It may be incorporated into sewn pockets designed to contain the electrode and prevent skin contact. The pads should be saturated by soaking in the electrolyte solution before application to the patient's skin.</li> <li>▪ Hybrid systems, e.g. Wescor electrodes with aqueous electrolyte solutions, or Wescor gel discs used with non-Wescor electrodes should not be used.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>B</b></p>

<p><b>Iontophoresis - time, current</b></p> <ul style="list-style-type: none"> <li>▪ When aqueous electrolyte solutions are applied on pad supports a current of 0.5 mA should be applied, and increased gradually to a maximum of 4 mA. Once 4 mA is attained the current should be maintained for a minimum of 3 minutes and a maximum of 5 minutes. Longer times should not be necessary to increase sweat production provided good electrical contact is maintained, by use of well maintained electrodes and suitably saturated pads.</li> <li>▪ When Wescor systems are used, the manufacturer's current and time recommendations should be followed. This will depend on the specific model used.</li> <li>▪ For both systems, the patient must be kept under close supervision throughout the iontophoresis period.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p>
<p><b>Medium of Collection</b></p> <ul style="list-style-type: none"> <li>▪ During collection sweat must be protected from contamination and evaporation (see example SOP).</li> <li>▪ Sweat should be collected onto preweighed sodium chloride free filter paper or Wescor disposable collectors.</li> <li>▪ The size of the filter paper should be approximately equal to the area stimulated, i.e. the size of the electrolyte support pads</li> <li>▪ Filter paper should be covered with a sheet of impervious material at least 1cm larger in all dimensions than the filter paper.</li> <li>▪ The impervious material must be completely sealed to the skin surface using a suitable adhesive tape.</li> <li>▪ Filter paper and the inner side of the impervious material must never come into direct contact with the operator's hands.</li> <li>▪ Wescor collectors should be used according to the manufacturer's instructions, taking precautions to avoid direct contact of the sweat collecting surface with the operator's hands.</li> </ul>	<p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p>
<p><b>Collection Time</b></p> <ul style="list-style-type: none"> <li>▪ Sweat should be collected for not more than 30 minutes and not less than 20 minutes.</li> <li>▪ The Orion electrode should not be used.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>B</b></p>
<p><b>Sweat Analysis</b> <b>Pre-analytical</b></p>	
<p><b>Storage before analysis</b></p> <ul style="list-style-type: none"> <li>▪ Throughout sweat collection, transport and analysis every effort should be made to minimise evaporation of the sample.</li> </ul>	<p style="text-align: center;"><b>C</b></p>



<p><b>Report format</b></p> <p>The report format should include:-</p> <ol style="list-style-type: none"> <li>i. Full patient identification</li> <li>ii. Date and time of test and date and time of report</li> <li>iii. Sweat weight/volume collected and minimum weight/volume acceptable for local sweat test parameters</li> <li>iv. Analytical results (mmol/L) <b>It should be explicit on the report form which analyte(s) have been measured.</b> i.e. chloride sodium conductivity (sodium chloride equivalent)</li> <li>v. Reference ranges (see section 6)</li> <li>vi. Interpretation of results (see section 6)</li> <li>vii. Recommendations for repeat testing if appropriate (see section 6.9)</li> </ol>	<b>C</b>
<p><b>Quality</b></p> <ul style="list-style-type: none"> <li>▪ Sweat which has been subject to evaporation and/or contamination must not be measured.</li> <li>▪ The analytical range of the methods used must cover the concentration ranges found in normals and subjects with cystic fibrosis.</li> <li>▪ The analytical methods must be fully documented as standard operating procedures (SOP) to comply with Clinical Pathology Accreditation (or equivalent standard). The SOP must include the analytical method(s), quality control procedures, reporting, interpretation and safety aspects. An example SOP is provided (Appendix Documents 2a and 2b)</li> <li>▪ There must be an internal quality procedure (which differs from the calibration/standardisation procedure) at two concentrations (normal and intermediate or abnormal) for each analysis.</li> <li>▪ The analytical methods should each have a between batch CV of 5% (or less) at a concentration of 40-50 mmol/L.</li> <li>▪ The laboratory must participate in a suitable external quality assessment scheme.</li> <li>▪ If chloride and sodium concentrations are widely discrepant, the test should be repeated.</li> <li>▪ Results which are not physiological should be questioned, i.e. chloride or sodium &gt; 150 mmol/L.</li> <li>▪ For conductivity a provisional upper physiological limit of 170 mmol/L may be used pending further evidence</li> </ul>	<b>C</b>  <b>C</b>  <b>C</b>  <b>C</b>  <b>B</b>  <b>C</b>  <b>B</b>  <b>B</b>  <b>C</b>



<b>Responsibility for Testing and Training</b>	
<ul style="list-style-type: none"> <li>▪ Sweat collection must be performed by fully trained and experienced personnel:-               <ul style="list-style-type: none"> <li>- training schedules should be fully documented</li> <li>- the procedure should be documented as a standard operating procedure</li> <li>- appropriate revalidation procedures should be in place</li> </ul> </li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat collection can be undertaken by a variety of health professionals</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat analysis should be performed by qualified and experienced biomedical scientists or clinical scientists who are fully trained with regular validation:-               <ul style="list-style-type: none"> <li>- training and validation schedules should be fully documented</li> </ul> </li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ A consultant (or equivalent) clinical chemist should have responsibility for training, assessment of competence and revalidation for all staff undertaking sweat tests.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ A minimum number of 50 sweat tests per annum should be performed in any one centre.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ A minimum number of 10 collection procedures should be performed per person per annum</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ The <b>responsibilities</b> for sweat testing, both collection and analytical, should rest with a consultant (or equivalent) clinical chemist and should be clearly understood by all operators and users; a mechanism for reporting any concerns about performance should be in place and clearly understood.</li> </ul>	<b>C</b>

## GUIDELINES REPORT

### INTRODUCTION

#### Background/Need for a Guideline

Cystic Fibrosis (CF) is the most common life limiting autosomal recessive disease in Northern European populations with an incidence of 1:2500 live births <sup>(1)</sup>. It is less common in the American black population (1:15,000) <sup>(2)</sup> and rare in Oriental populations (1:90,000) <sup>(3)</sup>. The incidence in the Asian population is less well known but probably around 1:10,000 <sup>(4)</sup>.

The typical or classic clinical manifestations of respiratory infection and exocrine pancreatic insufficiency with elevated sweat electrolytes, result from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7. Over 1000 mutations at this locus are associated with CF and other CFTR related disorders.

Clinical features that can be associated with atypical presentation include sino-pulmonary disease, pancreatic sufficiency, idiopathic pancreatitis, isolated obstructive azoospermia due to absence of the vas deferens and heat stroke. Such patients may have only one identified mutation.

The sweat test, a quantitative measurement of electrolytes in sweat, remains vital in supporting the clinical diagnosis of cystic fibrosis. Indications for sweat testing include:-

- \* Phenotype suggestive of CF
- \* Family history of CF
- \* A positive newborn screening test
- \* Suspicion of an atypical phenotype

In the majority of CF patients with typical features and identified mutations, the sweat test is diagnostic. In atypical CF where CF mutations have been identified, the sweat test can be intermediate, but is usually helpful in making a diagnosis <sup>(5,6)</sup>. The diagnosis of CF can remain uncertain in those patients with suggestive clinical features, an intermediate sweat test and no identified mutations. Very rarely, the sweat test is normal in a patient with a genotype of CF <sup>(7,8,9)</sup>.

The sweat test remains the key laboratory test to support the diagnosis of CF. It is of critical importance that sweat testing is carried out accurately with measurement of relevant analytes to allow clinical interpretation of results. Sweat testing is currently performed in approximately 180 laboratories across the UK. In most cases, sweat collection and analysis is performed by a clinical chemistry department.

A UK audit demonstrated wide variability in practice and standards<sup>(10)</sup>. Particular concerns were lack of appropriate quality control for analytical methods, a substantial number of laboratories measuring sodium alone, variability in reference ranges, lack of audit and sporadic reports of adverse patient events.

These findings stimulated the Specialist Advisory Group for Paediatric Investigations of UK NEQAS to establish an external quality assessment scheme for sweat analysis, and the need for guidelines for performance of the sweat test.

## **OBJECTIVE**

To produce guidelines on how to perform the sweat test for the investigation of cystic fibrosis in the UK.

To include:

- ◆ organisation/delivery for patient care (including patient/parent information)
- ◆ sweat collection (including subject suitability)
- ◆ sweat analysis
- ◆ quality of the testing
- ◆ interpretation of results (including false positives; indications for repeat analysis; use of other tests)
- ◆ who should do the collection and analysis
- ◆ assessment of competence and training needs

## **SCOPE**

The guidelines cover the following aspects of the sweat test and apply to subjects of all ages.

### **Patient information**

#### **Subject suitability**

- physiology
- clinical state
- exclusions/restrictions

#### **Sweat Collection**

- site of collection
- stimulation methods and equipment
- collection medium/time/containers

### **Sweat Analysis**

- weighing
- elution
- analytes
- analytical methods
- reporting

### **Quality**

- internal quality control
- external quality assessment
- audit

### **Reference Values and Interpretation**

- definitions
- false positives
- repeat testing
- use of other tests

### **Responsibility for testing and training**

- responsibility for sweat testing
- who should perform sweat testing?
- competence/training

## THE EVIDENCE AND RECOMMENDATIONS

### 1. Patient information

#### Evidence

Systematic searching failed to find any randomised, controlled or evidence based publications regarding the value of patient information material in preparation for a sweat test. There are no studies that look at the content or format of patient information.

A recent survey of laboratories in the United States due for publication this year<sup>(11)</sup> revealed that of 800 laboratories doing sweat testing in the United States: 10% provide written information, 1% have video formats and 30% have formalised verbal information.

**Evidence level IV**

An informal enquiry by the working group of major centres indicated that in the UK approximately 50% have local information sheets<sup>(12)</sup>.

Information sheets are considered good clinical practice and an accepted means of distributing information although they may have shortcomings:-

- patients/parents may not be in a situation or environment to integrate the information
- the personnel given the responsibility to hand out the sheets may not be equipped to answer the questions that arise
- there may be language difficulties

**Evidence level IV**

Patient Information	Grade
<ul style="list-style-type: none"> <li>▪ It is good clinical practice to prepare the patient and, where appropriate, parent effectively before testing. Informed consent should be obtained in accordance with local policy. Pre-test information appropriate for the individual should include why the test is being done, how it will be performed, risks associated with the test, what the subject will experience, and contact details regarding the testing and final result. An example leaflet for patients/parents is provided (see Appendix document 1).</li> </ul>	<p><b>C</b></p>

## 2. Subject Suitability

There are a number of subject factors that can affect sweating and sweat test results. In situations where sweat testing has been shown to be unreliable, genotyping may be the diagnostic test of choice.

### Evidence

Preterm infants do not sweat in the first 7-14 days, but most term infants are able to sweat from the first day<sup>(13)</sup>.

**Evidence level III**

In term infants, sweat sodium (and chloride) can be high in the first 7 days, particularly in the first 48 hours<sup>(14)</sup>.

**Evidence level III**

The consensus from current clinical practice is that it can be difficult to collect adequate quantities of sweat from very young infants, especially below 3 kgs in weight.

**Evidence level IV**

Sweat electrolytes can be elevated in underweight or dehydrated infants and lowered in infants on systemic corticosteroids or with oedema<sup>(15,16)</sup>.

**Evidence level IV**

Sweat electrolytes can be elevated in subjects if the stimulation site has active eczema<sup>(17)</sup>.

**Evidence level IV**

Sweat electrolytes are not affected by flucloxacillin<sup>(18)</sup>. There are no data for other antibiotics

**Evidence level IIb**

Sweat electrolytes are not affected by diuretics or intravenous fluids as long as the patient is stable<sup>(15)</sup>.

**Evidence level IV**

NCCLS guidelines<sup>(19)</sup> state 'Iontophoresis should not be performed on a patient receiving oxygen by an open delivery system. While the possibility of an explosion due to the generation of an electrical spark is remote, it cannot be ignored.' No evidence is given to support this statement.

**Evidence level IV**

Subject Suitability	Grade
<ul style="list-style-type: none"> <li>▪ Sweat tests can be performed after 2 weeks of age in infants greater than 3 kg who are normally hydrated and without significant systemic illness</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat testing can be attempted in term infants after 7 days of age if clinically important, but will need repeating if insufficient quantity of sweat is collected</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests should be delayed in subjects who are dehydrated, systemically unwell or who have eczema affecting the potential stimulation sites</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests should be delayed in subjects who are oedematous and/or on systemic corticosteroids</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests should not be performed in subjects who are on oxygen by an open delivery system. This would not apply to an infant in headbox or on nasal prong oxygen.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat tests can be performed in subjects on flucloxacillin</li> </ul>	<b>B</b>

### 3. Sweat Collection

The following sections give recommendations for practice and the evidence on which they are based. The practical procedure is covered in detail in the example standard operating procedures (see Appendix 2a and 2b).

#### Evidence

##### 3.1 Site of collection

The UK laboratory subgroup confirmed that the majority of testing centres use the flexor surface of the forearm as the sweat collection site<sup>(12)</sup>. The cathode may be placed in a variety of locations on the flexor or extensor surface of the same forearm, or on the upper arm. The back, chest and thigh are used successfully by some centres<sup>(20)</sup>.

**Evidence level IV**

##### 3.1.1 Avoidance of Contamination

The NCCLS guidelines<sup>(19)</sup> give detailed instructions as follows:

Do not stimulate sweat from any area of diffuse inflammation, serous or bloody discharge. Use gauze or filter paper that is low in sodium and chloride content. Wash and dry the patient's skin thoroughly. Do not directly handle the weighing vial, the paraffin wax film, the collection site, or the collection filter paper with the fingers. Always use forceps or powder free gloves.

**Evidence level IV**

### 3.1.2 Number of Collections

A few UK centres always or sometimes carry out two separate collections in response to a request for a sweat test<sup>(20)</sup>; this is also recommended in the NCCLS guidelines<sup>(19)</sup>. The reason given is quality control - a discrepancy between the two results suggesting unacceptable precision in the performance of the duplicate tests. An audit of 158 paired left and right arm chloride concentrations from patients attending a UK centre revealed no CF patient would have been missed if testing had been carried out on one arm only. An Altman Bland difference plot showed no bias between the arms, and a confidence interval for 95% limits of agreement for chloride of 20mmol/L<sup>(12,20)</sup>.

**Evidence level III**

Testing in duplicate will not identify systematic or contamination errors affecting reagents or equipment in use on that day.

**Evidence level IV**

**Carrying out the sweat test procedure in duplicate increases the time taken and the discomfort for all patients without improving the diagnostic rate.**

**Overall Evidence level III**

## 3.2 Stimulation (Methods and Equipment)

### 3.2.1 Equipment - Power Supply and Electrodes

The power supply, electrodes, electrolyte solutions and supports must be capable of supplying an adequate current to induce sweating by iontophoresis while not compromising patient safety due to excessive current density.

Some early power sources used AC current, with a risk of cardiac arrhythmias<sup>(21)</sup>, leading to the recommendation that electrodes should never cross the trunk, and that the right arm should be used rather than the left. Other power sources consisting of a simple battery and rheostat had no safety cut-out, allowing current increase if skin resistance dropped eg as the result of blistering<sup>(21,22)</sup>. They therefore depended on manual adjustment of current throughout the iontophoretic procedure. Incorporation of a fixed resistor, in addition to the variable resistor, can limit the maximum current even when the power supply is shorted. Circuit diagrams have been published<sup>(21)</sup>, some of which incorporate a safety circuit<sup>(23)</sup>. In house manufacture by Medical Physics or an electrician's workshop without independent testing may no longer be considered to meet acceptable safety standards. Wescor systems incorporate both a fail safe current cut-out, and a high resistance cut-out<sup>(24,25)</sup>, as does the C&S Electronics power supply<sup>(26)</sup>.

**Evidence level IV**

CPA standards<sup>(27)</sup> state that: All equipment must be safe, clean and cared for. All equipment must be checked for compliance with "Safety Requirements for electrical equipment for measurement, control and laboratory use". There are standard operating procedures for the regular maintenance of equipment. The department must have a procedure for reporting adverse incidents relating to medical devices to the appropriate authority.

**Evidence level IV**

**Existing Consensus Guidelines state:**

a. NCCLS <sup>(19)</sup>: If current source is manually controlled a milliammeter should be supplied to enable the operator to keep current below 4mA. Equipment should be battery powered. 22.5V is sufficient, although automatically controlled power supplies may require higher voltage. Electrodes should be made of copper or stainless steel. If larger than 2.5x2.5cm they should be sufficiently pliable to bend to fit the patients forearm or leg. Surface should be smooth and free of irregularities. If gel discs are used these should fit snugly into the recessed electrode. Copper electrodes should be cleaned with emery cloth with each use.

**Evidence level IV**

b. Welsh <sup>(28)</sup>: The equipment used should be EMS or Wescor. The equipment should be maintained as per manufacturer's recommendations with particular reference to safety aspects. Records of electrode inspection and maintenance must be kept for independent inspection if required.

**Evidence level IV**

Electrodes have been made from a variety of materials. Schwarz showed penetration of electrode metal ions into the pads during iontophoresis <sup>(22)</sup>. This was of most concern for lead and copper but could be minimised by increasing the electrolyte pad thickness.

**Evidence level IIb**

**Power supplies must be battery powered and should incorporate a safety cut-out circuit. Power supplies and electrodes must be regularly checked and maintained, and records kept.**

**Overall Evidence level IV**

**3.2.2 Electrolyte Solutions:**

Price <sup>(29)</sup> showed increasing pilocarpine nitrate concentration from 0.5-5.0g/L increased the sweat yield and decreased the chloride result. The effect was most marked at <1g/L.

**Evidence level IIb**

Szabo <sup>(30)</sup> showed no effect on chloride results using 2.5, 5.0 or 7.5g/L pilocarpine nitrate.

**Evidence level IIb**

Pilocarpine nitrate is universally used as the pilocarpine source at the anode, replacing pilocarpine hydrochloride <sup>(31)</sup> in earlier literature. Concentrations used vary from 0.64g/L <sup>(31)</sup> to 15g/l <sup>(32)</sup>, with a single case report at 100g/L <sup>(33)</sup>.

**Evidence level IV**

The cathode electrolyte solution is arbitrary, serving to complete the electrical circuit. 0.07N Sodium bicarbonate <sup>(34)</sup>, 0.02N sulphuric acid <sup>(31)</sup>, 3 or 10 g/l potassium sulphate <sup>(22,35)</sup>, 10g/l sodium nitrate <sup>(36)</sup> have all been used. All Wescor Pilogel systems use the same pilocarpine nitrate electrolyte at both anode and cathode <sup>(32,37)</sup>. Avoidance of sodium and chloride containing electrolytes to reduce the possibility of contamination of the anode site would seem sensible, but has not been systematically studied.

**Evidence level IV**

The use of alkaline solutions such as sodium bicarbonate was demonstrated to lessen the likelihood of acid burns <sup>(22,29)</sup>.

**Evidence level IIb**

Respondents to a survey in the UK <sup>(38)</sup> used 2-5g/l pilocarpine nitrate on gauze, lint or filter paper, or 5g/l pilocarpine nitrate on gel discs. As cathode electrolyte virtually all used varying concentrations of magnesium sulphate (from 0.05 to 2.0 moles/L) or pilocarpine nitrate as for the anode.

**Evidence level IV**

**Existing Consensus Based Guideline state:**

a) NCCLS <sup>(19)</sup>. Pilocarpine nitrate 2-5g/L in aqueous solution or as 5g/l in gel disc at anode. 0.05moles/L magnesium sulphate or 0.01M sulphuric acid or pilocarpine gel disc at cathode.

**Evidence level IV**

b) Welsh <sup>(28)</sup>. Pilocarpine nitrate 2-5g/L in aqueous solution at anode, 0.1moles/L magnesium sulphate at cathode, or as for anode. Wescor gel discs according to manufacturer's instructions.

**Evidence level IV**

**Pilocarpine nitrate at 2-5g/l is recommended for use at both electrodes. Alternative solutions such as magnesium sulphate may be used at the cathode.**

**Overall Evidence level IIb**

Pilocarpine and other electrolyte solutions, when used for iontophoresis may be:-

1. Supplied as part of a manufacturer's system, e.g. as Wescor Pilogel discs.
2. Obtained entirely separately from the power pack used to iontophorese the drug into the sweat glands. This is the case for pad absorption sweat collection systems. The manufacturer of the power pack for these systems explicitly states that they do not manufacture or supply electrolyte solutions.

In the UK, pilocarpine and other electrolytes used with such systems fall into the category of unlicensed relevant medicinal products (commonly described as "specials") (Ref A). They may only be supplied under strictly limited conditions, by manufacturers who hold a "specials" licence (Ref B). "Specials" manufacturers within the UK may not advertise special products, although they may respond to requests for information on specific products. They include a number of hospital pharmacy department classed as production pharmacies as well a commercial companies. A full list of contact details is published regularly (Ref C).

Clinical biochemistry laboratories do not meet the legal requirements of a "specials" manufacturer, and should not manufacture electrolyte solutions for iontophoresis in-house.

**Evidence level IV**

## References

- A. Appelbe GE, Wingfield J. Chapter 2. Medicines Act 1968. The Licensing System. P13-34 in Pharmacy law and Ethics 1998 (\*6<sup>th</sup> edition). The Pharmaceutical Press, London.
- B. The supply of unlicensed relevant medicinal products for individual patients. MCA Guidance Note 14. Revised February 2000. Policy Unit, Inspection & Enforcement Division, Medicines Control Agency, Market Towers, 1 Nine Elms Lane, London, SW8 5NQ.
- C. Special-order Manufacturers. British National Formulary latest edition. British Medical Association and Royal Pharmaceutical Society of Great Britain (e.g. No. 42 September 2001, p 767).

### 3.2.3 Iontophoresis - time, current

The earliest iontophoresis reference <sup>(34)</sup> specified 2mA/4.9cm<sup>2</sup>, (after a slow increase) for 5 min if filter paper supports used, 15 min if gauze used. Other early papers used 2-5mA for up to 15 minutes, without investigating the effect of variation <sup>(21,22,30,31,35,39)</sup>. The first Webster sweat collection system model 3500 <sup>(24,36)</sup> used gauze pads and suggested 1.5mA for 5 min after a slow rise, from a power supply capable of settings from 1-5mA.

**Evidence level IIb**

Price <sup>(29)</sup> collected insufficient sweat by the Orion system using a total time of 5 min. He varied stimulation times from 5-15 min once the current had stabilised within the iontophoresis zone and found no effect on sweat volume or chloride result.

**Evidence level IIb**

Webster <sup>(40)</sup> reviewed the theoretical basis of calculation of pilocarpine delivery per square centimeter of skin surface, and concluded the heterogeneous medium of the dermis, sweat glands and capillaries was too complex for this to be valid. He concluded that conditions could only be established empirically. He demonstrated that increasing iontophoresis time from 1-3 min at 1.5mA increased sweat production rate, however further increase of iontophoresis time from 3-7 min produced no further increase in sweat rate. Kirk <sup>(41)</sup> demonstrated that using a current of 1, 2 or 4mA had no effect on sodium or osmolality results.

**Evidence level IIb**

Webster calculated that optimal sweating occurred when 47 mC of electrical charge was delivered per square centimeter of skin surface <sup>(40)</sup>. A lower current was therefore sufficient to induce adequate sweating using the smaller Wescor electrodes. Survey of practice in 1998-9 of 30 UK laboratories <sup>(38)</sup> identified the use of currents of 1.5 to 4mA applied for 4-10 minutes. Only 2 centres used >5.5 min.

The current density at the stimulation site will depend on the area over which the current is applied. In practice, a current of 4 mA is used with a wide variety of electrode sizes without any reports of adverse affects.

**Evidence level IV**

The Wescor instruction manuals state: With the introduction of Pilogel pilocarpine discs <sup>(37)</sup> the Wescor model 3600 power supply was set to deliver 1.7mA for 5 min <sup>(25)</sup>. Operator variation was not possible. The most recent Wescor Nanoduct system <sup>(32)</sup> uses 0.5mA for 2 min once current attained through smaller electrodes, and increases the pilocarpine concentration threefold in buffered citrate gel.

**Evidence level IV**

Existing Consensus Based Guidelines state:

- a. NCCLS <sup>(19)</sup>: For filter paper sweat collections NCCLS recommends beginning with a current of 0.5mA, increasing gradually to 4mA and maintaining 4mA for 5 min. Current is decreased slowly. For Microbore tubing collections (Wescor) follow the manufacturers instructions.

**Evidence level IV**

- b. Welsh <sup>(28)</sup> Current should be increased slowly to a maximum of 4mA and manually controlled at this level by output control for 5 minutes. Current is decreased slowly. Wescor - programmed automatically to 1.5mA for 5 minutes, then decreased slowly.

**Evidence level IV**

To provide adequate sweat stimulation iontophoresis should be carried out for at least 3 minutes, but for no longer than 5 minutes. The current should not exceed 4 mA.

**Overall Evidence level 11b**

### 3.2.4 Safety:

1. The definitive paper by Schwarz <sup>(22)</sup> examined hazards in detail. A 50mA current passed from one forelimb to the other in a rabbit caused respiratory arrest by tetanic muscular spasm. Application of a current of 1.5mA per square centimetre to a healthy adult's forearm (i.e. greatly in excess of the density of 0.07mA/cm<sup>2</sup> used in the standard test) did not cause blisters or burns provided plenty of electrolyte was present <sup>(22)</sup>. Schwarz carried out a series of experiments to assess factors that predisposed to blisters and burns. He showed atropine injection produced an oedematous swollen area and reduced skin resistance by a factor of 40-100. Iontophoresis at 4mA for 5 min to introduce atropine into the skin resulted in pain, pitting and oedematous swelling. Skin resistance over areas of frank blisters was even further reduced. He noted that with constant current iontophoresis skin resistance could be observed to drop markedly as blisters formed. The following measures increased the risk of complications:-

- ◆ Tightly strapped electrodes produced worse blisters than loosely fitted ones with movement of electrode to equalise current flow.
- ◆ The lowest pH and worst blisters were found with unbuffered hydrochloric acid at concentrations down to 0.01 mmol/L. With Potassium chloride blisters formed if fewer than 12 filter papers were used. Blisters formed during iontophoresis when skin pH < 2.6. 0.1N citrate and HCl/glycine buffers caused blisters when the initial buffer pH was < ~2.0. 0.9% sodium chloride and 1.2% sodium nitrate generated pH of 3.4 and 3.2 next to the electrode. Using 4.5g/L pilocarpine nitrate and 12 filter papers the pH of the papers nearest the skin was 5.9, and of those nearest the electrode was 4.2 after 4mA for 5 min.
- ◆ Less filter papers led to more blistering
- ◆ Dryer filter papers led to more blistering

**Evidence level IIb**

The original iontophoresis paper <sup>(34)</sup> refers to the rare occurrence of burns, attributed to skin- electrode contact. A large-scale survey <sup>(31)</sup> of 7,200 tests carried out by an experienced technician reported superficial burns at the cathode at a rate of <1:200.

**Evidence level IV**

2. Reports of adverse effects are all small number studies
  - a. One anecdotal report <sup>(42)</sup> details a burn suffered during a sweat test carried out by an inexperienced SHO without monitoring the patient during iontophoresis.

**Evidence level IV**

- 1) b. Rattenbury <sup>(43)</sup> reported two cases of burns in tests carried out by experienced technicians. In one case locally modified button electrodes had been applied without pilocarpine gels in place. In the second the infant had eczema, although not at the test site. The burn was attributed to electrode skin contact. A questionnaire returned by 6/10 paediatric labs indicated that all had seen burns at some time. Skin reddening was seen frequently - spectrum of normal response to pilocarpine iontophoresis; blisters were associated with acid burns due to inadequately dampened pads or uneven electrode surface; frank electrical burns were due to electrode-skin contact.

**Evidence level IV**

- c. Two incidents <sup>(44)</sup> ascribed to use of buckled, corroded and poorly maintained electrodes were reported to the MDA and led to production of a safety notice. This recommends that pads be slightly larger than the electrodes to minimise the chance of electrode- skin contact. No incidents were reported to the Scottish Incident Reporting & Investigation Centre <sup>(45)</sup>.

**Evidence level IV**

3. The UK surveys <sup>(38)</sup> showed 21/30 centres had observed reddening or urticaria and 7 had observed blistering or burns. Regional surveys using the same or similar questionnaires showed variability in whether or not burns had ever been reported <sup>(46,47)</sup>.

**Evidence level IV**

4. An early consensus document <sup>(21)</sup> recommended that pads should be the same size as electrodes, as larger pads led to higher current density immediately below the electrode, especially if tightly fastened. The Wescor 3600 system electrodes are recessed <sup>(25,37)</sup> reducing the potential for the edge of the electrode to make skin contact. This advantage is lost if pilogel discs are used with non Wescor electrodes. The use of gel discs 6mm thick also provides a uniform current density and the thickness reduces acid penetration from the electrode to the skin.

**Evidence level IV**

5. Existing Consensus Guidelines state:

- a. **NCCLS** <sup>(19)</sup>: Gauze squares or filter paper, slightly larger than the electrodes but smaller than the patient's extremity. Should be larger than the electrode. Eg 3.8x3.8cm electrode should use 5.1x5.1cm gauze square.

**Evidence level IV**

- b. **Welsh** <sup>(28)</sup>: Hospital Lint (BPC Plain 500gram 4-8 thicknesses, or Whatman 42/44 filter paper 10-15 papers)

**Evidence level IV**

**A theoretical risk of atrial fibrillation has never been documented. Burns or blisters are sporadically reported resulting from electrode-skin contact or inadequate reservoir of electrolyte solution between skin and electrode.**

**Overall evidence level IV**

**Risk can be minimised by using well saturated lint pads of a suitable size and thickness, and by observing the patient for signs of distress or disturbance of the electrodes and pads throughout the iontophoresis.**

**Overall Evidence level IIb**

All evidence in this section has been incorporated into recommendations under the headings of Sweat Collection, Electrolyte Solutions, Electrolyte Supports and Iontophoresis - time, current.

### **3.3 Collection Medium/Time**

Sweat must be collected in sufficient quantity for accurate and precise analysis. During collection it must be protected from contamination and evaporation. At the end of the collection, all sweat produced, including any condensate on the waterproof covering, must be transferred back to the filter paper for analysis. See example SOP (appendix 2a and 2b) for details.

**Evidence level IV**

#### **3.3.1 Medium of Collection**

1. Gibson and Cooke <sup>(34)</sup> described collection of sweat onto filter paper circles 2.5cm in diameter or gauze 3x3 inch squares. The only advantage of the larger gauze squares coupled with a longer collection time was to increase the volume of sweat collected, necessary for the sensitivity of early chloride methods.

**Evidence level IV**

2. Concern about possible sodium chloride contamination led some authors to wash and dry filter papers or gauze before use <sup>(39)</sup>.

**Evidence level IV**

3. A number of authors experimented with collectors that allowed liquid sweat to be pooled or collected in sequential aliquots. Identification of problems of condensation on cold cups <sup>(48)</sup> led Webster to develop first his heated cup system <sup>(36)</sup> and subsequently the Macroduct <sup>(37)</sup> and conductivity <sup>(32)</sup> collectors.

**Evidence level IV**

4. Filter papers are covered with an impervious sheet of material, and secured in place with adhesive tape. Suitable materials include polythene, parafilm and oiled silk.

**Evidence level IV**

5. Current Consensus Guidelines:

- a. **NCCLS** <sup>(19)</sup>: recommend use of gauze squares or filter papers. These should be low in sodium and chloride content. If filter papers are used they should be of a type sufficiently absorbent to collect all of the stimulated sweat. Use the same size gauze or filter paper for stimulation and collection. Alternatively sweat may be collected into microbore tubing.

**Evidence level IV**

- b. **Welsh** <sup>(28)</sup>: recommend Whatman No42/44 preweighed paper or alternatively, Wescor microbore tubing.

**Evidence level IV**

**Sweat should be collected onto low sodium filter paper of approximately equal size to the stimulated area (ie the lint pads used in iontophoresis) or into Wescor collectors. Filter paper must be sealed into position with impervious material such as polythene or parafilm and waterproof adhesive tape. Care must be taken to ensure the seal is intact throughout the collection.**

**Overall Evidence level IV**

### 3.3.2 Collection Time:

There is a wealth of experimental data on the effect of length of collection time on sweat secretion and sweat concentration.

1. The Orion electrode was designed to measure sweat conductivity as soon as sweat appeared on the skin surface. Price <sup>(29)</sup> showed that this gave erratic readings due to insufficient sweat secretion, and modified the method to collect sweat under a cup for 10 minutes before measurement in situ.

**Evidence level IIb**

2. Differential sweat collections have been carried out by collecting for 5 min periods onto different filter papers<sup>(31)</sup>, collecting onto Macroduct<sup>(49)</sup> or other tubing<sup>(50)</sup> and sectioning the tubing, or measuring conductivity continuously<sup>(32)</sup>. All authors concluded that stimulated sweat secretion is initially low, then rises, but once established (after approximately 2 minutes), falls steadily with time.

**Evidence level IIb**

3. Often using measurements of osmolality or conductivity for differential sweat collection it was also shown that sweat concentration decreased in tandem with sweat secretion rate<sup>(29,32,36,49,50,51)</sup>. Price<sup>(29)</sup> found sweat chloride fell steadily, reaching a plateau 10 minutes after iontophoresis ended. Over the range of times that might be used in practice Kirk<sup>(41)</sup> found no significant difference in osmolality or sodium collected from 2 adult volunteers on separate occasions for 10, 20 or 40 minutes.

**Evidence level IIb**

4. Hjelm considered that lower sweat weight (collected over 30 min) resulted in higher sweat sodium concentration and used a graph of sweat weight versus sweat sodium to aid interpretation<sup>(52)</sup>.

**Evidence level III**

5. Decreasing sweat collection time from 60 to 30 minutes led to a statistically insignificant decrease in the mean weight of sweat collected from 520 to 490 mg<sup>(20)</sup>. Extending the sweat collection time beyond 30 minutes will produce little, if any, additional weight/volume.

**Evidence level III**

6. Current Consensus Guidelines state:
  - a. **NCCLS**<sup>(19)</sup>: The collection time for sweat is generally 30 minutes. Extension of this time can result in a sample taken from less than maximally stimulated glands and can lead to a false negative result.
  - b. **Welsh**<sup>(28)</sup>: The sweat should be collected at ambient temperature for 30 minutes.

**Evidence level IV**

**Sweat secretion is low immediately after iontophoresis, increases to a maximum between 10-30 minutes, and then decreases rapidly. Sweat should be collected for not less than 20 minutes and not more than 30 minutes. Measurements in situ (Orion electrode) should not be used<sup>(53)</sup>.**

**Overall Evidence level IIb**

**Addendum: Availability of replacement Equipment in UK:**

The widely used EMS power supply is no longer manufactured, although the company still services existing units. The only identified supplier of a battery powered, current limited "Gibson-Cooke" power supply is C&S Electronics Inc, 2565 16<sup>th</sup> Ave, Columbus, NE 68601<sup>(54)</sup>. This is widely used in the USA.

Wescor systems are supplied in the UK by Chemlab Scientific Products, Hornchurch, Essex.

<b>Sweat Collection</b>	
<ul style="list-style-type: none"> <li>▪ The flexor surface of either forearm is the preferred site for sweat collection. Consideration may be given to other sites if both arms are eczematous, too small or otherwise unsuitable. Other sites used successfully include the upper arm, thigh and back.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Great care must be taken at all stages of the procedure to avoid contamination (see example SOP)</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ In response to a sweat test request it is sufficient to carry out one sweat collection only.</li> </ul>	<b>B</b>
<ul style="list-style-type: none"> <li>▪ The power supply used must be battery powered and should include a safety cutout.               <ul style="list-style-type: none"> <li>- monitoring of the current must be carried out throughout iontophoresis where possible. Wescor systems from model 3600 onwards have no ammeter but have an appropriate safety cut out system</li> <li>- the power supply and electrodes must be regularly checked, maintained and records kept.</li> <li>- electrical safety of all power supplies must be checked annually.</li> </ul> </li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Electrodes should be of a suitable size and curvature to fit snugly on the patient's limb.               <ul style="list-style-type: none"> <li>- they are most commonly made of copper or stainless steel.</li> <li>- electrodes should be firmly secured in position to the electrolyte support pads or gels using straps that are adjustable to fit the patient (eg Velcro or rubber).</li> </ul> </li> <li>- electrodes must be regularly cleaned and inspected, and discarded if they show pitting or irregularities.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Selection of new equipment, and maintenance of existing equipment must comply with CPA Accreditation (or equivalent standard).</li> </ul>	<b>C</b>

<p><b>Electrolyte Solutions</b></p> <ul style="list-style-type: none"> <li>▪ Aqueous solutions or Wescor gel discs containing Pilocarpine nitrate at 2-5g/l are recommended for use at both electrodes. Alternative solutions (e.g. magnesium sulphate) may be used at the cathode.</li> <li>▪ Solutions containing sodium and/or chloride should be avoided because of the risk of contamination of the collection</li> <li>▪ Unbuffered acid solutions should not be used because of the increased risk of burns.</li> <li>▪ Electrolytes used for iontophoresis must either be obtained as part of a medical device (e.g. Wescor Pilogel discs) or from a recognised manufacturer of unlicensed medical products. Solutions must not be produced in-house by hospital laboratories.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>C</b></p>
<p><b>Electrolyte Supports</b></p> <ul style="list-style-type: none"> <li>▪ Suitably thick pads must be used for the electrolyte solutions to minimise the risk of acid burns. Pads of Hospital Lint BPC Plain 500 gram folded to provide 4-8 thicknesses (greater than 1cm thick) are recommended as an electrolyte reservoir with filter paper collection systems. The pad should be at least 1cm larger than the electrode in all directions to prevent electrode-skin contact. It may be incorporated into sewn pockets designed to contain the electrode and prevent skin contact. The pads should be saturated by soaking in the electrolyte solution before application to the patient's skin.</li> <li>▪ Hybrid systems, e.g. Wescor electrodes with aqueous electrolyte solutions, or Wescor gel discs used with non-Wescor electrodes, should not be used.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>B</b></p>
<p><b>Iontophoresis - time, current</b></p> <ul style="list-style-type: none"> <li>▪ When aqueous electrolyte solutions are applied on pad supports a current of 0.5 mA should be applied, and increased gradually to a maximum of 4 mA. Once 4 mA is attained the current should be maintained for a minimum of 3 minutes and a maximum of 5 minutes. Longer times should not be necessary to increase sweat production provided good electrical contact is maintained, by use of well maintained electrodes and suitably saturated pads.</li> <li>▪ When Wescor systems are used, the manufacturer's current and time recommendations should be followed. This will depend on the specific model used.</li> <li>▪ For both systems the patient must be kept under close observation throughout the iontophoresis period.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p>

<p><b>Medium of Collection</b></p> <ul style="list-style-type: none"> <li>▪ During collection sweat must be protected from contamination and evaporation (see example SOP).</li> <li>▪ Sweat should be collected onto preweighed sodium chloride free filter paper or Wescor disposable collectors</li> <li>▪ The size of the filter paper should be approximately equal to the area stimulated, i.e. the size of the electrolyte support pads</li> <li>▪ Filter paper should be covered with a sheet of impervious material at least 1cm larger in all dimensions than the filter paper.</li> <li>▪ The impervious material must be completely sealed to the skin surface using a suitable adhesive tape.</li> <li>▪ Filter paper and the inner side of the impervious material must never come into direct contact with the operator's hands.</li> <li>▪ Wescor collectors should be used according to the manufacturer's instructions, taking precautions to avoid direct contact of the sweat collecting surface with the operator's hands.</li> </ul>	<p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p> <p style="text-align: center;"><b>C</b></p>
<p><b>Collection Time</b></p> <ul style="list-style-type: none"> <li>▪ Sweat should be collected for not more than 30 minutes and not less than 20 minutes.</li> <li>▪ The Orion electrode should not be used.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>B</b></p>

#### 4. Sweat Analysis

##### 4.1 Pre analytical aspects

##### 4.1.1. Storage before analysis

Evaporation of sweat during collection, transfer and transport is a potential source of error in the sweat test <sup>(19)</sup>.

Liquid sweat may be stored in 100 µl capacity glass capillary tubes sealed with plasticine, provided an air gap is left between the sweat and the plasticine, for up to 6 hours <sup>(55)</sup>.

**Evidence Level IIb**

LeGrys <sup>(56)</sup> found no change in chloride concentration when sweat samples collected on gauze pads were stored at 4°C in tightly sealed containers for up to three days, with or without diluent.

**Evidence level IIb**

Macroduct capillary tubing is not entirely impermeable. Evaporation was evident within 48 hours on storage at room temperature, but there was no change in conductivity measurements in sodium chloride solutions stored in heat sealed Macroduct tubing at 4°C for up to 72 hours <sup>(57)</sup>.

Evidence Level IIb

**Sweat may be collected at remote sites and transported to the laboratory for analysis provided there is attention to storage details.**

Overall evidence level IIb

#### **4.1.2. Weighing of sweat collected**

In order to accurately weigh sweat to the nearest mg, a balance sensitive to 0.0001g is required as recommended by NCCLS <sup>(19)</sup>.

The same balance must be used to weigh the container and filter paper before and after collection. Re-weighing should take place as soon as practicable after collection.

Evidence level IV

#### **4.1.3 Definition of adequate sample**

Gibson and Cooke's original method <sup>(34)</sup> described a mean sweat rate of 5.17g/m<sup>2</sup>/min with a range from 1.22-9.18 in 55 subjects tested. Many authors have shown that sweat concentration varies with sweat secretion rate (see section 3.3.2) <sup>(29,32,49,51,52,58)</sup>.

Evidence level IIb

Earlier literature <sup>(58)</sup> stated that this did not cause difficulties in interpretation, as it was limited by the minimum volume of sweat required for analysis.

Evidence level IV

As analysis volumes fell, a minimum sweat secretion rate of 1g/m<sup>2</sup>/min averaged over the collection period was proposed <sup>(19)</sup>. This rate is achieved by the vast majority of subjects <sup>(12)</sup>.

Evidence level IV

Hjelm <sup>(52)</sup> showed an inverse correlation between sweat weight and sodium concentration for normal and CF children. He used a graph of sodium versus weight to fully separate the two populations, but did not report on chloride measurements.

Evidence level III

Data collected by the UK laboratory subgroup showed some evidence of an increase in intermediate chloride results when collections of less than  $1\text{g}/\text{m}^2/\text{min}$  were analysed, although this varied from centre to centre <sup>(12)</sup>. Although the figure is relatively arbitrary, collections of less than  $1\text{g}/\text{m}^2/\text{min}$  indicate either that suboptimal sweating has occurred or that a significant amount of the sweat produced has been lost by leakage or evaporation. Pooling separate sweat collections increases the weight available for analysis, but does not change whatever factors led to the poor yield being obtained, potentially influencing the result.

**Evidence level III**

A locally derived minimum sweat weight/volume should be calculated that equates to an average sweat rate of  $1\text{g}/\text{m}^2/\text{min}$  over the collection period. This minimum weight/volume should be quoted on the report. Collections below this minimum weight/volume should not be analysed. Insufficient sweat collections should not be pooled. The full sweat test should be repeated.

**Overall Evidence level III**

**Addendum:-**

Calculation of average sweat rate over the collection period

The average sweat production rate over the collection area is calculated in  $\text{g}/\text{m}^2/\text{min}$  to assess whether sweat production has been adequate. The collection area equates to the area covered by the sweat collector, i.e. the area of the filter paper or Macroduct collector. It should be approximately equal to the stimulated area over which pilocarpine has been delivered to the sweat glands by iontophoresis, i.e. the area covered by the pilocarpine soaked electrode supports or pilogel discs.

Calculate the collection area of the filter paper collector in  $\text{cm}^2$  as  $\pi r^2$  where r is the radius.

Pilogel discs and the collection area of a Macroduct have a diameter of 2.8cm.

The average sweat rate over the collection period ( $\text{g}/\text{m}^2/\text{min}$ ) =

$$\frac{10000}{\text{area (cm}^2\text{)}} \times \frac{\text{weight (mg or ul)}}{1000} \times \frac{1}{\text{collection time (min)}}$$

$$= \frac{10 \times \text{weight (mg or ul)}}{\text{collection area (cm}^2\text{)} \times \text{collection time (min)}}$$

A locally derived minimum sweat weight or volume should be used. This must be demonstrated to be equivalent to an average rate of  $1\text{g}/\text{m}^2/\text{min}$  e.g., for a 30 minute collection on 5.5cm diameter filter paper  $1\text{g}/\text{m}^2/\text{min} = 71\text{mg}$

For a 20 minute Macroduct collection  $1\text{g}/\text{m}^2/\text{min} = 12\text{uL}$ . For a 30 minute Macroduct collection  $1\text{g}/\text{m}^2/\text{min} = 18\text{uL}$ . The manufacturer's recommendation of about 15uL as a minimum volume appears to be the mean of these. This emphasises the importance of collection time in interpreting minimum acceptable rates, and the futility of increasing the collection time in an attempt to improve yield.

#### **4.2 Elution of sweat from filter paper**

There is very little evidence on which to base best practice. If laboratories collecting sweat on filter paper treat their internal QC appropriately (i.e. apply the QC material to filter paper) the results of internal QC will provide evidence of satisfactory elution. The NCCLS guidelines <sup>(19)</sup> recommend 40 mins without shaking (to avoid detaching fibres from the filter paper which might interfere in the analysis) for elution from filter paper. We are aware that many laboratories successfully employ a roller mixer. In the audit of laboratory practice in the Trent Region <sup>(46)</sup>, the most common time period allowed for elution was 30 minutes.

**Evidence level IV**

#### **4.3 Analyte(s)**

##### **4.3.1 Chloride**

Sweat chloride is the measured analyte most directly related to the abnormal function of the cystic fibrosis transmembrane regulator (CFTR), the chloride channel that is defective in cystic fibrosis patients <sup>(59,60,61)</sup>.

When constituents of sweat in populations with and without CF are compared, chloride consistently shows the greatest discrimination when compared to sodium and osmolality <sup>(62,63,64,65,66)</sup>.

**Evidence level IIb**

Chloride is recommended as the analyte of choice by NCCLS guidelines <sup>(19)</sup> and the Welsh Standard <sup>(28)</sup>.

**Evidence level IV**

##### **4.3.2. Sodium**

Sweat sodium does not discriminate as well as chloride between control and CF populations <sup>(62,63,64,65,66)</sup>. The value of sodium:chloride ratios as a discriminating test is not clear (see section 6.4). Some laboratories measure sodium in addition to chloride as a quality assurance procedure (see section 5.3.1).

**Evidence level IIb**

### 4.3.3 Potassium

Potassium is rarely analysed in sweat and discriminates poorly between populations with and without CF <sup>(66)</sup>.

**Evidence level IIb**

### 4.3.4. Conductivity

In CF other constituents of sweat, notably sodium and potassium, are also increased, as are measures of the total concentration of ions (conductivity and osmolality). Because conductivity measurements reflect the concentration of sweat chloride plus all other ions such as sodium which bear less or no relation to CFTR function it may be expected that they would not be as effective in discriminating between CF and non CF populations.

Data from UK NEQAS Sweat Testing Surveys <sup>(67)</sup> provides evidence of better-between-laboratory agreement for conductivity measurements compared to chloride or sodium (section 5.4).

**Evidence level III**

The US Cystic Fibrosis Foundation advises that it is not appropriate to perform the sweat test using conductivity <sup>(68)</sup>. The NCCLS guidelines <sup>(19)</sup> only accept conductivity measurement as a 'screening test' as does the Welsh Standard <sup>(28)</sup>. The role of a screening test is questionable.

**Evidence level IV**

Two large scale studies comparing sweat conductivity and chloride have been published <sup>(69,70)</sup> showing excellent discrimination between normal and cystic fibrosis populations. A recent study of 118 CF patients and 200 controls demonstrated no misdiagnoses using conductivity alone, but 7 intermediate results by conductivity that were normal or abnormal by chloride measurement <sup>(71)</sup>.

**Evidence level IIb**

However, because conductivity measurements are rarely used by tertiary and referral centres, who all measure sweat chloride, there is a lack of comparative data on conductivity measurements in adults and in patients with intermediate sweat chloride results who may have atypical cystic fibrosis. Further collection of data in conjunction with chloride measurements is necessary before recommendations on interpretation of conductivity measurements in these groups of patients can be made. This evidence is required to resolve the current controversy in the literature on the relative merits of measurement of sweat chloride and conductivity <sup>(70,72,73,74)</sup>.

**It remains to be shown whether the improved analytical performance of conductivity over chloride is sufficient to outweigh the theoretical disadvantages in its measurement.**

**Overall evidence level IIb**

#### 4.3.5 Osmolality

Osmolality is a measure of total sweat solute concentration, including uncharged molecules. Therefore it may be expected to be less effective in discriminating between CF and non CF populations than chloride or conductivity.

Osmolality correlates well with sweat sodium<sup>(55)</sup>. It has a poor discriminatory power compared to chloride in distinguishing between cystic fibrosis and normal individuals which parallels sweat sodium<sup>(70)</sup>.

**Evidence level IIb**

In one centre sweat osmolality was measured in addition to sodium. After sweat chloride was introduced, osmolality was found to add no further information, and to provide no better discrimination than sweat sodium in addition to chloride. It was abandoned in favour of sweat chloride<sup>(55)</sup>.

**Evidence level IIb**

In the UK use of osmolality has been largely superseded by conductivity; as a result there is a lack of external QA data available (UK NEQAS Sweat Testing Surveys) on osmolality<sup>(67)</sup>.

**Evidence level IIb**

#### 4.4 Methodology

These guidelines do not provide recommendations on which method(s) to use.

##### 4.4.1 Chloride

The following methods for sweat chloride are widely used in the UK:

- a. Colorimetry<sup>(65,75)</sup>
- b. Coulometry<sup>(76)</sup>
- a. Indirect Ion Selective Electrode<sup>(77,78)</sup>
- b. Direct Ion Selective Electrode (ISE). A small number of laboratories report in the UK NEQAS<sup>(67)</sup> using a variety of methods which they classify as 'Direct ISE'. There is insufficient data to assess the performance of these methods.

The NCCLS<sup>(19)</sup> guidelines recommend coulometry, and caution about the use of systems employing ISEs intended for analysis of serum.

**Evidence level IV**

Replacing and/or maintaining an analyser such as a chloride meter or a flame photometer solely for sweat measurements may not be possible. There is no scientific reason why standard laboratory spectrophotometers or ISEs should not be used for sweat analysis, provided appropriate standards and internal quality control materials are used, and the methodology is validated for the specific equipment used.

Given appropriate attention to ensure analytical validity over a wide range of chloride concentration (0-150 mmol/L), including the use of appropriate internal quality control procedures, there seems to be no theoretical reason why ISEs should not be used for sweat analysis; a medium which is simpler than serum and less variable than urine.

Colorimetry, coulometry and ISEs are the major method groups in UK NEQAS<sup>(67)</sup> with CV of approximately 10% at 40 mmol/L chloride concentration. Local QA schemes<sup>(57)</sup> have shown that all these methods give satisfactory performance. For the colorimetric method a between batch coefficient of variation of 2.5% has been reported<sup>(70)</sup> at a concentration of 50 mmol/L (see also section 5.3.1).

**Evidence level III**

Measurements of chloride by the Orion direct ISE in situ on the stimulated site have been shown to be much less reliable than analysis of sweat collected over 30 minutes, and should not be used<sup>(29,53)</sup>.

**Evidence level IIb**

#### **4.4.2 Sodium**

Flame photometry<sup>(79)</sup> and indirect ISE<sup>(77,78)</sup> are reported in UK NEQAS Sweat Testing surveys. All methods give similar performance in the UK NEQAS<sup>(67)</sup> with a CV of approximately 10% at 40 mmol/L sodium (see also section 5.3.1).

**Evidence level III**

#### **4.4.3 Conductivity**

Measurement of sweat conductivity in the UK is limited to users of Wescor equipment who achieve CV <5% at all concentrations of electrolyte circulated in the UK NEQAS Sweat Testing Surveys<sup>(67)</sup>.

**Evidence III**

### **4.5. Reporting**

#### **4.5.1 Report format**

A report format is detailed in the Welsh Consensus Guidelines<sup>(28)</sup> which form the basis of the recommendations for reporting.

**Evidence level IV**

<b>Sweat Analysis</b>	
<b>Pre-analytical</b>	
<p>Storage before analysis</p> <ul style="list-style-type: none"> <li>▪ Throughout sweat collection, transport and analysis, every effort should be made to minimise evaporation of the sample. <b>C</b></li> <li>▪ If storage is necessary before analysis sweat collections on paper pads should be kept at 4 °C for a maximum of 3 days, and in appropriately sized, air tight containers which do not allow leakage or evaporation. <b>B</b></li> <li>▪ Liquid sweat from Macroduct collections can be stored in sealed Macroduct tubing for up to 72 hours at 4°C. Haematocrit tubes sealed with plasticine are suitable, provided an air gap is left between plasticine and sweat. <b>B</b></li> <li>▪ Sweat may be collected at remote sites and transported to the laboratory for analysis provided there is attention to storage details. <b>B</b></li> </ul>	
<b>Weighing</b>	
<ul style="list-style-type: none"> <li>▪ The same balance must be used throughout <b>C</b></li> <li>▪ A balance sensitive to 0.0001g must be used to weigh sweat <b>C</b></li> <li>▪ Sweat collections onto paper pads should be weighed and analysed as soon as practicable <b>C</b></li> </ul>	
<p><b>Definition of adequate sample</b></p> <ul style="list-style-type: none"> <li>▪ The sweat secretion rate measured as an average rate over the collection period should not be less than 1g/ m<sup>2</sup>/min. Collections below this rate should not be analysed. Insufficient sweat collections should not be pooled. The full sweat test should be repeated. <b>B</b></li> </ul>	
<p><b>Analysis</b></p> <p><b>Elution of sweat from filter paper</b></p> <ul style="list-style-type: none"> <li>▪ When sweat is collected onto filter paper (section 3.2.1) it should be eluted for a minimum of 40 minutes. <b>C</b></li> </ul> <p><b>Analytes</b></p> <ul style="list-style-type: none"> <li>▪ Sweat chloride concentration must be measured. <b>B</b></li> <li>▪ Sweat sodium must not be the only or primary analyte measured. <b>B</b></li> <li>▪ Sweat potassium measurement is not recommended. <b>B</b></li> <li>▪ Sweat conductivity measurement for the investigation of CF requires further study. If conductivity is measured sweat chloride should also be measured until the relative merits of conductivity have been established. <b>B</b></li> <li>▪ Sweat osmolality measurement is not recommended. <b>B</b></li> </ul>	

<p><b>Methodology</b></p> <ul style="list-style-type: none"> <li>▪ Colorimetry, coulometry and ISEs are satisfactory methods for analysis of sweat chloride.</li> <li>▪ Flame photometry or ISEs are satisfactory methods for analysis of sweat sodium.</li> <li>▪ Conductivity measurement using the Wescor equipment is a satisfactory method of analysis.</li> </ul>	<p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>B</b></p> <p style="text-align: center;"><b>B</b></p>
<p><b>Report format</b></p> <p>The report format should include:-</p> <ol style="list-style-type: none"> <li>i. Full patient identification</li> <li>ii. Date and time of test and date and time of report</li> <li>iii. Sweat weight/volume collected and minimum weight/volume acceptable for local sweat test parameters</li> <li>iv. Analytical results (mmol/L) <b>It should be explicit on the report form which analyte(s) have been measured.</b> i.e. chloride sodium conductivity (sodium chloride equivalent)</li> <li>v. Reference ranges (see section 6)</li> <li>vi. Interpretation of results (see section 6)</li> <li>vii. Recommendations for repeat testing if appropriate (see section 6.9)</li> </ol>	<p style="text-align: center;"><b>C</b></p>

## 5.0 Quality

### Evidence

There are published reports <sup>(80,81,82,83,84)</sup> and personal communications which report clinical experience of the poor performance of sweat testing leading to an incorrect diagnosis. False negative results are of particular concern due to the potential for diagnostic delay <sup>(85)</sup>. There is concern about the competency of the operator performing the collection, the need for quality control and external assessment to assess method performance, the competency of the analyst and interpretation.

The causes of false positive and false negative results can arise from one or more of the following reasons:-

- ◆ patients' physiology
- ◆ inadequate sweat collection
- ◆ poor/unreliable methodology
- ◆ poor operator technique
- ◆ misinterpretation

This section relates to the performance of the sweat collection and the analytical methods for chloride, sodium, and conductivity measurements.

### 5.1 *Sweat Contamination*

If sweat collected has been subjected to evaporation or contamination, it should not be measured. One indication of this may be insufficient sweat measured as an average rate of  $<1\text{g/m}^2/\text{min}$ . (see section 4.1.3). Any other collection where the operator detects or strongly suspects evaporation or a contamination problem (e.g. seal broken during sweat collection or filter paper dropped on floor) should not be analysed.

**Evidence level IV**

### 5.2 *Analytical Methods*

Suitable methods (see section 4.4) should be used which enable measurement of analytes in the concentration ranges likely to be measured for both normals and subjects with cystic fibrosis (i.e. for chloride and sodium analytical range 0-150 mmol/L). The lower limit of detection should be ascertained for the method in use and be no greater than 10 mmol/L. CPA standards and guidelines state that performance of each test should be fully documented in the form of a standard operating procedure (SOP) <sup>(27)</sup>. The SOP should include the analytical method(s) quality procedures, reporting, interpretation and all safety aspects.

**Evidence level IV**

### 5.3 *Internal Quality Control (IQC)*

The Welsh Standards recommend an internal quality control material (sodium chloride 60-70mmol/L) for the analysis step with an acceptance criteria of  $\pm 2$  mmol/L <sup>(28)</sup>.

**Evidence level IV**

The NCCLS Guidelines <sup>(19)</sup> recommend the use of an aqueous electrolyte solution (e.g. sodium and/or potassium chloride) of known composition which is spotted onto pre-weighed filter paper or gauze and then processed in parallel with the unknown. When analysing sweat collected onto paper or gauze, it is insufficient to perform direct analysis of QC material. If undiluted sweat (i.e. Wescor System) is used, then it is acceptable to directly analyse the QC solutions. The NCCLS recommend that both a 'low' and a 'high' QC solution should be processed simultaneously with each patient sample or batch of patient samples. These solutions should be different from the calibrators. Acceptable limits for each analyte should be established for each QC material. Greater imprecision can be expected with smaller weights and low concentrations.

**Evidence level IV**

An audit in the Trent Region <sup>(46)</sup> showed that only 50% of laboratories used IQC at clinically important concentrations. Only 3 out of 15 laboratories detailed their IQC procedure as including material spotted onto weighed filter paper. 50% of laboratories had CVs of 5% or less for sodium and chloride analysis. A South West Regional Audit <sup>(47)</sup> showed a variety of QC materials in use; most of the commercially available QC's were applicable to elevated results but were not relevant to the normal range. A UK Audit including tertiary Paediatric Centres and District General Hospitals revealed wide variation in standardisation and internal quality control <sup>(10)</sup>. Quality control materials should be at clinically important concentrations and should differ from standards.

**Evidence level III**

CPA Standards and Guidelines state that quantitative analyses require multiple levels of internal quality control <sup>(27)</sup>.

**Evidence level IV**

#### 5.3.1 *Chloride and Sodium*

Published data on the precision of sweat chloride and sodium analysis demonstrate CV% of approximately 3-10%. Between batch CV% of 3.4 and 3.2% at a chloride concentration of 70 mmol/L were obtained by manual colorimetric analysis of Gibson and Cooke and Wescor collections respectively <sup>(86)</sup>. The corresponding CV% for a sodium concentration of 60 mmol/L analysed by flame photometry and Wescor were 2.1 and 1.2% <sup>(86)</sup>. Coefficients of variation of 11%, 8% and 4% at sodium and chloride concentrations of 30, 60 and 120 respectively, were reported by using an ion selective electrode <sup>(87)</sup>. Between batch CV% at 10, 20 and 50 mmol/L chloride were 7.1, 6.1 and 6.0 using an enzyme method <sup>(88)</sup>. Heeley <sup>(70)</sup> using a colorimetric method for chloride quoted CV% of 2.5% at a concentrations of 50 mmol/L and 4.3% for sodium (53 mmol/L) using flame photometry. Between batch CVs for IQC material of 2.4% for sodium (51 mmol/L), using flame photometry, and 3.4% for chloride (100 mmol/L), using coulometry have been reported, <sup>(63)</sup>.

**Evidence level III**

#### 5.3.2. *Conductivity*

A between batch coefficient of variation of 1.0% is reported by Hammond <sup>(69)</sup> at a concentration of 67 mmol/L NaCl equivalents.

**Evidence level III**

**All analytical methods should have some measure of assessing method precision at two different concentrations. Material at clinically important concentrations separate from calibrators should be treated in exactly the same way as patient specimens.**

**Overall Evidence level IV**

**Based on current performance of methods, a target between batch CV should be 5% (or less) at a concentration of 40-50 mmol/L.**

**Overall evidence level III**

#### 5.4 *External Quality Assessment (EQA)*

External quality assessment is essential in order to:-

- identify weighing errors
- identify poorly performing analytical methods, including standardisation problems
- identify calculation errors
- identify interpretation problems

EQA will not identify errors arising from inadequate stimulation or poor collection techniques.

The College of American Pathologists commenced a proficiency testing programme in 1994. Aqueous solutions are distributed. The target value is the all method mean  $\pm$  10 mmol/L (or 15%) whichever is greater. Data collected over 1994-1998 showed that the CV% of chloride and conductivity remained consistent at 5.1-5.5% and 1.8-2.6% respectively <sup>(83)</sup>.

**Evidence level III**

The Royal College of Pathologists, Australasia (RCPA) have a Quality Assurance programme for sweat electrolytes <sup>(89)</sup>. Allowable limits of performance for chloride (and sodium) are  $\pm$  2 mmol/L up to a concentration of 20 mmol/L and  $\pm$  10% above 20 mmol/L.

**Evidence level IV**

A UK External QA Scheme commenced on a pilot basis in June 1999 <sup>(67)</sup>. Following the pilot phase, there have been five circulations over a one year period (May 2000 - May 2001). The scheme currently uses aqueous 'salt' solutions of sodium chloride, potassium chloride and potassium dihydrogen phosphate in order to try and mimic real sweat. A wide range of concentrations have been used from 5 to 90 mmol/L.

193 (of which 180 are within the UK) laboratories are performing sweat test analyses.

98	-	Na and Cl
24	-	Cl only
21	-	Conductivity only
20	-	Na only
20	-	Na, Cl and conductivity
6	-	Cl and conductivity
3	-	Na and conductivity
1	-	Osmolality only

There is a 60:40 split in the proportion of laboratories collecting sweat into the Wescor system versus filter paper.

With the limited data set so far, it has been shown that the measured analyte concentrations for sodium and chloride agree with the weighed in values for sodium chloride. Conductivity does not equate to chloride concentrations equivalents. Conductivity has the best between laboratory agreement with CV of < 5% compared to chloride 5-10%, and sodium 5-7% at concentrations of 50 mmol/L.

Preliminary data of bias and consistency confirms that conductivity is measured the most consistently; there is a wider spread of performance for both sodium and chloride.

**Evidence level III**

CPA Standards and Guidelines <sup>(27)</sup> state that the department must participate in approved External Quality Assessment schemes corresponding to its repertoire, and evidence of satisfactory performance will be sought. Where no such accredited scheme exists for a particular test, laboratories are encouraged to participate in non approved schemes or pilot schemes.

**Evidence level IV**

**The laboratory must participate in a suitable external quality assessment scheme.**

**Overall evidence level IV**

#### **5.5 Sodium and Chloride Concentrations and Ratios**

The NCCLS guidelines <sup>(19)</sup> state that both analytes should be proportionally increased or decreased. Discordant values can indicate problems with collection or analysis.

Generally sweat sodium and chloride concentrations agree within 15 mmol/L.

**Evidence level IV**

Data from the UK Laboratory Subgroup and data subsequently collected from 8 large centres showed that for 1571 non cystic fibrosis patients the mean bias of sodium - chloride was 5.2 mmol/L; the 95% limits of agreement were - 8.4 to + 18.9 mmol/L. Out of 2409 results the highest reported chloride in a cystic fibrosis patient was 143 mmol/L, the highest sodium was 135 mmol/L<sup>(12)</sup>.

**Evidence level III**

Sodium and chloride concentrations in sweat gland fluid do not exceed 160 mmol/L<sup>(90)</sup>.

**Evidence level IIb**

Causes of concentrations above include laboratory error, Munchausen syndrome (including 'by proxy')<sup>(91)</sup> and pseudohypoaldosteronism<sup>(92)</sup>.

**Evidence level IV**

**Results which are not physiological should be questioned, i.e. chloride or sodium > 150 mmol/L.**

**Overall evidence level IIb**

**For conductivity a working upper limit of 170 mmol/L was calculated by substituting a chloride of 150 mmol/L in Hammond's regression equation Chloride = 0.974 x conductivity - 15.2.**<sup>(69)</sup>

**Evidence level I**

## 5.6 Failure Rates

Insufficient sweat may be collected because of an inadequate collection process or because of subject variability, including age (see section 2), race, and skin condition. A high % failure rate may also suggest poor operator technique. The weight (or volume) of sweat collected should be routinely monitored to determine the proportion of infants from whom adequate sweat collection cannot be obtained, and any trends noted (see section 4.1.3 for definition of adequate sweat weight). This proportion may vary with the patient population. Differences in skin resistance because of ethnicity or individual patient variability may lead to insufficient sample. Le Grys found<sup>(93)</sup> an insufficient rate of 4.7% in the neonatal period, and 1.6% across all age groups. Collection systems may vary in performance. Hammond<sup>(69)</sup> quoted a 0.7% failure rate for collection on to gauze or filter paper compared with 6.1% using the Wescor Macoduct, although Heeley<sup>(70)</sup> reported only 1.4% with the latter. Denning reported a failure rate of 1.9% using iontophoresis and collection on gauze<sup>(53)</sup>.

**Evidence level III**

The NCCLS <sup>(19)</sup> state that the proportion of inadequate collection should not exceed 5% (unless many patients tested are < 1 month of age).

Most laboratories (79.5%) are successful in achieving this goal <sup>(94)</sup>. When failure rate was evaluated relative to the collection method used, 83.2% of the gauze or filter paper users and 73.5% of the Macroduct users had a failure rate of 5% or less.

**Evidence level III**

Data from 12 large UK centres demonstrated failure rates for first sweat tests, corrected to 1g/m<sup>2</sup>/min, varying from <1 to 28%. All centres except one achieved failure rates of <10%, 3/12 centres achieved a rate of <5% <sup>(12)</sup>.

**Evidence level III**

Repeat rates may vary with the subject, in particular age. Tertiary referral centres may have higher repeat rates due to a higher proportion of very young or difficult patients, (i.e. who do not sweat easily) <sup>(12)</sup>.

According to Le Grys <sup>(94)</sup>, a large cystic fibrosis centre reported a 25% failure rate in patients tested at 2-4 weeks of age compared to an all-age failure rate of 3.6%.

**Evidence level IV**

**Based on current UK performance, a target failure rate of 5% is not unreasonable. 10% should be achieved by all.**

**Centres should monitor repeat rates and investigate any significant increase in failure rate.**

**Overall evidence level IV**

## 5.7 Assessment of Performance/Audit

Quality of sweat testing can be assessed by collecting data on population means and range, % repeat collections (and by operator) and final outcome of all positive and intermediate results. Population means and ranges can be calculated to identify any shifts in method performance with time. External quality assessment is an important way of assessing performance (see section 5.4).

**Evidence level IV**

It is good clinical practice to follow up all positive and intermediate results. (See recommendations for audit section 8).

**Evidence level IV**

<b>Quality</b>	<b>GRADE</b>
<ul style="list-style-type: none"> <li>▪ Sweat which has been subject to evaporation and/or contamination must not be measured.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ The analytical range of the methods used must cover the concentration ranges found in normals and subjects with cystic fibrosis.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ The analytical methods must be fully documented as standard operating procedures (SOP) to comply with Clinical Pathology Accreditation (or equivalent standard). The SOP must include the analytical method(s), quality control procedures, reporting, interpretation and safety aspects. An example SOP is provided (Appendix documents 2a and 2b)</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ There must be an internal quality procedure (which differs from the calibration/standardisation procedure) at two concentrations (normal and intermediate or abnormal) for each analysis.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ The analytical methods should each have a between batch CV of 5% (or less) at a concentration of 40-50 mmol/L.</li> </ul>	<b>B</b>
<ul style="list-style-type: none"> <li>▪ The laboratory must participate in a suitable external quality assessment scheme.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ If chloride and sodium concentrations are widely discrepant, the test should be repeated.</li> </ul>	<b>B</b>
<ul style="list-style-type: none"> <li>▪ Results which are not physiological should be questioned, i.e. chloride or sodium &gt; 150 mmol/L.</li> </ul>	<b>B</b>
<ul style="list-style-type: none"> <li>▪ For conductivity a provisional upper physiological limit of 170 mmol/L may be used pending further evidence</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Failed sweat collections (i.e. insufficient weight or volume) should not exceed 10% of the tested population (excluding repeats and tests carried out in sick/very young patients). There should be a target of 5%.</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Performance of sweat testing should be reviewed on a regular basis. This should include:-               <ul style="list-style-type: none"> <li>- insufficient collections - as % of total tests</li> <li style="padding-left: 40px;">- per operator</li> <li>- analytical failure rate (i.e. % outside accepted QC range)</li> <li>- external quality assessment performance</li> </ul> </li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ The laboratory should work with clinicians to audit sweat test results, in particular repeat collections, diagnoses and outcome of positive and intermediate results on a regular basis (see section 8).</li> </ul>	<b>C</b>

## 6. Reference Values and Interpretation

A sweat chloride concentration of more than 60 mmol/L is consistent with a diagnosis of cystic fibrosis<sup>(95,96)</sup>. This should however, be interpreted in the context of the patients age and phenotype. The diagnosis is made when there is elevation of sweat chloride by greater than 60 mmol/L in a patient with one or more clinical features consistent with the cystic fibrosis phenotype, a positive neonatal screening test or history of cystic fibrosis in a sibling<sup>(96)</sup>. Sweat sodium measurements are less reliable as concentrations of 60 - 80 mmol/L are seen in patients without cystic fibrosis, especially in adults. The presence of two mutations of the CFTR gene known to cause CF may provide confirmatory evidence but the demonstration of mutations is not necessary to make a diagnosis of CF. The genetic diagnosis of CF by the demonstration of two CFTR mutations (known to be associated with clinical disease) does not require confirmation by a sweat test.

### 6.1 Sweat Chloride and Sweat Sodium Concentrations

A number of studies have published ranges for sweat electrolytes for individuals with and without CF. The following are major studies which report sweat chloride concentrations in patients with CF and in various groups of healthy controls. Many of these studies were carried out prior to genotyping being available and so some control individuals could have had CF or CF related disorders. Only the first two studies report genetic mutations and sweat chloride concentrations.

#### (a) Wisconsin Study<sup>(97)</sup>

In a study of infants sweat testing was successfully performed in 99.3% of 725 infants and a mean (95% confidence interval of the mean) normal sweat chloride of 10.6 (9.9 - 11.3) mmol/L was found.

Cystic Fibrosis patients who were DF508 homozygotes, DF508 compound heterozygotes, or had two other mutant alleles, had mean sweat chloride concentrations of 100, 97.6 and 99.6 mmol/L (Table 1). CF heterozygote carriers had a mean chloride concentration of 14.9 (13.4 - 16.4) mmol/L which is significantly higher compared to infants not carrying a CF mutation. From these data they calculated in 184 normal infants with no DF508 alleles that 30 mmol/L is the upper limit of normal using a mean plus 2 SDs. The mean plus 3 standard deviations for the group of 128 CF heterozygote carriers was 40 mmol/L. The authors concluded that the upper limit of normal sweat chloride in infants should be revised to 40 mmol/L.

**Evidence level IIB**

**(b) Genotype/Phenotype Study<sup>(98)</sup>**

Sweat chloride concentrations were reported in 798 patients studied by the Genotype/Phenotype Consortium. This study included patients over a wide range of ages. In 328 patients who were homozygous for DF508 they reported a mean (SD) sweat chloride concentration of 106 (22) mmol/L (table 2). DF508 compound heterozygotes, apart from DF508 /R117H, had mean sweat chloride concentrations ranging from 100 (20) to 110 (18) mmol/L. These were not significantly different from DF508 /DF508 patients. Sweat chloride concentrations were significantly lower in DF508 /R117H heterozygotes with a mean of 82 (19) mmol/L. No values for healthy individuals are reported in this study.

**Evidence level III**

**(c) Birmingham Study<sup>(63)</sup>**

Twenty adult patients with CF had a mean (range) sweat chloride concentration of 106 (81-122) mmol/L compared to 31 (14 - 48) mmol/L in healthy controls and 33 (13 - 52) in patients with chest disease. Sodium concentrations were also measured and it was demonstrated that a chloride concentration of >70 mmol/L was more specific than a sodium concentration of >60 mmol/L for the diagnosis of CF.

**Evidence level IIb**

**(d) Ohio Study<sup>(99)</sup>**

This study examined sweat chloride concentrations in 187 adults who did not have CF. 166 had a respiratory disorder and 21 were healthy. These were compared to 13 adults with CF. The mean (SD) sweat chloride concentrations in the adults with CF was 101 (7) mmol/L, significantly higher than the other groups which ranged from 20.9 (15.4) mmol/L in asthma to 38.2 (28.7) mmol/L in patients with pancreatitis (Idiopathic pancreatitis has subsequently been identified as a CFTR related disorder). This study was performed prior to the discovery of the CF gene and may have included patients with a CF genotype or who were heterozygotes. In this study 99% of the patients assumed to be non-CF had a sweat chloride concentration of <70 mmol/L and 96% <60 mmol/L.

Medications such as B<sub>2</sub> agonists, corticosteroids, antibiotics and theophyllines did not affect sweat chloride concentration in the non-CF groups.

**Evidence level III**

**(e) Bristol and Birmingham Study<sup>(62)</sup>**

This study retrospectively examined 1390 tests in 1100 children between 1 month and 16 years. Mean values were not reported. 105 tests were from patients with CF. Ten tests from 7 patients with CF had a sweat sodium of <60 mmol/L while none had a chloride concentration of <70 mmol/L.

**Evidence level III**

**(f) New York Study<sup>(100)</sup>**

This early study examined sweat chloride concentrations in 50 patients with CF and 50 controls. The patients with CF had a mean chloride concentration of 106 mmol/L and controls 32 mmol/L. No SDs or SEs were reported. Three controls had a sweat chloride concentration of >60 mmol/L and 3 patients with CF a concentration of <60 mmol/L. Considerably more overlap was demonstrated in sweat sodium concentrations compared to chloride.

**Evidence level III**

**(g) Hopkins Study<sup>(34)</sup>**

In the original study describing the sweat test mean (range) sweat chloride concentrations were reported in 39 controls of 21.1 (7 - 49) mmol/L, 5 relatives of a CF patient, 32.5 (16 - 47) mmol/L and 11 patients with CF, 94.8 (80 - 122) mmol/L. None of the controls had a chloride concentration of >60 mmol/L.

**Evidence level III**

**(h) Collection of data from 12 large UK centres<sup>(12)</sup>**

Data was collected for 2409 sweat tests. After exclusion of outliers the upper limit of normal for sweat chloride could be defined as follows: 2 Standard deviations above the mean = 44 mmol/L, 3 Standard deviations above the mean = 56, 97.5 centile = 52, and 2 standard deviations above the mean of log transformed data = 54. There was good agreement between the centres on the mean sweat chloride of the trimmed data, but more variability in the number of patients who were in the upper percentiles. This is likely to reflect the referral of patients with intermediate or abnormal sweat tests at their local hospital. A cut-off of 40 mmol/L is suggested as a conservative upper limit of normal that would minimise false negatives in cystic fibrosis patients. Results >60 mmol/L are clearly abnormal, and those in the intermediate 40-60 region require repeat sweat testing and further investigation.

**Evidence level III**

**6.2 Variation in Chloride Concentrations with Age**

A study<sup>(86)</sup> of 112 matched control and 112 cystic fibrosis individuals from 0-40 years demonstrated a statistical increase in sweat chloride for normal children aged 1-12 years, but no increase for cystic fibrosis children. In normal subjects over 12 years of age there were no age related change in chloride, while the older cystic fibrosis patients showed a fall with age. The magnitude of the changes with age was insufficient to cause any diagnostic confusion - cystic fibrosis patients of all ages had chloride >60 mmol/L. Of a subgroup of 9 patients with intermediate chloride results in the 50-70 mmol/L region several have subsequently been shown to have CF mutations and atypical disease<sup>(101)</sup>. None of these patients would have been missed by using an upper limit of chloride of 40 mmol/L. An upper limit of 40 mmol/L for sweat chloride is appropriate for subjects of all ages.

**Evidence level III**

### 6.3 Studies Measuring Sweat Sodium only

A number of other studies have reported sweat sodium concentrations. As this measurement is not considered sufficiently specific as the primary analyte for the diagnosis of CF<sup>(62)</sup>, these are not considered further. Sodium does increase with age, may be affected by other conditions and drugs and is in general less discriminatory. (see section 4.3.2).

**Evidence level III**

### 6.4 Sweat Sodium:Chloride Ratios

Patients with cystic fibrosis usually have a Na : Cl ratio less than one<sup>(62,86)</sup>. The Na : Cl ratio increases with age in both CF and normals as expected as sodium concentration increases more with age than chloride<sup>(86)</sup>.

Potential use of the Na/Cl ratio as a diagnostic aid has been investigated by several groups<sup>(62,66,102,103,104)</sup>.

A small study comparing Na : Cl ratios from adults with cystic fibrosis, normal adults and those with chest disease showed good discrimination<sup>(63)</sup>.

**Evidence level IIb**

However Kirk showed that combining sodium and chloride did not improve discrimination in 9 adolescent/adult patients with intermediate chloride results<sup>(86)</sup>.

**Evidence Level III**

Augarten<sup>(5)</sup> suggested that the Na/Cl ratio is genetically determined and may be of help in establishing the diagnosis of CF in patients with a borderline sweat test i.e. for intermediate results with a Na/Cl ratio less than 1.0 the diagnosis of CF should be considered.

**Evidence level III**

Recently Massie examined the relationship between Cl and Na in  $\Delta$  F508 homozygous and heterozygous individuals identified by newborn screening<sup>(105)</sup>. The ratio of Cl : Na had a PPV of 37% and therefore was not helpful in the diagnosis of screened infants in this situation.

**Evidence level III**

**The value of the Na : Cl ratio as a discriminating test for CF patients with intermediate/normal chloride results is currently unclear.**

**Overall Evidence Level III**

## 6.5 Sweat Conductivity

Hammond<sup>(69)</sup> carried out a large scale study comparing sodium, chloride and conductivity. The best linear correlation is of conductivity vs sodium + potassium. The relationship of chloride vs conductivity shows a conductivity of 57 mmol/L equates to a chloride of 40 mmol/L while the 50 mmol/L conductivity cut-off recommended by the NCCLS Guidelines<sup>(19)</sup> and the Cystic Fibrosis Foundation would equate to a chloride of 33 mmol/L and include a significant number of the normal population.

**Evidence level 11b**

The study by Heeley<sup>(70)</sup> comparing simultaneous measurements of sodium, chloride, conductivity and osmolality showed that a Chloride concentration of 38 mmol/L is 3SDs above the mean for the normal population. For conductivity the corresponding figure is 67 mmol/L.

**Evidence level 11b**

The manufacturer's manual dated 1997<sup>(106)</sup> states that mean conductivity 33 mmol/L, 3SDs above = 67 mmol/L. (n=471). Their recommendation is that "the majority of normal values will fall below 60 mmol/L with the majority of positive values above 90 mmol/L. Use caution in interpreting any result in the intermediate region between 60 and 90". In a manual update issued in April 2000, 80mmol/L was substituted for 90mmol/L.

**Evidence level IV**

A set of 1732 conductivity measurements supplied by 13 UK laboratories demonstrated excellent agreement with published data. The mean conductivity of trimmed results was 39mmol/L. Two standard deviations above the mean equated to a conductivity of 61mmol/<sup>(54)</sup>.

**Evidence level III**

Repeated measurements of sweat conductivity in 20 healthy infants, 20 healthy adults and 15 diagnosed cystic fibrosis patients to assess within and between subject variation, supported a cut-off point of 60mmol/L to minimise unnecessary repeats, while not missing any cystic fibrosis patients<sup>(107)</sup>.

**Evidence level IIb**

**A value below 60 mmol/L (NaCl equivalents) is unlikely to be associated with cystic fibrosis. Values above 90 mmol/L support a diagnosis of cystic fibrosis. Cystic fibrosis should not be diagnosed based on conductivity measurement alone. Confirmation should be sought using sweat chloride or genotyping (See also Section 4.3.4). Intermediate values (i.e. 60-90 mmol/L) require further investigation by sweat chloride and/or genotyping.**

**Overall Evidence level 11b**

## 6.6 Genotypes and Intermediate Sweat Electrolytes

A number of studies have examined the relationship of intermediate sweat tests to the CF genotype. Desmarquest et al, studied 24 individuals with sweat chloride concentrations in the range of 40 - 60 mmol/L<sup>(108)</sup>. The mean age that the sweat tests were performed was 4.8 years and the patients were followed up for 10 years. Of the 24 patients a total of 15 mutations of the CFTR gene were identified. Three patients were carriers of 2 definite CF mutations. The 5T allele was identified in a further 4 children. Mutations associated with intermediate/low sweat chloride levels are the 3849+10kbC→T mutation and P67L<sup>(7,101,109)</sup>. DF508 heterozygotes plus splice and missense mutations are associated with chloride concentrations significantly lower than DF508 homozygotes<sup>(110)</sup>.

A further study suggests that in a patient with a chloride concentration of 40 - 60 mmol/L a Cl<sup>-</sup>/Na<sup>+</sup> ratio of one or greater is supportive of a diagnosis of CF. However a ratio of less than one does not exclude the diagnosis<sup>(86)</sup>. In a neonatal screening programme 122 DF508 heterozygotes with an elevated immunoreactive trypsinogen test were identified with a sweat chloride <60 mmol/L. Extended mutation analysis was then undertaken. Four infants had a chloride concentration of 40 - 60 mmol/L and two of these had phenotypic features of CF by 12 months<sup>(111)</sup>.

**Evidence level IV**

## 6.7 Other Disorders Associated with CF Mutations

A number of conditions have now been identified which are associated with mutations of one or two mutations at the CF locus. These include some patients with congenital bilateral absence of the vas deferens, idiopathic pancreatitis and possibly chronic sinusitis<sup>(112-116)</sup>. In these conditions sweat chloride concentrations may be intermediate or abnormal. The decision to label such conditions as cystic fibrosis remains with the clinician managing the individual patient. A classification has been suggested by the World Health Organisation<sup>(117)</sup>.

## 6.8 Other Diseases Associated with Increased Sweat Electrolyte Concentrations

A large number of conditions have been associated with abnormalities of sweat concentrations of sodium and chloride. In most of these studies only one or two patients are reported and in many the sweat electrolytes return to normal values when the acute condition was treated. Few of these diseases are phenotypically similar to cystic fibrosis and usually do not represent a problem in differential diagnosis. This has been reviewed by a number of authors<sup>(95,118)</sup> and is not considered in the scope of these guidelines and there is no recommendation.

## 6.9 Indications for a Repeat Sweat Test, i.e. when to

It has been traditional teaching that a sweat test needs to be repeated before the diagnosis of CF is confirmed. However, if the genotype confirms the diagnosis of CF then a repeat sweat test may not be necessary.

A repeat sweat test is recommended when the result is abnormal or borderline and the genotype is not confirmatory.

If there is doubt that a negative test is not in keeping with the clinical picture and the genotype is inconclusive the sweat test should be repeated. All borderline values not confirmed by genotype should be repeated.

**Evidence level IV**

## 6.10 Further Investigations

### (a) Genotype Analysis

Genotype analysis can establish the diagnosis of cystic fibrosis. Finding two abnormal genes in a patient with an intermediate sweat test confirms a diagnosis of cystic fibrosis. An intermediate level of chloride (40 - 60 mmol/L), the presence of one mutation and one phenotypic expression of cystic fibrosis may be enough to confirm the diagnosis. It is impractical to screen for all mutations using PCR and most genetic laboratories only examine 15 - 20 mutations. This should account for 90-95% of mutations in most Northwest European populations.

**Evidence level III**

### (b) Nasal Potential Difference Measurement

The epithelial lining of the nose expresses CFTR, and in cystic fibrosis where CFTR function is abnormal, sodium and chloride transport is therefore also abnormal. This results in a greater trans-epithelial electro-potential difference (PD) in patients with cystic fibrosis compared to those with normal epithelium. The measurement of nasal PD with the soft catheter is sometimes a useful confirmatory test for cystic fibrosis<sup>(95,119)</sup>. There are 3 specific features which distinguish cystic fibrosis patients from healthy individuals:

- a) more negative basal PD
- b) greater inhibition of potential difference after perfusion with amiloride.
- c) little or no change following perfusion with a chloride free solution with isoproterenol indicating an absence of CFTR mediated chloride secretion.

This method is technically more challenging than the sweat test and should only be carried out in laboratories with considerable experience of this methodology. This test may be of value in determining the diagnosis in patients with intermediate sweat tests. Basal PD measurements in a group of 18 CF patients with a sweat chloride < 70 mmol/L were all less than -25 mV, significantly less than healthy controls but not significantly different from CF controls<sup>(120)</sup>.

**Evidence level III**

(c) Mineralocorticoid Suppression

In healthy individuals but not in cystic fibrosis patients, the administration of oral fludrocortisone (5 mgs) causes a reduction in sweat sodium concentration<sup>(121)</sup>. There are no reports on the effect on chloride concentrations. This test is not widely used.

**Evidence level IV**

**Recommendations:**

<b>Interpretation of sweat electrolytes</b>	<b>GRADE</b>
<p>The following definitions are recommended for interpretation:-</p> <ul style="list-style-type: none"> <li>▪ A sweat chloride concentration of &gt; 60 mmol/L supports the diagnosis of CF.</li> <li>▪ Intermediate chloride concentration of 40 - 60 mmol/L is suggestive but not diagnostic of CF.</li> <li>▪ A sweat chloride of less than 40 mmol/L is normal and there is a low probability of CF.</li> <li>▪ Sodium should not be interpreted without a chloride result.</li> <li>▪ Pending further data on conductivity measurements a value below 60 mmol/L (NaCl equivalents) is unlikely to be associated with cystic fibrosis. Values above 90 mmol/L support a diagnosis of cystic fibrosis.</li> <li>▪ Cystic fibrosis should not be diagnosed based on conductivity measurement alone.</li> </ul>	<p><b>B</b></p> <p><b>B</b></p> <p><b>B</b></p> <p><b>B</b></p> <p><b>B</b></p> <p><b>B</b></p>
<p><b>Repeat Testing</b></p> <p>A repeat sweat test is recommended when the sweat test result is not in keeping with the clinical phenotype and/or genotype.</p>	<p><b>C</b></p>
<p><b>Further Investigations</b></p> <ul style="list-style-type: none"> <li>• Mutation analysis can be a useful diagnostic test, particularly in patients with a mild or atypical phenotype where sweat chloride concentration may be intermediate</li> <li>• Nasal potential difference may be helpful as a confirmatory investigation for the diagnosis</li> <li>• There is no routine place for the use of the mineralo corticoid suppression adaptation of the sweat test</li> </ul>	<p><b>B</b></p> <p><b>B</b></p> <p><b>B</b></p>

**Table 1**

**Wisconsin Study**

	<b>n</b>	<b>Age (Weeks) Mean (SD)</b>	<b>Sweat Chloride (mmol/L) Mean [95% CI]</b>
Healthy infants	184	9.3 (5.3)	10.6 [9.9 - 11.3]
DF508 heterozygote carriers	128	8.8 (5)	14.9 [13.4 - 16.4]
DF508 /DF508	61	10.5 (9.9)	100 [97.6 - 102.4]
DF508 /another	47	9.3 (9.3)	97.6 [93.1 - 102.1]
Another/another	7	16.5 (17.3)	99.6 [86.5 - 112.7]

**Table 2**

**Genotype/Phenotype Study**

	<b>n</b>	<b>Age (Years) Mean (SD)</b>	<b>Sweat Chloride (mmol/L) Mean (SD)</b>
DF508 / DF508	328	13 (9)	106 (22)
DF508 / G542X	147	12 (9)	109 (23)
DF508 / R117H	20	24 (10)	82 (19)+
X / X	16	12 (10)	105 (19)

+ p<0.001 Vs DF508

## 7. Responsibility for Testing and Training

### 7.1 Who should perform the sweat test? Skills and Training Needs

Collection of sweat is performed by a variety of different professionals including laboratory, medical, nursing, phlebotomists, physiotherapists and respiratory measurement technical staff. The analytical procedures for measurement of sweat electrolytes are usually performed by clinical chemistry departments.

Sweat testing requires attention to detail and the commonest cause of an incorrect diagnosis is inaccuracy in performing or interpreting the test, - most likely where the test is done only occasionally <sup>(80,82)</sup>.

Shwachman and Mahmoodian <sup>(82)</sup> state that 'the greatest error in sweat testing is probably attributable to the inexperience of the technician who is improperly trained and is requested to do the test infrequently, perhaps three to five times a month'.

**Evidence level IV**

#### UK Position

In the UK there is a huge range of workloads. Data from a UK audit <sup>(10)</sup> and four Regional audits <sup>(46,47,57,122)</sup> are shown in the table below.

	<b>U.K. 2000</b>	<b>N.W. Thames 1996</b>	<b>Trent 1999</b>	<b>South West 1998</b>	<b>West Midlands 1995</b>
Number of centres providing data	30	39	15	14	19
Number of sweat tests per year	30-400 (median 100)	Not available	7-150	15-200 (median 55)	30-200
Number of sweat tests per diagnosis	5-152 (median 30)	Not available	7.5-100	Not available	Not available
Number of sweat collections/operator per year	5-268 (median 50)	all > 10	1-76 (median 15)	9-200 (median 33)	3-50 (median 11)

In most centres, laboratory staff (n = 22) who carried out the sweat collection, ranged from the most junior member to the head of department with the majority using medical laboratory scientific officers (n = 12). Based on the UK Audit, most centres will have to carry out an average of 30 normal sweat tests for 1 positive. Most centres restricted sweat collection to one or two trained staff.

**Evidence level III**

### **Consensus Guidelines**

The Welsh Standards state that the analysis should be performed by fully trained laboratory staff. Collection may be performed by laboratory or nursing staff, but must be fully trained by the laboratory. A minimum of 10 procedures per annum per staff member should be performed to maintain expertise <sup>(28)</sup>.

**Evidence level IV**

The NCCLS Guidelines recommended that sweat testing be performed only in those institutions where there is a sufficiently large testing volume to maintain quality <sup>(19,123)</sup>, although there is no statistically significant data which correlates performance with workload volume.

**Evidence level IV**

### **UKNEQAS**

Data from UKNEQAS <sup>(67)</sup> shows that approximately 15,000 sweat tests are performed in the UK each year. The mean number of sweat tests per annum carried out by individual centres in the UK is 100 (median - 60) with a range of 2 - 500.

While there is no direct correlation between workload and error rate of interpretation (some laboratories with small workloads have made no errors of interpretation) there is a tendency for the higher error rates to be associated with the laboratories with smaller workloads. Two or more errors have not occurred to date in laboratories performing more than 100 tests per annum. 50% of those laboratories making two or more errors performed 50 sweat tests or less. Of the 75 errors of interpretation, 39 were based on 'analytically correct' numeral results confirming that different criteria for interpretation are being used by different laboratories. (Unpublished data courtesy of UKNEQAS) <sup>(67)</sup>.

**Evidence level III**

**In conclusion, therefore, there are no scientific data which relate performance to the level of training/skills/frequency of analysis/workload volume. However, it is likely that familiarity with the procedure and frequency of analysis will affect performance.**

**For this reason it is not acceptable for an organisation or an individual to perform very few sweat tests. Sweat collections may be performed satisfactorily by a variety of different health professionals. However, collection of a minimum number per annum is required to maintain quality, with a minimum number of 50 tests per annum and 10 collections per person per annum. All staff performing the sweat test must be fully trained and re-validated.**

**Overall Evidence level IV**

## 7.2 Responsibilities

### Analysis

Sweat testing is a chemical test and the analytical procedure is most appropriately part of the repertoire of a clinical chemistry department.

Compliance with CPA standards <sup>(27)</sup> require that staff must be appropriately qualified for the work they are performing.

The responsibility of performing sweat tests, including training and revalidation of staff for both the collection and analysis should rest with a consultant (or equivalent) clinical chemist.

**Evidence level IV**

### Recommendations

<b>Responsibility for Testing and Training</b>	<b>GRADE</b>
<ul style="list-style-type: none"> <li>▪ Sweat collection must be performed by fully trained and experienced personnel:-               <ul style="list-style-type: none"> <li>- training schedules should be fully documented</li> <li>- the procedure should be documented as a standard operating procedure</li> <li>- appropriate revalidation procedures should be in place</li> </ul> </li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat collection can be undertaken by a variety of health professionals</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ Sweat analysis should be performed by qualified and experienced biomedical scientists or clinical scientists who are fully trained with regular validation:-               <ul style="list-style-type: none"> <li>- training and validation schedules should be fully documented</li> </ul> </li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ A consultant (or equivalent) clinical chemist should have responsibility for training, assessment of competence and revalidation for all staff undertaking sweat tests</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ A minimum number of 50 sweat tests per annum should be performed in any one centre</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ A minimum number of 10 collections procedures should be performed per person per annum</li> </ul>	<b>C</b>
<ul style="list-style-type: none"> <li>▪ The <b>responsibilities</b> for sweat testing, both collection and analytical, should rest with a consultant (or equivalent) clinical chemist and should be clearly understood by all operators and users; a mechanism for reporting any concerns about performance should be in place and clearly understood.</li> </ul>	<b>C</b>

## **8. Recommendations for Audit**

The key points for audit at local level are:-

### **8.1 Test performance (see also quality section 5)**

- To monitor internal quality control performance
- To monitor external quality assessment performance
- To monitor test failure rates/repeat rates

### **8.2 Relationship of sweat test results to diagnosis**

- To collect data on all positive and intermediate sweat test results and relate to diagnosis. This should include data arising from neonatal screening programmes.

### **8.3 Adverse events**

- Nature and number of any events (e.g. burns, blisters) associated with the iontophoresis procedure.

## **9. Recommendations for further Research**

The following are suggested as national/multicentre initiatives which require further research:-

- To establish a national data base for intermediate and atypical sweat test results and correlate with genotype in collaboration with the UK CF database.  
In particular to:-
  - collect data from the UK population
  - obtain data on intermediate sweat test results and atypical CF patients
  - obtain data on the very young and older age groups
- To undertake a multicentre study to establish an evidence based algorithm for investigation of intermediate sweat test results
- To undertake a multicentre study to evaluate the measurement of sweat conductivity versus sweat chloride ( $\pm$  sodium) as a test for diagnosis of cystic fibrosis
- To evaluate the delivery of patient information

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## PROCESS OF GUIDELINE DEVELOPMENT

The Working Group was formed in October 2000 and met on 6 occasions throughout 2000/1/2. The process for guideline development undertaken by the Working Group is summarised as Figure 1.

### GRADING OF EVIDENCE AND RECOMMENDATIONS

The Working Group has undertaken a systematic review of evidence in accordance with SIGN methodology and the evidence and recommendations have been graded according to this <sup>(i)</sup>. The working group note that the SIGN grading system had been revised <sup>(ii, iii)</sup> since work on these guidelines commenced. It was felt that the revised version offered no advantage in this instance and, therefore, after consultation with the Royal College of Paediatrics & Child Health, the original SIGN version has been used.

The group felt that the SIGN criteria, particularly IIb and III, are sometimes difficult to interpret in the context of the performance of a laboratory diagnostic test. The working group have interpreted these levels as follows:-

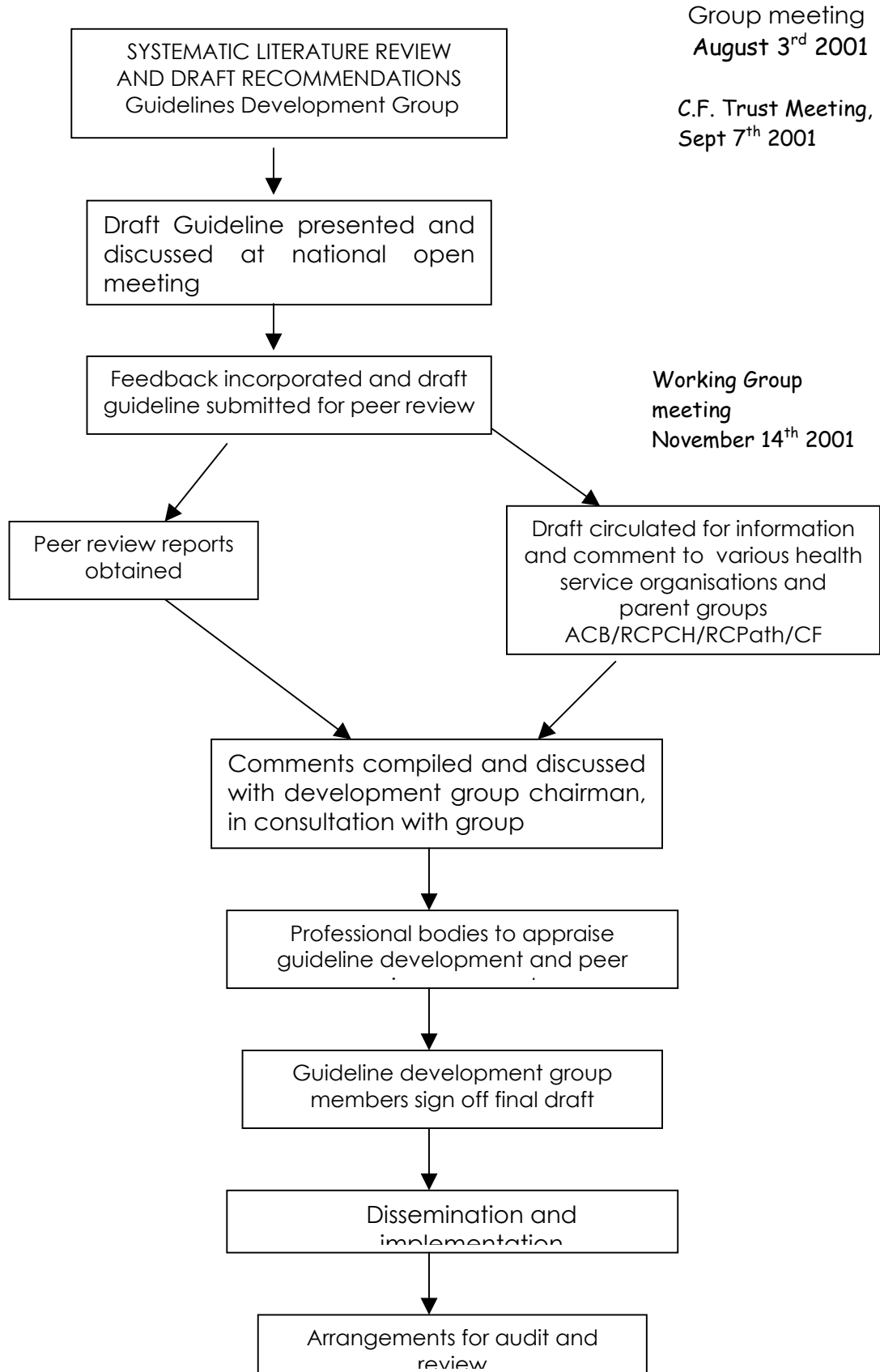
Evidence Level	Grading of Evidence
IIb	<ul style="list-style-type: none"> <li>- a planned scientific study with hypothesis</li> <li>- a study not controlled</li> <li>- an experimental study, with a low risk of bias</li> </ul>
III	<ul style="list-style-type: none"> <li>- non-experimental/observational study</li> <li>- investigation of a standard procedure</li> </ul>

Additional points of note are:-

- publication is not essential to be considered as good evidence.
- where several pieces of evidence relate to the some topic, an 'overall evidence' level has been assessed.
- because it is unethical to undertake controlled trials (randomised or otherwise) to evaluate variability in the performance of the sweat test, there are little data which qualify as grade I or II evidence.
- formulation of recommendations was reached by consensus agreement of the working group members.

Figure 1

## Performance of the Sweat Test Process for Guideline Development



## References

- i. *SIGN Guidelines: An introduction to SIGN methodology for the development of evidence-based clinical guidelines.* Scottish Intercollegiate Network (SIGN). SIGN Publication Number 39, July 1999.
- ii. Scottish Intercollegiate Guidelines Network. *SIGN 50: a guideline developers' handbok.* Edinburgh: SIGN, 2001.
- iii. Harbour R, Miller (for Scottish Intercollegiate Guidelines Network Grading Review Group). *A new system or grading recommendations in evidence based guidelines.* *BMJ* 2001; 323: 334-336.

## SYSTEMATIC REVIEW

The search process comprised the following:-

- **Searching of computerised data bases**  
Medline 1965-2001  
Human studies  
Children 0-18 years  
All types  
Reviews, meta-analyses, searched on sweat tests, editorials, clinical trials, letters, etc.
- **Hand searching**
  - text books and review articles
  - review of existing literature assembled by expert group members
  - selected articles pre 1965
  - personal contact with recognised national and international experts - UK, USA, Australia
- **Specific searching**  
For particular sections of the report, specific searching as detailed below was undertaken:-

Sweat collection

1. Published articles on sweat test combined with:-
  - Iontophoresis
  - Burns
  - Urticaria
  - Apparatus
  - Equipment

2. Review of questionnaire data collected for sweat test workshops from 30 centres (Association of Clinical Biochemists National Meeting 1998 and UK National External Quality Assessment Schemes Workshop 1998)
3. Wescor instruction manuals (Webster sweat collection system model 3500. 1979; Macroduct sweat collection system model 3600-sys 1983) and Website (<http://www.wescor.com>)
4. Data collected by Internet enquiry (Association of Clinical Biochemists Mailbase) and personal contact with colleagues in UK and USA.
5. Information supplied by Wescor Inc, via Chemlab Scientific Products, Astra House, Christy Close, Southfield Business Park, Laindon, Essex, SS15 6TQ, in response to enquiry.

#### Sweat Analysis

1. Searched on Medical Subject Headings (MeSH) vocabulary for Sweat Test. No exact match, except for the following:-
  - Sweat
  - Sweating
  - Gland, Sweat
  - TestingSearched on: Iontophoresis, burns, urticaria, equipment and supplies  
Used a combination of MeSH and keyword or textword searching
2. UK National External Quality Assurance Schemes Sweat Test External Quality Assurance Surveys

#### Quality

1. UK Audits on Sweat Testing (unpublished data <sup>(46,47,57,122)</sup>).
  2. UK National External Quality Assessment Schemes Data from Sweat External Quality Assessment Surveys<sup>(67)</sup>
- **Review of existing Consensus Based Guidelines**
    - NCCLS 2000 <sup>(19)</sup>
    - Welsh Sweat Standard 1999 <sup>(28)</sup>
  - **National UK Laboratory Sweat Test Subgroup** <sup>(12)</sup>
    - this comprises evidence from a 'Consensus of experts' collected from the National UK Laboratory Subgroup

Under the chairmanship of Dr. J. Kirk, the subgroup was made up of: Birmingham Children's Hospital (Dr. A. Green), Edinburgh Royal Hospital for Sick Children (Dr. J. Kirk), Great Ormond Street (Dr. Tony Reynolds), Sheffield Children's Hospital (Dr. J. Bonham), Southend Hospital (Mr. M. Fahie Wilson), UKNEQAS (Mr. Finlay McKenzie). The group discussed data that had been collected by a pre-circulated questionnaire. Data was collected from the meeting participants and also from Belfast Sick Children's Hospital (Ms. G. Roberts), Bristol Children's Hospital (Dr. J. Stone), University Hospital, Cardiff (Ms H. Losty), Glasgow Royal Hospital for Sick Children (Mrs. M. Rae), St James University Hospital, Leeds (Dr. L Shapiro), Liverpool Children's Hospital (Dr. D. Isherwood) and Manchester Children's Hospital (Dr. M. Addison). Data was collected on site of collection, number of collections, equipment, collection time, minimum sweat rate, definition of upper limit of reference range and lower limit of CF range and methodology.

The evidence base for the guidelines was updated during the course of the guideline development process to take account of newly published evidence and evidence arising from the open review meeting/consultation process.

## **CONSULTATION AND PEER REVIEW**

### **i) Discussion Forum**

The first draft of the guidelines were presented to the Cystic Fibrosis Trust Directors (September 7<sup>th</sup> September 2001) and at an open meeting for all professionals/patient group representatives on November 13<sup>th</sup> 2001 (Appendix 3).

Comments and new evidence resulting from these meetings were subsequently considered by the working group at a meeting on November 14<sup>th</sup>, 2001.

### **ii) Web**

Draft guidelines were made available on the following Web sites during November and December 2001 and January 2002:-

Association of Clinical Biochemists

The Royal College of Pathologists informed members of the availability of the guidelines with an invitation for comment

UKNEQAS

### **iii) Consultation**

Views of interested parties not on the working group have been addressed by circulation of the draft guidelines to:

Wescor Inc.,  
LOGAN,  
Utah,  
U.S.A.

Clinical Pathology Accreditation (UK) Ltd.,  
45 Rutland Park,  
Botanical Gardens,  
SHEFFIELD,  
S10 2PB.

UK NEQAS,  
P.O. Box 3909,  
BIRMINGHAM,  
B15 2UE.

Comments arising from this consultation period were addressed by the working group chairman in consultation with group members. A consensus agreement was reached by the group for each comment.

**iv) Specialist Independent Peer Reviewers**

The guidelines were reviewed by a panel of independent expert peer reviewers. Comments were addressed by the working group at a meeting on February 19<sup>th</sup>, 2002 and consensus agreement reached. The draft guidelines were modified in response to the reviewers' suggestions.

The peer reviewers were:-

- |                       |   |
|-----------------------|---|
| Professor John Dodge  | Professor of Child Health,<br>Department of Child Health, Singleton Hospital,<br>Swansea, SA2 8QA.                        |
| Miss Monica Goldfinch | Clinical Scientist,<br>Department of Clinical Biochemistry, Royal Victoria<br>Infirmary, Newcastle, NE1 4LP.              |
| Mr. Paul Griffiths    | Consultant Biochemist,<br>Wansbeck General Hospital, Ashington,<br>Northumberland.  |
| Dr. Margaret Hodson   | Cystic Fibrosis Consultant,<br>Royal Brompton and Harefield NHS Trust, Sydney<br>Street, London, SW3 6NP.                 |
| Ms. Helen Losty       | Clinical Scientist,<br>Department of Medical Biochemistry, University<br>Hospital of Wales, Heath Park, Cardiff, CF4 4XW. |

- Dr. Bryan Stack                      Consultant Physician,  
President of the British Thoracic Society,  
Gartnavel General Hospital, 1053 Great Western Road,  
Glasgow, G12 OYN.
- Dr. Maurice Super                      Consultant Paediatric Geneticist,  
Paediatric Genetics Unit, Royal Manchester Children's  
Hospital, Hospital Road, Pendlebury, Manchester, M27  
4HA.
- Dr. Janet Stone                      Clinical Scientist,  
Chemical Pathology, Bristol Royal Infirmary,  
Marlborough Street, Bristol, BS2 8HW.

## APPENDIX 1

### Example Sweat Test information sheet for patients/parents

This fact sheet has been produced to provide information for people who have been referred for a sweat test. In addition, it explains how to find your way to the department, what the results may mean and how you can get the results of your test.

---

#### What is a sweat test?

A sweat test measures the amount of salt (usually as chloride) that is in the sweat.

---

#### Why does this need to be carried out?

The test is carried out on children or adults who are having recurrent chest infections, those that have frequent and unexplained pale stools, those that are having problems gaining weight or growing properly or as part of a screening programme. There are also other rarer indications for a sweat test. A positive result may mean that you or your child has cystic fibrosis (CF) but a final diagnosis will take into account other symptom, clinical findings and test results. People with CF have a high amount of salt in their sweat. A normal result can be helpful in ruling out CF. It is important to diagnose this condition as soon as possible in order to begin appropriate treatment.

---

#### Who does this test?

(insert according to local arrangements)

---

#### Does the test hurt?

Some people experience a tingling sensation on the arm or leg where the sweat has been collected. No needles are involved.

---

#### How is the test carried out?

Special pads soaked in a chemical called Pilocarpine that stimulates sweat production are placed on the lower arm or leg. These are secured in place and a small electric current is passed through the pad from a battery box to further stimulate the sweating process. The test is not painful, although a tingling sensation may occur. The pads are left in place for about 5 minutes and then removed. There should be a red mark where the pilocarpine has stimulated the skin. This is a usual phenomenon and should fade within a few hours. The skin is then carefully washed with pure water and dried. A piece of filter paper or sometimes a plastic coil is placed over the stimulated area and secured. You will then be asked to wait for about 30 minutes for the sweat to be absorbed into the filter paper or coil device. During that time you (or your child) is free to read (play) (or eat, although salty foods such as crisps should be avoided to minimise any risk of contamination. The filter paper or coil is then removed and sent to the laboratory for analysis.

**The results**

In most cases the results will clearly show either a high (abnormal) or normal salt level in the sweat. Sometimes the results can be borderline and the test will need to be repeated. In a few cases the test may need to be repeated for technical reasons such as not enough sweat has been collected. Many doctors like to confirm an abnormal sweat test with a second sweat test.

---

**How long will it take to get results?**

(insert paragraph according to local arrangements)

---

**Who will inform me of the results?**

(insert paragraph according to arrangements)

---

**Further questions**

If you have questions about the process of doing the sweat test, please contact (please insert local details).

If you have further questions regarding the need for a sweat test in yourself or your child, please speak to the doctor who has referred you for this test as they can give you further information.

---

## APPENDIX 2a

# SWEAT TESTING PROCEDURE *WESCOR MACRODUCT*

## Sweat Collection

### INTRODUCTION:

Cystic fibrosis is the most common serious genetic disease in Caucasians, with a UK incidence of approximately 1 in 2,500 live births. The primary defect affects chloride ion transport across membranes, producing excessively viscous exocrine secretions. The major presenting symptoms are failure to thrive, recurrent respiratory infections and pancreatic insufficiency resulting in malabsorption. The increased secretion of chloride (and to a lesser extent other) ions in sweat is the basis of a diagnostic test for the condition.

### PRINCIPLE:

Pilocarpine is delivered to a small area of sweat glands on the arm by iontophoresis. The stimulated sweat produced from this area is collected directly into a Macroduct for chloride analysis.

### HAZARDOUS SUBSTANCES AND NATURE OF HAZARD:

REFER TO LABORATORY COSHH GUIDELINES FOR EACH

Pilocarpine	<b>TOXIC</b>
Sweat	<b>BIOHAZARD</b>
Electrode contact	<b>BURNS</b>

### PRECAUTIONS:

Wash hands before and after procedure.

In case of contact of pilocarpine with eyes, mouth or large areas of skin, flush area with copious amounts of water.

Check electrodes before each test. Replace if they show signs of pitting or buckling. Do not use Pilogel discs that are beyond their expiry date, cracked, or showing any evidence of deterioration. Never leave the patient at any time during iontophoresis and investigate any complaint of "stinging" or "burning" at once. At the end of the test

the stimulated area should appear red. If there is any evidence of blistering or burning seek medical assistance.

### **PATIENT AND SPECIMEN REQUIREMENTS:**

Reliable sweat test results are most likely to be obtained when the test is carried out with care by an experienced operator. ONLY staff who have been trained in the use of the Wescor system should perform this test.

The sweat test should be deferred in babies <7days old and/or <3 kgs in weight, subjects who are dehydrated, systemically unwell or who have marked eczema or oedema. Sweat tests should not be performed in subjects who are on oxygen by an open delivery system (this does not apply to headbox or nasal prong oxygen).

### **INSTRUMENTATION AND APPARATUS:**

1. Wescor sweat collection system (Model 3600, 3700 etc).  
Check the following components.
  - (a) Power supply box (also charging stand and transformer if using model 3600).
  - (b) 2 iontophoresis electrodes (red and black) each with Velcro strap.
  - (c) Velcro straps in different sizes, to fit Macroduct.
  - (d) Sweat extractor tool and scissors or nippers.
2. Sealable tubes or cups for sweat transport and storage. Eg. Autoanalyser cups and caps, 100 $\mu$ l Haematocrit capillary tubes and Plasticine Miniseal block.
3. Mediswabs
4. Kleenex medical wipes or cotton wool balls.
5. Adhesive labels for sample identification.

### **REAGENTS:**

1. *Macroduct Test Kit*. Chemlab Cat no WE55032 consisting of 12 Pilogel discs and 6 collectors. Store according to manufacturer's instructions.
2. *Distilled or deionised water in wash bottle.*

### **PROCEDURE:**

1. Visually check condition of power pack, connections and electrodes. Carry out any routine maintenance described in the instrument manual.
2. Explain the procedure to the patient/parents. Depending on local protocols this may include provision of a patient leaflet. Patients may already have received a leaflet together with their appointment.

3. Pour distilled or deionised water into clean container and soak the cotton wool balls.
4. Ask parent/guardian to remove patient's clothes to expose arm or alternative collection site. Either arm can be used.
5. Select sites for iontophoresis. The inner surface of the forearm is almost always the most satisfactory site. Either arm can be used. The skin should be hairless and wrinkle free and should not be broken or irritated. In very small babies, with insufficient area on the forearm, the upper arm or outside of the thigh may be used as collection sites.
6. Swab the area selected with a Mediswab, and then using a cotton wool ball soaked in distilled or deionised water. Dry with a clean tissue.
7. Moisten the skin with a fresh tissue dampened with distilled or deionised water to ensure good current flow.
8. Place a pilocarpine gel disc on top of each electrode and rotate the disc to ensure good contact.
9. Strap both electrodes in position. Sweat collection will take place at the red (positive) electrode site. Select this site to give the best possible contact for the Macroduct ie farthest from wrist, on the area with best subcutaneous tissue. Ensure the electrodes are at least 2 cm apart to prevent any bridging of current on the skin surface between them. If necessary the negative electrode may be placed on the outer surface of the forearm, or on the upper arm.
10. Although the principle is the same for all versions of the Wescor power supply the details vary slightly. The manufacturer's instructions supplied with each unit should be followed in all cases.

Example for the 3600 SYSTEM:

- a. Plug 3600 unit into charger unit in case. Connect transformer to unit and to power supply. Wait for status light to change to green.
- b. Remove 3600 unit from charger. Plug electrodes into 3600 unit and press start button. "In Process" should light immediately and current flow light come on dimly at first.
- c. If 'chirrup' alarm sounds either:
  - i. there is a break in the circuit or
  - ii. the power supply is inadequately charged.Action is the same in both cases.  
Recharge power supply (chirrup will stop when power supply is returned to charger).  
Check electrode attachments.
- d. After iontophoresis is complete "chirrup" will sound briefly and "In Process" light will go out.

11. Remove electrodes from arm. Usually the stimulated area is visibly pink or red. Swab area under red (positive) electrode thoroughly using tissues soaked in distilled or deionised water. Do this at least three times and then dry the area with tissues.
12. Open packet containing Macroduct. Keeping surface covered with polythene, thread Velcro strap through. (Furry side faces OUT).
13. Immediately strap Macroduct firmly into position on patient's arm over stimulated area. Be careful not to nip skin when tightening. For small babies or other subjects who may disturb the collection strap Macroduct into position with elastic bandage.
14. Roll down child's sleeve and leave Macroduct in position for 20 min. It may be left longer if insufficient sweat (less than 2 turns = approximately 15uL) has collected after 20 min but should never be left longer than 30 minutes. Although suggested in the manufacturer's instruction manuals (1) extending the collection time beyond 30 minutes does not increase the weight collected by a significant amount.
15. Label autoanalyser cup with patient name and laboratory number.
16. Leaving Macroduct in position on arm remove perspex cover. Attach extraction tool (white end) to outer end of coil and unravel. Do NOT squeeze the dispenser tubing as this may cause loss of sweat. Cut as close to Macroduct as possible. Put cut end into auto-analyser cup and then squeeze extraction tool to transfer sweat, avoiding bubbles. Remove extraction tool from liquid before releasing pressure on tubing. Cap cup and keep upright.
17. The minimum acceptable volume of sweat, corresponding to  $1\text{g}/\text{m}^2/\text{min}$  is 12ul or mg for a 20 minute collection, or 18ul or mg for a 30 minute collection. (See guidelines 4.1.3) This may be assessed approximately using the insert from the Macroduct packs. Weighing is not necessary, but if preferred may be assessed by transferring the sweat into a weighed labelled autoanalyser cup and reweighing immediately. Assess sweat samples for adequacy immediately after collection. Collections of less than 12uL or mg in 20 minutes or 18ul or mg in 30 minutes should not be analysed. The collection time must be taken into account when assessing adequacy. Extending the collection time in an attempt to increase yield also increases the minimum volume required. As sweat production falls off rapidly after 30 minutes a collection that is insufficient at 30 minutes is highly unlikely to increase in volume sufficiently to produce an adequate collection at times longer than 30 minutes. Insufficient sweat collections should not be pooled to provide sufficient volume for analysis. The full sweat test should be repeated.
18. If analysis is not to be carried out immediately, or if the sample has to be transported to a different site for analysis it is recommended that sweat is transferred to a labelled plain glass capillary tube. An air gap should be left at both ends, which are then sealed with plasticine.

## Sweat Analysis

### PRINCIPLE:

The sweat produced is collected directly into a Macroduct and analysed for chloride (sweat sodium and conductivity may also be measured). Sweat chloride may be analysed by a colorimetric, coulometric or ion selective electrode procedure.

### HAZARDOUS SUBSTANCES AND NATURE OF HAZARD:

Human Sweat

**BIOHAZARD**

Chemicals

**Appropriate to method used**

### PRECAUTIONS:

Avoid contamination or evaporation of the sweat sample.

### SAMPLE REQUIREMENTS:

On return to laboratory immediately proceed to analysis or transfer to 100ul glass capillary tube (or other sealable container of appropriate size). Seal ends with plasticine and label with patient name and laboratory number. Store for up to 6 hours before analysis.

### INSTRUMENTATION AND APPARATUS:

Appropriate to methodology. See separate SOPs for analysis & equipment maintenance.

### REAGENTS:

Appropriate to methodology. See separate SOPs.

### STANDARDS:

The method should be standardised using commercial or in-house materials at concentrations appropriate for sweat samples. ie 0-150mmol/L.

The linearity and sensitivity of the method must be determined to establish its working range. The detection limit should be determined for each analyte measured. It should be no greater than 10mmol/L.

### **INTERNAL QUALITY CONTROL:**

Two concentrations of Internal quality control material should be analysed with each sweat sample batch. One concentration should be within the normal range, and the second within the intermediate or abnormal range. These may be commercial or in-house.

### **PROCEDURE:**

1. Sample directly from analyser cup or score the ends of the glass capillary and carefully break open. After sampling transfer remaining sweat to glass capillary and seal ends.
2. In duplicate prepare sweat samples and internal quality control material for analysis. This must include any pre-dilutions of the sweat sample.
3. Analyse IQC and sweat samples. If any result lies outside the working range of the method it must be repeated at an appropriate dilution.

### **CALCULATION:**

Calculate the sweat chloride concentration, allowing for any dilution factors.

All calculations must be independently checked.

### **QUALITY CONTROL VERIFICATION:**

IQC results should be within locally defined limits.

Methods should be capable of producing a between batch CV of <5%. Acceptable limits for IQC should reflect this.

### **RESULT REPORTING**

The report form should include:

- i. Full patient identification
- ii. Date and time of test and date and time of report
- iii. Sweat weight/volume collected and minimum weight/volume acceptable for local sweat testing parameters. This must have been demonstrated to be equivalent to an average sweat rate of 1 g/m<sup>2</sup>/min over the collection period used.
- iv. Analytical results (chloride, conductivity, sodium) in mmol/l. It should be explicit on the report form which analyte(s) have been measured.
- v. Reference ranges ie
  - A sweat chloride concentration of > 60 mmol/l supports the diagnosis of CF
  - Intermediate chloride concentration of 40 - 60 mmol/l are suggestive but not diagnostic of CF
  - A sweat chloride of less than 40 mmol/l is normal and there is a low probability of CF.

- Sweat sodium should not be interpreted without a chloride result. It adds nothing to chloride interpretation in clearly normal or abnormal cases, but may occasionally be useful in interpretation of intermediate chlorides.
  - Pending further data on conductivity measurements a value below 60mmol/L (NaCl equivalents) is unlikely to be associated with cystic fibrosis. Values above 90 mmol/L support a diagnosis of cystic fibrosis.
- vi. Interpretation of results, based on the above reference ranges, and any further information supplied about the patient (eg pancreatic sufficient, unusual CF mutation etc)
- vii. Recommendations for repeat testing if appropriate:
- Patient unsuitable for sweat stimulation
  - Insufficient collection
  - First abnormal or intermediate result
  - Non-physiological result ie chloride or sodium >150mmol/L, conductivity > 170mmol/L, discrepancy of >20mmol/l between sodium and chloride.

## REFERENCES

1. Instruction Manuals - Macroduct Sweat Collection System appropriate to model used.
2. Medical Devices Agency. Safety Notice. MDA SN1999(05)
3. Guidelines for the performance of the Sweat Test for the investigation of Cystic Fibrosis in the UK.

## APPENDIX 2b

# ***SWEAT TESTING PROCEDURE GIBSON AND COOKE FILTER PAPER COLLECTION***

## **Sweat Collection**

### **INTRODUCTION:**

Cystic fibrosis is the most common serious genetic disease in Caucasians, with a UK incidence of approximately 1 in 2,500 live births. The primary defect affects chloride ion transport across membranes, producing excessively viscous exocrine secretions. The major presenting symptoms are failure to thrive, recurrent respiratory infections and pancreatic insufficiency resulting in malabsorption. The increased secretion of chloride (and to a lesser extent other) ions in sweat is the basis of a diagnostic test for the condition.

### **PRINCIPLE:**

Pilocarpine is delivered to a small area of sweat glands on the arm by iontophoresis. The stimulated sweat produced from this area is collected onto filter paper for chloride analysis.

### **HAZARDOUS SUBSTANCES AND NATURE OF HAZARD:**

**REFER TO LABORATORY COSHH GUIDELINES FOR EACH**

Pilocarpine	<b>TOXIC</b>
Cathode electrolyte	<b>POTENTIALLY TOXIC</b>
Sweat	<b>BIOHAZARD</b>
Electrode contact	<b>BURNS</b>

### **PRECAUTIONS:**

Wash hands before and following procedure.

In case of contact of pilocarpine or cathode electrolyte with eyes, mouth or large areas of skin, flush area with copious amounts of water.

Check electrodes before each test. Replace if they show signs of pitting or buckling. Never leave the patient at any time during iontophoresis. Increase and reduce the current gradually and investigate any complaint of "stinging" or "burning" at once. At

the end of the test the stimulated area should appear red. If there is any evidence of blistering or burning seek medical assistance.

### **PATIENT AND SPECIMEN REQUIREMENTS:**

Reliable sweat test results are most likely to be obtained when the test is carried out with care by an experienced operator. ONLY staff who have been trained in the use of the Gibson & Cooke system should perform this test.

The sweat test should be deferred in babies <7days old and/or <3 kgs in weight, subjects who are dehydrated, systemically unwell or who have marked eczema or oedema. Sweat tests should not be performed in subjects who are on oxygen by an open delivery system (this does not apply to headbox or nasal prong oxygen).

### **INSTRUMENTATION AND APPARATUS:**

1. Sodium chloride free filter paper eg Whatman No 41/42/44/541, similar in size to the electrolyte supports.
2. Airtight container for weighing and eluting filter paper eg Universal container
3. Laboratory balance sensitive to 0.0001g
4. Iontophoresis power supply eg C & IS Electronics Gibson - Cooke Power Supply
5. 2 electrodes
6. Rubber or Velcro electrode straps of adjustable size.
7. Electrolyte support pads. eg Pads of Hospital Lint BPC Plain 500 gram folded to provide 4-8 thicknesses (greater than 1cm thick) The pad should be at least 1cm larger than the electrode in all directions to prevent electrode-skin contact. It may be incorporated into sewn pockets designed to contain the electrode and prevent skin contact.
8. Mediswabs
9. Cotton wool balls.
10. Paper towels or tissues
11. Container suitable for holding distilled or deionised water for washing eg Traysin.
12. 2 Containers suitable for soaking lint pads in electrolyte solutions eg Gallipots
13. Plastic forceps
14. Sheet of impervious material at least 1cm larger in all dimensions than the filter paper, e.g. polythene or parafilm
15. Waterproof strapping, e.g. Sleaf or Setonplast

### **REAGENTS:**

- (1) *Pilocarpine nitrate solution (0.2 - 0.5%). Pharmaceutical Grade*
- (2) *Cathode electrolyte, if different eg magnesium sulphate. Pharmaceutical grade.*
- (3) *Distilled or Deionised water*

## PROCEDURE:

1. Visually check condition of power pack, connections and electrodes. Carry out any routine maintenance described in the instrument manual.
2. Label container to be used. Using forceps place a filter paper in the labelled container. Zero the balance and record the weight of the container and filter paper to 4 decimal places.
3. Transport weighed filter paper and container in a polythene bag.
4. Place lint electrolyte support in container. Add pilocarpine solution to saturate the lint.
5. Place second lint electrolyte support in separate container. Add pilocarpine or alternative cathode electrolyte solution to saturate the lint.
6. Explain the procedure to the patient/parents. Depending on local protocols this may include provision of a patient leaflet. Patients may already have received a leaflet together with their appointment.
7. Pour deionised water into clean container and soak the cotton wool balls.
8. Ask parent/guardian to remove patient's clothes to expose arm or alternative collection site. Either arm can be used.
9. Select sites for iontophoresis. The inner surface of the forearm is almost always the most satisfactory site. The skin should be hairless and wrinkle free and should not be broken or irritated. In very small babies, with insufficient area on the forearm, the upper arm or the thigh may be used as collection sites.
10. Swab the area selected with a Mediswab and then using cotton wool balls soaked in deionised water. Dry with a clean tissue.
11. Moisten the skin with a fresh tissue dampened with distilled water to ensure good current flow.
12. Place soaked pilocarpine lint pad in position and strap red (positive) electrode in place. As sweat collection will take place at this site choose the best possible surface ie farthest from wrist, on the area with best subcutaneous tissue.
13. Place soaked cathode electrolyte lint pad in position and strap black (negative) electrode in place.
14. Ensure the electrodes are sufficiently far apart to prevent any bridging of current on the skin surface between them. Dry skin between electrodes with tissues. If necessary the negative electrode may be placed on the outer surface of the forearm, or on the upper arm.
15. Check position of both electrodes and pads, and that there is a sufficient margin of well saturated pad around both electrodes to prevent electrode-skin contact.
16. Connect the electrodes to the iontophoresis box :
17. positive (red) = pilocarpine, negative (black) = cathode electrolyte.

18. Switch on and set current to 0.5mA
19. Slowly (over 10-15 seconds) turn the current up to 4mA. Time for 5 minutes. Due to high skin resistance some patients, usually adults, will very occasionally trigger the power pack safety cutout at a current of less than 4 mAmps. If this occurs check all connections and resite the pads and electrodes. If the problem recurs note the current at which the cutout occurs and carry out iontophoresis at a current just below this point.
20. Slowly (over 10-15 seconds) reduce current, and switch off.
21. Remove electrodes and pads from arm. Usually the stimulated area is visibly pink or red. Swab area under red (positive) electrode thoroughly using tissues soaked in distilled water. Do this at least three times and then dry the area with tissues.
22. Remove the filter paper from the sweat bottle using sterile forceps and place on stimulated area.
23. Immediately cover with parafilm or polythene and tape in place, taking care to completely seal the parafilm or polythene to the skin surface. Avoid touching the inner surface of the polythene or parafilm.
24. Replace clothes and leave patient for 30 min. Extending the collection time beyond 30 minutes does not increase the weight collected by a significant amount.
25. Rub the outside surface of the strapping to transfer any sweat condensate onto the filter paper. Remove strapping carefully and use sterile forceps to transfer filter paper to weighed sweat bottle.
26. Replace lid tightly. Transport bottle in polythene bag. Re-weigh using same balance to 4 decimal places.
27. Assess sweat samples for adequacy immediately after collection. Calculate the minimum acceptable weight of sweat, corresponding to 1g/m<sup>2</sup>/min as follows: (See guidelines 4.1.3).

Calculate the area of the filter paper collector in cm<sup>2</sup> as  $\pi r^2$  where r is the radius.

Then sweat rate (g.m<sup>2</sup>/min) =

$$\frac{10000}{\text{area (cm}^2\text{)}} \times \frac{\text{weight (mg or ul)}}{1000} \times \frac{1}{\text{collection time (min)}}$$
$$= \frac{10 \times \text{weight (mg or ul)}}{\text{area (cm}^2\text{)} \times \text{collection time (min)}}$$

e.g., for a 30 minute collection on 5.5cm diameter filter paper 1g/m<sup>2</sup>/min = 71 mg

Collections of less than the locally derived minimum sweat weight should not be analysed. The collection time must be taken into account when assessing adequacy. Extending the collection time in an attempt to increase yield also increases the minimum weight required. As sweat production falls off rapidly after 30 minutes a collection that is insufficient at 30 minutes is highly unlikely to increase in weight sufficiently to produce an adequate collection at times longer than 30 minutes. Insufficient sweat collections should not be pooled to provide sufficient volume for analysis. The full sweat test should be repeated.

28. Collections of less than  $1\text{g}/\text{m}^2/\text{min}$  should not be analysed. To relate weight to rate use the following equation:

A or volume may be used routinely. This must be demonstrated to be equivalent to a minimum rate of  $1\text{g}/\text{m}^2/\text{min}$ .

Insufficient sweat collections should not be pooled to provide sufficient weight for analysis. The full sweat test should be repeated.

## Sweat Analysis

### PRINCIPLE:

The sweat produced is eluted from the filter paper into a suitable diluent and analysed for chloride (sweat sodium may also be measured). Sweat chloride may be analysed by a colorimetric, coulometric or ion selective electrode procedure.

### HAZARDOUS SUBSTANCES AND NATURE OF HAZARD:

Human Sweat  
Chemicals

**BIOHAZARD**  
**Appropriate to method used**

### PRECAUTIONS:

Avoid contamination or evaporation of the sweat sample.

### SAMPLE REQUIREMENTS:

Immediately proceed to analysis or store weighed sweat collections on filter paper pads at  $4^{\circ}\text{C}$ , for a maximum of 3 days in appropriately sized, air tight containers which do not allow leakage or evaporation. Sweat collections may be transported for analysis at this stage.

### **INSTRUMENTATION AND APPARATUS:**

Appropriate to methodology. See separate SOPs for analysis & equipment maintenance.

### **REAGENTS:**

Appropriate to methodology. See separate SOPs.

### **STANDARDS:**

The method should be standardised using commercial or in-house materials at concentrations appropriate for sweat samples ie 0-150mmol/L.

The linearity and sensitivity of the method must be determined to establish its working range. The detection limit should be determined for each analyte measured. It should be no greater than 10mmol/L.

### **INTERNAL QUALITY CONTROL:**

Two concentrations of Internal quality control material should be analysed with each sweat sample batch. One concentration should be within the normal range, and the second within the intermediate or abnormal range. These may be commercial or in-house.

1. Using forceps place a filter paper in a labelled container. Zero the balance and record the weight of the pot and filter paper to 4 decimal places.
2. Pipette internal quality control material onto the filter paper to give approximately the same weight as the patient sample.
3. Reweigh the container and filter paper to four decimal places
4. Treat patient samples and internal QC material in parallel for all remaining steps of the procedure.

### **PROCEDURE:**

#### **ELUTION:**

1. Add diluent (volume is method dependent) to the patient and IQC filter papers.
2. Cap containers and elute for a minimum of 40 minutes. Mixing may be achieved using a roller mixer.
3. Centrifuge containers to remove any fibres of filter paper. Use "supernatant " for analysis.

#### **ANALYSIS**

1. In duplicate prepare sweat samples and internal quality control material for analysis. This must include any pre-dilutions of the sweat sample.

2. Analyse IQC and sweat samples. If any result lies outside the working range of the method it must be repeated at an appropriate dilution.

#### **CALCULATION:**

Calculate the sweat chloride concentration in the IQC and patient samples, allowing for sweat weight and dilution factors.

All calculations must be independently checked.

#### **QUALITY CONTROL VERIFICATION:**

IQC results should be within locally defined limits.

Methods should be capable of producing a between batch CV of <5%. Acceptable limits for IQC should reflect this.

#### **RESULT REPORTING**

The report form should include:

- i. Full patient identification
- ii. Date and time of test and date and time of report
- iii. Sweat weight/volume collected and minimum weight/volume acceptable for local sweat testing parameters. This must have been demonstrated to be equivalent to an average sweat rate of 1 g/m<sup>2</sup>/min over the collection period used.
- iv. Analytical results (chloride, conductivity, sodium) in mmol/l. It should be explicit on the report form which analyte(s) have been measured.
- v. Reference ranges i.e.
  - A sweat chloride concentration of > 60 mmol/l supports the diagnosis of CF
  - Intermediate chloride concentration of 40 - 60 mmol/l are suggestive but not diagnostic of CF
  - A sweat chloride of less than 40 mmol/l is normal and there is a low probability of CF.
  - Sweat sodium should not be interpreted without a chloride result. It adds nothing to chloride interpretation in clearly normal or abnormal cases, but may occasionally be useful in interpretation of intermediate chlorides.
- vi. Interpretation of results, based on the above reference ranges and any further information supplied about the patient (eg pancreatic sufficient, unusual CF mutation etc)
- vii. Recommendations for repeat testing if appropriate:
  - Patient unsuitable for sweat stimulation
  - Insufficient collection
  - First abnormal or intermediate result
  - Non physiological result ie Chloride or sodium >150mmol/L, discrepancy of >20mmol/l between sodium and chloride.

## REFERENCES

1. LE Gibson & RE Cooke A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilising pilocarpine by iontophoresis. *Pediatrics* 1959: 23; 545-549
2. Instruction Manual - sweat power supply
3. Medical Devices Agency. Safety Notice. MDA SN1999(05)
4. Guidelines for the performance of the Sweat Test for the investigation of Cystic Fibrosis in the UK.

## APPENDIX 3

### FORTHCOMING NATIONAL MEETING

#### **National Guidelines for the Performance of the Sweat Test for the Investigation of Cystic Fibrosis**

A multi-disciplinary group, under the aegis of the Royal College of Paediatrics & Child Health, the Association of Clinical Biochemists, the Royal College of Pathologists, the Cystic Fibrosis Trust, the British Thoracic Society and the British Paediatric Respiratory Society, has been working on producing draft guidelines on how to perform the sweat test for the purposes of investigation of cystic fibrosis in the UK.

This initiative arose from the Specialist Advisory Group for Paediatrics of the National External Quality Assessment Scheme (NEQAS). The group is keen to ensure there is full and open discussion about the recommendations being proposed and, in order to aid this process, NEQAS is organising a meeting on November 13<sup>th</sup> 2001 in Birmingham (see attached programme). The aim of the meeting is to consult widely about the guideline draft recommendations and to get feedback from a wide range of healthcare professionals and patients. The meeting is open to all professionals and patient group representatives.

Anne Green  
Consultant Clinical Biochemist  
on behalf of the Sweat Test Guidelines Development Group

If you are interested in attending the meeting, please contact:-

Mrs. Mary Dowling,  
Department of Clinical Chemistry,  
Birmingham Children's Hospital,  
Steelhouse Lane,  
Birmingham. B4 6NH.  
Tel: (0121) 333 9916  
Fax: (0121) 333 9911  
Email: [secretary.anneg@bhamchildrens.wmids.nhs.uk](mailto:secretary.anneg@bhamchildrens.wmids.nhs.uk)  
Closing date for registration:- 31<sup>st</sup> October 2001

APPENDIX 3 contd..

**PRESENTATION OF DRAFT RECOMMENDATIONS  
NOVEMBER 13<sup>TH</sup> 2001  
Postgraduate Centre, University Hospital (Queen Elizabeth)  
Birmingham**

**This is a meeting open to all professionals and parent group representatives  
Chairman: Anne Green**

1000h	Registration and coffee	
1030h	Introduction <ul style="list-style-type: none"> <li>- Background</li> <li>- Scope of guidelines</li> <li>- Guideline Group Working Arrangements</li> </ul>	Anne Green, Birmingham Children's Hospital
1100-1115h	Presentation of Draft Guidelines with Discussion <ul style="list-style-type: none"> <li>▪ Patient information</li> <li>▪ Subject suitability</li> <li>▪ Sweat collection and analysis</li> </ul>	Peter Weller, Birmingham Children's Hospital.
1115-1130h		Peter Weller, Birmingham Children's Hospital
1130-1230h		Jean Kirk, Royal Hospital for Sick Children, Edinburgh Mike Fahie-Wilson, Southend Hospital
1230-1330h	LUNCH	
<b>Chairman: Peter Weller</b>		
1330-1430h	Presentation of Draft Guidelines continued <ul style="list-style-type: none"> <li>▪ Quality <ul style="list-style-type: none"> <li>- UK NEQAS data</li> <li>- quality standards</li> <li>- responsibility</li> </ul> </li> </ul>	Finlay MacKenzie Anne Green
1430-1500h	<ul style="list-style-type: none"> <li>▪ Reference values for testing and interpretation</li> </ul>	Stuart Elborn, Belfast City Hospital
1500h	Panel discussion and next stages	Anne Green
1515h	TEA AND CLOSE	

**The meeting is sponsored by UK NEQAS Birmingham**

*UK NEQAS Birmingham is a not-for-profit organisation which is part of the NHS, and is dedicated to improving the comparability of laboratory test results and so improve patient care*