

Guidelines for the Performance of the Sweat Test for the Investigation of Cystic Fibrosis in the UK 2nd Version

(These guidelines supersede the 2003 guidelines)

**An Evidence Based Guideline
March 2014**



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Sweat Test Guidelines: www.acb.org.uk

ABBREVIATIONS

ACB	Association for Clinical Biochemistry & Laboratory Medicine
BTS	British Thoracic Society
CPA	Clinical Pathology Accreditation (UK) Ltd.
CFNG	Cystic Fibrosis Nurses Group
CFT	Cystic Fibrosis Trust
IBMS	Institute of Biomedical Science
CLSI	US Clinical and Laboratory Standards Institute
NBS	Newborn Screening
NEQAS	National External Quality Assurance Schemes
NCFSB	Neonatal Cystic Fibrosis Screening Board
RCPATH	Royal College of Pathologists
RCPCH	Royal College of Paediatrics and Child Health
SOP	Standard Operating Procedure
UKNSPC	UK Newborn Screening Programme Centre
WAG	Welsh Assembly Government

CONTENTS

	Page
Guidelines Development Group	6
Consultation and peer review.. .. .	8
Stakeholder organisations.. .. .	10
Notes for Users	11
Review and Updating	12
Summary of Recommendations	13
 Guidelines Report	
 <i>Introduction</i>	 24
 <i>Objective</i>	 26
 <i>Scope</i>	 26
 Guideline presentation convention	 28
 <i>The Evidence and Recommendations</i>	
1. What patient information needs to be provided?...	29
2. Which patients are suitable to a have sweat test?	31
3. How should sweat be collected?	34
4. How should sweat by analysed?	46
5. What are the quality requirements for a sweat testing service.. .. .	54
6. What reference values and interpretive comments should be used?.. .. .	63
7. Who has responsibility for testing and training? ..	72

References	75
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Process of Guideline Review

Methodology	88
Grading of Evidence and Recommendations	91
Grading Scheme for Recommendations	91
Systematic Review	95
Expected Implications of the New Guidelines.. ..	99

Appendices

1. Sweat test information leaflet (Cystic Fibrosis Trust) ..	101
2. Example standard operating procedures	
(a) WescorMacroduct®.. .. .	103
(b) Gibson and Cooke filter paper collection.. ..	111
3. Calculation of average sweat rate over the collection period	121

GUIDELINES DEVELOPMENT GROUP

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All members of the guideline development group have made declarations of interest and further details of these are available on request. No conflicts of interest were declared.

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Association for Clinical Biochemistry & Laboratory Medicine (funding received during previous guideline review)

Birmingham Children's Hospital NHS Foundation Trust

Institute of Biomedical Science

North West Wales NHS Trust

CONSULTATION AND PEER REVIEW

i) Discussion Forum

The first draft of the guidelines were at an open meeting for all professionals/patient group representatives on June 15th 2009

Comments and new evidence resulting from this meeting were subsequently considered by the working group at a meeting on June 15th 2009 and subsequently following additional literature search

ii) Web

Draft guidelines were made available on the following Web sites during February/March 2011:-

Association for Clinical Biochemistry

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.,
(An Elitech Group Company)
370 W 1700 S
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Utah 84321,
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C&S Electronics Inc
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Clinical Pathology Accreditation (UK) Ltd.,
21 – 47 High Street,
Feltham,
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TW13 4UN.

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 following the consultation period and consensus agreement reached regarding changes and amendments. The draft guidelines were modified in response to the reviewers' suggestions.

The peer reviewers were:-

Jean Kirk, Consultant Clinical Scientist, Edinburgh, UK

Kevin Southern, Reader and Honorary Consultant in Paediatric Respiratory
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Ronda Greaves, Senior Lecturer, Victoria, Australia

Lisa Shelley is a parent of a child diagnosed with cystic fibrosis and has commented on the guideline from the viewpoint of the 'target population'.

STAKEHOLDER ORGANISATIONS

The following organisations were consulted on the draft guideline:

Association for Clinical Biochemistry & Laboratory Medicine

British Paediatric Respiratory Society

British Thoracic Society

Cystic Fibrosis Nurses Group

Cystic Fibrosis Trust

Institute of Biomedical Science

National External Quality Assurance Schemes

Neonatal Cystic Fibrosis Screening Board

Royal College of Pathologists

Royal College of Paediatrics and Child Health

Welsh Assembly Government

NOTES FOR USERS

It is intended that the recommendations contained in this updated 2nd version of the guidelines will be adopted for local use in the UK wherever sweat testing for the investigation of cystic fibrosis is performed.

The guidelines are aimed at the following staff involved in the pathway for the diagnosis of cystic fibrosis by sweat test:

laboratory staff (medical laboratory assistants, biomedical scientists, clinical scientists and chemical pathologists

clinicians requesting the test and overseeing patient care

nursing staff involved in patient care.

It is important that discussion takes place between all relevant health professionals (clinical chemists, paediatricians, chest physicians, CF nurses and biomedical scientists), 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. Chair of the guideline review group holds editorial responsibility for the guideline.

For details of where to obtain copies, contact:-

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REVIEW AND UPDATING

This guideline was issued from the Working Group in draft form initially during January 2011, and in final form in 2014 after incorporation of comments from the formal appraisal by the Royal College of Paediatrics and Child Health.

Where the grading of a recommendation has changed following the appraisal process, this is highlighted.

Royal College of Paediatrics & Child Health Appraisal

It is planned to review the Guidelines in 2020.

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

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SUMMARY OF RECOMMENDATIONS

1. What patient information needs to be provided?

- Informed consent should be obtained in accordance with local policy.
- It is good clinical practice to prepare the patient and, where appropriate, the parents effectively before testing by providing pre-test information appropriate for the individual. This 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 (appendix 1), and specifically for infants identified by newborn screening the 'UKNSPC CF Suspected' leaflet may be accessed via their website⁽¹⁴⁰⁾.

[Grade D]

2. Which patients are suitable to a have sweat test?

- Sweat tests can be performed after 2 weeks of age in infants greater than 2 Kgs at time of testing who are normally hydrated and without systemic illness [Grade C]
- 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 [Grade C]
- Sweat tests should be delayed in subjects who are oedematous or under topiramate treatment or receiving systemic 9-alpha fludrocortisone [Grade D]
- Sweat tests should be delayed in subjects who are dehydrated, underweight, systemically unwell or who have eczema affecting the potential stimulation sites where practicable [Grade D]
- Sweat tests can be performed in subjects on Flucloxacillin [Grade C]
- Sweat tests should not be performed in subjects who are on oxygen by an open delivery system (including headbox). This would not apply to an infant on nasal prong or face mask oxygen [Grade D]

2.1 When should sweat tests be done as part of newborn screening (NBS)?

- Sweat testing is a key investigation for confirmation of CF after a positive newborn screening test [Grade C]

- Sweat testing following newborn screening should only be undertaken in laboratories experienced in performing tests in patients less than 3 months of age [Grade D]
- Sweat testing results should be available at first consultation for infants identified in NBS programme [Grade D]

3. How should sweat be collected?

3.1 Which sites should be used for sweat collection?

- The flexor surface of either forearm is the preferred site for sweat collection. Consideration may be given to other sites, e.g. upper arm or thigh, if both arms are eczematous, too small or otherwise unsuitable. [Grade D]

3.1.1 How can sweat contamination be avoided?

- Great care must be taken at all stages of the procedure to avoid contamination (see Appendices for example Standard Operating Procedures SOP). Do not use chloride-containing solutions to clean the skin, nor apply local anaesthetic gel [Grade D]

3.1.2 How many sweat collections should be made?

- Carrying out the sweat test procedure by bilateral sequential stimulation with simultaneous sweat collection does not significantly increase time taken nor discomfort to the patient but decreases failure rate [Grade C]
- In response to a sweat test request it is sufficient to carry out one sweat collection only unless results suggest an abnormality in the collection or testing is suspected [Grade D]

3.2 Which methods and equipment are suitable for sweat stimulation?

3.2.1 What power supply and which electrodes should be used?

- The power supply used must be battery powered and should include a safety cut-out that limits the amount of current at 5mA or lower.
- Monitoring of the current must be carried out throughout iontophoresis where possible. Wescor® systems without an ammeter are current-limited with an appropriate safety cut-out system.
- The operator should carry out a visual check of the power supply for any damage or malfunction at each use. Electrical safety of all power supplies

must be regularly tested by an electrician and records kept [Grade D]

- Electrodes should be of a suitable size and curvature to fit snugly on the patient's limb:
 - they should be firmly secured in position but not too tightly strapped in order that circulation is not restricted.
 - they must be regularly cleaned and inspected, and discarded if they show pitting or irregularities [Grade D]
- Selection of new equipment and maintenance of existing equipment must comply with CPA Accreditation (or equivalent standard) [Grade D]

3.2.2 Which Electrolyte Solutions can be used?

- 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. [Grade D]
- Solutions containing chloride must not be used because of the risk of contamination of the collection [Grade D]
- Unbuffered acid solutions should not be used because of the increased risk of burns [Grade C]
- 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 [Grade D]

3.2.3 What current and duration should used for Iontophoresis?

- When aqueous electrolyte solutions are applied on pad supports a minimum current of 1.5 mA should be applied, and increased gradually up to a maximum of 4 mA. Once the maximum is attained the current should be maintained for a minimum of 3 minutes and a maximum of 5 minutes [Grade D]
- When Wescor® systems are used, the manufacturer's current and time recommendations should be followed. This will depend on the specific model used and is automatically controlled by all current models [Grade D]
- For Wescor® and C & IS Electronics Gibson – Cooke Power Supply

systems the patient must be kept under close observation throughout the iontophoresis period [Grade D]

- The WescorNanoduct® is not recommended for sweat stimulation and analysis [Grade D]

3.2.4 What safety issues need to be considered during iontophoresis?

- Suitably thick pads must be used for the electrolyte solutions to minimise the risk of acid burns [Grade C]
- Hybrid systems, e.g. Wescor®power supplies or electrodes with aqueous electrolyte solutions, or Wescor® gel discs used with non-Wescor electrodes, should not be used as this may lead to injury [Grade D]

3.3 Which collection medium should be used and how long should sweat be collected for?

3.3.1 Which collection medium should be used?

- Sweat should be collected onto pre-weighed chloride free filter paper or gauze of approximately equal size to the stimulated area (i.e. the lint pads used in iontophoresis) or into WescorMacroduct® disposable collectors [Grade D]
- Filter paper or gauze 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 [Grade D]
- During collection by any method, sweat must be protected from contamination, as well as condensation and evaporation (see example Standard Operating Procedures in Appendices 2a and 2b) [Grade D]

3.3.2 How long should sweat be collected for?

- Sweat should be collected for not less than 20 minutes (unless Macroduct tubing is full earlier) and not more than 30 minutes [Grade C]

3.3.3 How should the Macroduct® sample be removed?

- Collections with Macroduct® should be removed by stopping the free end of the tubing with a syringe or clamp and cutting the attachment to the Macroduct [Grade D]

4. How should sweat be analysed?

4.1 Which pre analytical aspects need to be considered?

4.1.1 How should samples be stored before analysis?

- Throughout sweat collection, transport and analysis, every effort should be made to minimise evaporation of the sample [Grade D]
- 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 [Grade C]
- Liquid sweat from Macroduct® collections can be stored in either sealed Macroduct® tubing or capped PCR tubes 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. [Grade D]
- Storage of liquid sweat for up to 72 hours at higher temperatures should be in capped PCR tubes [Grade C] Sweat may be collected at remote sites and transported to the laboratory for analysis provided there is attention to storage details [Grade D]

4.1.2 How should sweat weight/volume be determined?

- The same balance must be used throughout [Grade D]
- A balance sensitive to 0.0001g must be used to weigh sweat [Grade D]
- Sweat collections onto paper pads should be weighed and analysed as soon as practicable [Grade D]

4.1.3 What is an adequate sample?

- The sweat secretion rate measured as an average rate over the collection period should not be less than 1g/ m²/min. Collections below this rate should not be analysed. Insufficient sweat collections must not be pooled. The full sweat test should be repeated [Grade D]

4.2 How should the sweat sample be prepared for analysis?

- When sweat is collected onto filter paper (section 3.2.1) elution time should be greater than 1min and less than 3 hours [Grade C]
- Samples from filter paper should be homogenised and mixed thoroughly before analysis [Grade D]

- Sweat collected using the WescorMacroduct® system should be carefully expelled and mixed prior to analysis [Grade D]

4.3 Which analytes should be measured in sweat for diagnosis of CF?

- Chloride is the analyte of choice [Grade B]
- Sweat conductivity measurement alone is not an adequate diagnostic test for the investigation of CF. Conductivity may be used as a screening test [Grade D]
- In infants under the age of 6 months, sweat chloride concentration must be measured even if conductivity levels are normal [Grade D]
- In infants and children over the age of 6 months, chloride concentration must be measured if borderline or positive conductivity levels are obtained [Grade D]
- Sweat sodium/potassium/osmolality measurement are not recommended [Grade D]

4.4 What methodology should be used for measurement?

4.4.1 What methods are available for Chloride measurement?

- Colorimetry, coulometry and ISEs are satisfactory methods for analysis of sweat chloride. Where possible, duplicate analytical measurements of chloride should be undertaken [Grade D]

4.4.2 What methods are available for Conductivity measurement?

- Conductivity measurement using the Wescor Sweat-Chek® equipment is a satisfactory method of analysis [Grade D]
- The WescorNanoduct® is not currently recommended for routine sweat collection and analysis [Grade D]

4.5 What format should the report take?

The report format should include:-

- i. Full patient identification
Requester and delivery address
- ii. Date and time of test and date and time of report

iii. Analytical results (mmol/L)

It should be explicit on the report form which analyte(s) have been measured.

i.e. chloride

conductivity

Reason if no result is available/performed

iv. Reference interval (see section 6)

v. Interpretation of results (see section 6)

[Grade D]

5. What are the quality requirements for a sweat testing service?

5.1 Should sweat be analysed if contamination or evaporation are suspected?

- Sweat which has been subject to evaporation and/or contamination must not be measured. [Grade C]

5.2 What considerations should be given when selecting analytical methods?

- The analytical range of the methods used must cover the concentration ranges found in normals and subjects with cystic fibrosis [Grade C]
- The analytical methods must be fully documented as standard operating procedures (SOPs) to comply with Clinical Pathology Accreditation (or equivalent standard).

5.3 What Internal Quality Control (IQC) should be used?

- 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 [Grade C]

5.3.1 What imprecision should be achieved for chloride measurement?

- The chloride method used should have a between batch CV of 5% (or less) at a concentration of 40-50 mmol/L [Grade B]

5.3.2 What imprecision should be achieved for conductivity measurement?

- The conductivity method used should have a between batch CV of 2% (or less) at a concentration of 50 mmol/L [Grade B]

5.4 What considerations need to be given to External Quality Assessment (EQA)?

- The laboratory must participate in a suitable external quality assessment scheme [Grade C]

5.5 What are the expected upper limits for chloride and conductivity concentrations?

- Results which are not physiological should be questioned, i.e. chloride > 150 mmol/L [Grade B]
- For conductivity a provisional upper limit of 170 mmol/L may be used pending further evidence [Grade C]

5.6 What are acceptable failure rates?

- Centres should monitor repeat rates and investigate any significant increase in failure rate [Grade D]
- 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 less than 5% in children over 6 months of age [Grade C]
- In children under 6 months of age failed sweat collections should not exceed 20% of the tested population [Grade C]

5.7 How should service performance be assessed?

- Performance of sweat testing should be reviewed on a regular basis. This should include:-
 - insufficient collections - as % of total tests
 - per operator
 - analytical failure rate (i.e. % outside accepted QC range)
 - external quality assessment performance [Grade C]
- 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) [Grade C]

6. What reference values and interpretive comments should be used?

6.1 What reference values and interpretive comments should be used for sweat chloride concentrations?

- A sweat chloride concentration of > 60 mmol/L supports the diagnosis of CF [Grade C]
- A chloride concentration of 40 - 60 mmol/L or if less than 6 months of age 30-60 mmol/L is an intermediate result which requires further cystic fibrosis assessment such as a repeat test and /or further investigations [Grade C]
- A sweat chloride of less than 40 mmol/L (or less than 30 mmol/l in patients less than 6 months of age) makes CF unlikely but requires genetic and clinical correlation [Grade C]

6.2 What reference values and interpretive comments should be used for sweat conductivity?

- A value below 50 mmol/L (NaCl equivalents) is unlikely to be associated with cystic fibrosis. Values above 90 mmol/L support a diagnosis of cystic fibrosis [Grade C]
- Cystic fibrosis should not be diagnosed based on conductivity measurement alone. Confirmation should be sought using sweat chloride and/or genotyping (See also Section 4.3.3) in all patients with conductivity equal or greater than 50mmol/L. [Grade C]

6.3 What is the intra-individual biological variation of sweat analytes?

- Consideration should be given to intra individual biological variation during the interpretation of the sweat test, particularly around cut-off points. Biological variation is considerably greater than analytical imprecision [Grade D]

6.4 How does intermediate sweat chloride relate to genotype?

- Patients falling into this area of intermediate sweat chloride require further clinical evaluation by cystic fibrosis physicians prior to diagnostic labelling [Grade D]

6.5 What other disorders are associated with CF mutations?

No recommendations

6.6 What other diseases and conditions are associated with increased sweat electrolyte concentrations?

No recommendations

6.7 What are the indications for a repeating a sweat test?

- A repeat sweat test is recommended when the sweat test result is not in keeping with the clinical phenotype and/or genotype[Grade D]

6.8 What further investigations can be undertaken to diagnose cystic fibrosis?

No recommendations

7. Who has responsibility for testing and training?

7.1 Who should perform the sweat test - What skills are required and what are the training needs?

- 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 and collection of a sufficient number per annum is required to maintain competency and quality [Grade D]
- Sweat collection must be performed by fully trained and experienced personnel:-
 - training schedules should be fully documented
 - the procedure should be documented as a standard operating procedure
 - appropriate revalidation procedures should be in place [Grade C]
- Sweat collection can be undertaken by a variety of healthcare professionals [Grade C]
- Sweat analysis should be performed by qualified and experienced biomedical scientists or clinical scientists who are fully trained with regular validation:-
 - training and validation schedules should be fully documented [Grade C]

7.2 Who has responsibility for analysis and training?

- A consultant clinical scientist or consultant chemical pathologist should have responsibility for training, assessment of competence and revalidation for all staff undertaking sweat tests [Grade C]
- The responsibilities 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 [Grade C].

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 ⁽¹⁾. It is less common in the American black population (1:15,000) ⁽²⁾ and rare in Oriental populations (1:90,000) ⁽³⁾. The incidence in the Asian population is less well known but probably around 1:10,000 ⁽⁴⁾.

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 1800 mutations and polymorphisms at this locus are associated with CF and other CFTR related disorders ⁽¹⁵⁴⁾.

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 give an intermediate result, but is usually helpful in making a diagnosis ^(5,6). 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 ^(7,8,9).

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⁽¹⁰⁾.

Particular concerns were lack of appropriate quality control for analytical methods, a substantial number of laboratories measuring sodium alone, variability in reference intervals, 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. These were produced in 2003 and published by the RCPCH and ACB ^(150, 151 152 153).

The Need for Review

The first guidelines relating to sweat testing for cystic fibrosis were published in 2003⁽¹⁴¹⁾, the culmination of a process started during 2000. Since 2003 there has been global expansion of newborn screening for cystic fibrosis and it was anticipated a wealth of evidence-based literature becomes available as a result of this. Implementation of technology has also advanced with the introduction of conductivity measurement at a number of centres and therefore requires more detailed comment and review.

The 2003 guidelines were formulated based on literature review, but also relied on expert opinion. Users of the guideline were encouraged to critically review the content, but also to undertake local audit in order to increase the evidence base for a later review ⁽¹⁵¹⁾. As part of the RCPCH endorsement process certain shortcomings were identified, including the narrow computerised search criteria (only Medline was searched), a lack of published evidence being identified and consequently recommendations frequently being consensus from expert committee and/or clinical experience, and finally the lack of parent/patient representation during the guideline development process. The guideline suggested a review date of 2005 in order that these issues were addressed. This second version of the guidelines was not started until 2008 with finalisation of recommendations during 2014.

Issues raised above have been addressed within the guideline together with the expansion of the Working Group to include other professional bodies that play a key role in sweat testing, for example, IBMS and CF Nurses.

OBJECTIVE

To review and update the 2003 guidelines on how to perform the sweat test for the investigation of cystic fibrosis in the UK.

SCOPE

The guideline review covers the following aspects of the sweat test and although in the main is unchanged from the original has been extended to include use in newborn screening. The guideline applies to subjects of all ages from neonates, through infancy, childhood and adulthood. However, the guideline is primarily geared toward the paediatric population, where the majority of testing is undertaken.

What patient information needs to be provided?

Which patients are suitable to a have sweat test?

- physiology
- clinical state
- exclusions/restrictions
- newborn screening

How should sweat be collected?

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

How should sweat be analysed?

- pre-analytical factors
- weighing
- elution
- analytes
- analytical methods
- reporting

What are the quality requirements for a sweat testing service?

- internal quality control
- external quality assessment
- audit

What reference values and interpretive comments should be used?

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

Who has responsibility for testing and training?

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

GUIDELINE PRESENTATION CONVENTION

The guideline is presented such that evidence statements comprise the main body of text with the overall level of evidence included in parentheses (Evidence level 1++ - 4). Overall evidence level is arrived at following review of all evidence on an individual basis and consequent recording of an evidence level reflective of all evidence available. Classification is based on SIGN 2000⁽¹³⁷⁾ and is further detailed in Grading of Evidence and Recommendations.

All recommendations are presented in bold font in text boxes following evidence statements together with grading in capital letter in parentheses (Grade A-D) based on the evidence levels from which the recommendation is taken. Classification is based on SIGN 2000⁽¹³⁷⁾ and is further detailed in Grading of Evidence and Recommendations.

1. What patient information needs to be provided?

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 survey of parents preferences regarding counselling at the time of infants' sweat test in the newborn screening process for cystic fibrosis found that they valued the informational brochure as a resource⁽¹¹⁾. Another survey of parents given an information leaflet prior to a sweat test, in a general paediatric outpatient setting, showed parents valued an information leaflet⁽¹⁰⁾. It is important for parents to have the opportunity to ask questions after they had been given the information leaflet (Evidence level 3).

A National Audit of the UK Sweat Testing Guidelines was carried out in February 2006⁽¹²⁾. 73 laboratories returned questionnaires; 59 of these claimed compliance with the guideline of providing appropriate pre-test information.

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 assimilate 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 4)

The parents of children identified as 'CF suspected' through screening should be given the specific 'Cystic Fibrosis is Suspected' leaflet developed by the UKNSPC (UK Newborn Screening Programme Centre) which includes information about the sweat test⁽¹⁴⁰⁾. The leaflet was prepared by the Parent Support Research Team (Institute of Education, London), working for the UK Newborn Screening Programme Centre. A meeting of the working group (November 2004) was contributed to by parents and the CF Trust.

Recommendations

- Informed consent should be obtained in accordance with local policy.
- It is good clinical practice to prepare the patient and, where appropriate, the parents effectively before testing by providing pre-test information appropriate for the individual. This 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 (appendix 1), and specifically for infants identified by newborn screening the 'UKNSPC CF Suspected' leaflet may be accessed via their website⁽¹⁴⁰⁾.

[Grade D]

2. Which patients are suitable to have a sweat test?

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.

- Preterm infants do not sweat in the first 7-14 days, but most term infants are able to sweat from the first day ⁽¹⁾(Evidence level 2+).
- In term infants sweat sodium and chloride can be high in the first 7 days, particularly in the first 48 hours ⁽²⁾(Evidence level 3).
- There is a high likelihood of a successful sweat test in infants <6 weeks of age unless of African-American race or weighing less than 2 Kgs ^(3, 4), although insufficient sweat collection in infants <2.5Kg has been reported ⁽¹⁵⁶⁾(Evidence level 2+).
- 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 4).
- Sweat electrolytes can be elevated in dehydrated infants and children and lowered in infants on systemic 9 α fludrocortisone or with oedema ^(5,6) (Evidence level 3)
- Topiramate has been shown to decrease sweat production and may lead to falsely elevated sweat chloride concentration ^(158, 159, 165) (Evidence level 3)
- Sweat electrolytes can be elevated in underweight infants (height/weight ratio <75%) and children secondary to malnutrition (<3rd centile weight and <10th centile height)^(143,144,145) or psychosocial failure to thrive ^(145,146)(Evidence level 3)
- Sweat electrolytes can be elevated in subjects if the stimulation site has active eczema ⁽⁷⁾(Evidence level 3)

- Sweat electrolytes are not affected by diuretics or intravenous fluids as long as the patient is stable ⁽⁵⁾ (Evidence level 4).

Recommendations

- Sweat tests can be performed after 2 weeks of age in infants greater than 2 Kgs at time of testing who are normally hydrated and without systemic illness [Grade C]
- 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 [Grade C]
- Sweat tests should be delayed in subjects who are oedematous or under topiramate treatment or receiving systemic 9 α fludrocortisone [Grade D]
- Sweat tests should be delayed in subjects who are dehydrated, underweight, systemically unwell or who have eczema affecting the potential stimulation sites where practicable [Grade D]

See also Section 6.6

Sweat electrolytes are not affected in subjects on oral Flucloxacillin. There are no data for other antibiotics ⁽⁸⁾ (Evidence level 2+)

Recommendation

- Sweat tests can be performed in subjects on Flucloxacillin [Grade C]

CLSI guidelines ^(9, 26) state 'iontophoresis should not be performed on a patient receiving oxygen by an open delivery system due to a remote risk of spark causing explosion. This does not include patients receiving oxygen by face mask or nasal prong.

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 4).

Recommendations

Sweat tests should not be performed in subjects who are on oxygen by an open delivery system (including headbox). This would not apply to an infant on nasal prong or face mask oxygen [Grade D]

2.2 When should sweat tests be done as part of newborn screening (NBS)?

The sweat test is a key investigation for confirmation of the diagnosis of CF after a positive newborn screening test, as evidenced by Massie, Clements et al in review of the Australasian CF newborn screening programme ⁽¹³⁾. In this programme infants are classed as having a positive screening result if IRT is >99th centile and 1 or 2 CFTR mutations are identified. Sweat test analysis is used to further classify infants having 1 CFTR mutation as being either carriers or confirming a diagnosis of CF.(Evidence level 2++)

The sweat test should only be performed in laboratories experienced in performing sweat tests in infants under 3 months of age (Evidence level 4)

All infants identified in a NBS programme should have a sweat test result available at first consultation, especially those with only 1 mutation identified ⁽¹⁴⁾ (Evidence level 4).

Recommendations

- Sweat testing is a key investigation for confirmation of CF after a positive newborn screening test [Grade C]
- Sweat testing following newborn screening should only be undertaken in laboratories experienced in performing tests in patients less than 3 months of age [Grade D]
- Sweat testing results should be available at first consultation for infants identified in NBS programme [Grade D]

3. How should sweat be collected?

The following sections give recommendations for practice and the evidence statements on which they are based.

3.1 Which sites should be used for sweat collection?

The UK laboratory subgroup confirmed that the majority of testing centres use the flexor surface of the forearm as the sweat collection site ⁽¹⁵⁾. 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 chest and thigh are used successfully by some centres ⁽¹⁶⁾. (Evidence level 4)

Recommendations

- The flexor surface of either forearm is the preferred site for sweat collection. Consideration may be given to other sites, e.g. upper arm or thigh, if both arms are eczematous, too small or otherwise unsuitable. [Grade D]

3.1.1 How can sweat contamination be avoided?

The Clinical and Laboratory Standards Institute (CLSI) guidelines ⁽⁹⁾ 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 4).

The use of topical local anaesthetic gel (e.g. 4% amethocaine gel (Ametop)) has been reported as causing contamination in sweat collections ⁽¹⁷⁾. The use of any solution that contains chloride ions (e.g. Chlorhexidine) should be avoided (Evidence level 4).

Stringent measures should be put in place to avoid contamination at all stages of the sweat collection. The use of topical anaesthetic gels is not recommended.

Recommendations

- Great care must be taken at all stages of the procedure to avoid contamination (see Appendices for example Standard Operating Procedures SOP). Do not use chloride-containing solutions to clean the skin, nor apply local anaesthetic gel [Grade D]

3.1.2 How many sweat collections should be made?

A few UK centres always or sometimes carry out two separate collections in response to a request for a sweat test ⁽¹⁶⁾; this is also recommended in the Cystic Fibrosis Foundation Guidelines ⁽¹⁸⁾. The CLSI guidelines⁽⁹⁾ suggest that bilateral testing is useful. 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. A Bland Altman difference plot ⁽¹⁵⁵⁾ showed no bias between collections from different arms, and a confidence interval for 95% limits of agreement for chloride of 20 mmol/L ^(15, 16). Two other studies have also examined the necessity for performing two separate collections ^(19, 149)(Evidence level 2+) and found bilateral testing to reduce failure rate with little impact on cost but a reduction in repeat visits required, however stimulation should be sequential whilst collection may be simultaneous. Testing in duplicate will not identify systematic or contamination errors affecting reagents or equipment in use on that day (Evidence level 4).

Recommendations

- Carrying out the sweat test procedure by bilateral sequential stimulation with simultaneous sweat collection does not significantly increase time taken nor discomfort to the patient but decreases failure rate [Grade C]
- In response to a sweat test request it is sufficient to carry out one sweat collection only unless results suggest an abnormality in the collection or testing is suspected [Grade D]

3.2.1 What power supply and which electrodes should be used?

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.

It is recommended that electrodes should never cross the trunk, and that the right arm should be used rather than the left ⁽²¹⁾. There should be a safety cut-out to prevent increase of current if skin resistance drops, e.g. as a result of blistering ^(21, 22). Circuit diagrams have been published ⁽²¹⁾, some of which incorporate a safety circuit ⁽²³⁾. In house manufacture by Medical Physics or an electrician's workshop without independent testing is unlikely to be considered to meet acceptable safety standards. Wescor® systems incorporate both a failsafe current cut-out, and a high resistance cut-out ^(24, 25), as does the C&S Electronics power supply ⁽²⁶⁾(Evidence level 4).

Clinical Pathology Accreditation (UK) Ltd (CPA) standards ⁽²⁷⁾state that: Work areas shall be clean, uncluttered and well maintained and there shall be evidence of good housekeeping procedures (C5). In addition, there should be sufficient safety notices and labelling of the laboratory environment such that staff are aware of the risks and safe practice required. Standard operating procedures for the preventative maintenance, service and repair of equipment (Standard D1.2) should be available. The department must have a procedure for reporting adverse incident (and vigilance reporting) relating to medical devices to the appropriate authority (Evidence level 4).

In addition, the operator should carry out a visual check of the power supply for any damage or malfunction at each use. Electrical safety of all power supplies must be regularly tested by an electrician and records kept. The frequency of testing should comply with local risk assessment as part of a preventative maintenance policy ⁽¹⁴⁷⁾.

Electrodes have been made from a variety of materials. Schwarz showed penetration of electrode metal ions into the pads during iontophoresis⁽²²⁾. This was of most concern for lead and copper but could be minimised by increasing the electrolyte pad thickness (Evidence level 3)

Electrodes should be cleaned between each iontophoresis procedure. Wescor recommend the use of purified water for Macroduct® electrodes, and if necessary the use of a light duty cleaning pad to buff the electrodes – steel wool, sandpaper or emery cloth must not be used ⁽²⁵⁾.

Recommendations

- The power supply used must be battery powered and should include a safety cut-out that limits the amount of current at 5mA or lower..
Monitoring of the current must be carried out throughout iontophoresis where possible. Wescor® systems without an ammeter are current-limited with an appropriate safety cut-out system.
The operator should carry out a visual check of the power supply for any damage or malfunction at each use. Electrical safety of all power supplies must be regularly tested by an electrician and records kept [Grade D]
- Electrodes should be of a suitable size and curvature to fit snugly on the patient's limb:
 - they should be firmly secured in position but not too tightly secured in order that circulation is not restricted.
 - they must be regularly cleaned and inspected, and discarded if they show pitting or irregularities [Grade D]
- Selection of new equipment and maintenance of existing equipment must comply with CPA Accreditation (or equivalent standard) [Grade D]

3.2.2 Which Electrolyte Solutions can be used?

Price ⁽²⁸⁾ showed that increasing the pilocarpine nitrate concentration from 0.5-5.0 g/L increased the sweat yield – being lower at 0 and 0.5g/L , but plateauing at >1g/L. Conversely, the chloride concentration was thought to show an acceptable plateau concentration using pilocarpine at 0.5g/L, below this point chloride concentration was very much higher. (Evidence level 3)

Pilocarpine nitrate is universally used as the pilocarpine source at the anode, replacing pilocarpine hydrochloride ⁽²⁹⁾ in earlier literature. Concentrations used vary from 0.64 g/L ⁽²⁹⁾ to 15 g/L ⁽³⁰⁾, with a single case report at 100 g/L ⁽³¹⁾.(Evidence level 4).

The cathode electrolyte solution is arbitrary, serving to complete the electrical circuit ^(22, 29, 32, 33, 34). All Wescor Pilogel™ systems use the same pilocarpine nitrate electrolyte at both anode and cathode ^(30, 35). 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 4)

The use of alkaline solutions such as sodium bicarbonate was demonstrated to lessen the likelihood of acid burns ^(22, 28) (Evidence level 3)

Respondents to a survey in the UK ⁽³⁶⁾ used 2-5 g/L pilocarpine nitrate on gauze, lint or filter paper, or 5 g/L pilocarpine nitrate on gel discs. As for the cathode electrolyte, virtually all used varying concentrations of magnesium sulphate (from 0.05 to 2.0 mol/L) or pilocarpine nitrate, as for the anode (Evidence level 4)

Recommendations

- 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 [Grade D]
- Solutions containing chloride must not be used because of the risk of contamination of the collection [Grade D]
- Unbuffered acid solutions should not be used because of the increased risk of burns [Grade C]

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,
or
2. obtained entirely separately. This is the case for pad absorption sweat collection systems. The manufacturer of the power packs for iontophoresis 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')⁽³⁷⁾. They may only be supplied under strictly limited conditions, by manufacturers who hold a 'specials' licence⁽³⁸⁾. These include a number of hospital pharmacy department classed as production pharmacies as well as commercial companies. A full list of contact details is published regularly in the BNF⁽³⁹⁾.

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 4)

Recommendations

- Electrolytes used for iontophoresis must either be obtained as part of a medical device (e.g. WescorPilogel™ discs) or from a recognised manufacturer of unlicensed medical products. Solutions must NOT be produced in-house by hospital laboratories [Grade D]

3.2.3 What current and duration should used for Iontophoresis?

The earliest iontophoresis reference⁽³²⁾ specified 2 mA/4.9cm², (after a slow increase) for 5 min if filter paper supports used, 15 min if gauze used. Other early papers used 2-5 mA for up to 15 minutes, without investigating the effect of variation^(21, 22, 40, 29, 33, 41). The first Webster sweat collection system model 3500

^(24, 34) used gauze pads and suggested 1.5 mA for 5 min after a slow rise, from a power supply capable of settings from 1-5 mA (Evidence level 3)

Webster ⁽⁴²⁾ 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 1.5 mA increased sweat production rate, however further increase of iontophoresis time from 3-7 min produced no further increase in sweat rate. Kirk ⁽⁴³⁾ demonstrated that using a current of 1, 2 or 4mA had no effect on sodium or osmolality results (Evidence level 3)

Survey of practice in 1998-9 of 30 UK laboratories ⁽³⁶⁾ identified the use of currents of 1.5 to 4 mA applied for 4-10 minutes. Only 2 centres used >5.5 minutes.

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 4)

The WescorMacroduct® instruction manuals state: With the introduction of Pilogel™ pilocarpine discs ⁽³⁵⁾ the Wescor® model 3700 power supply is set to deliver 1.5 mA for 5 min ⁽²⁵⁾. Operator variation is not possible (Evidence level 4)

The WescorNanoduct®

The WescorNanoduct® system ^(30, 44, 45, 46, 47) uses a nominal current of 0.3 mA for 2.5 min, which is applied using smaller electrodes, and increases the pilocarpine concentration threefold in buffered citrate gel⁽¹³⁸⁾. The system is designed to measure sweat conductivity *in situ* following pilocarpine iontophoresis using a disposable conductivity sensor cell (Evidence level 4)

Recommendations

- When aqueous electrolyte solutions are applied on pad supports a minimum current of 1.5 mA should be applied, and increased gradually up to a maximum of 4 mA. Once the maximum is attained the current should be maintained for a minimum of 3 minutes and a maximum of 5 minutes [Grade D]
- When Wescor® systems are used, the manufacturer's current and time recommendations should be followed. This will depend on the specific model used and is automatically controlled by all current models[Grade D]
- For Wescor® and C & IS Electronics Gibson – Cooke Power Supply systems the patient must be kept under close observation throughout the iontophoresis period [Grade D]
- The WescorNanoduct® is not recommended for stimulation and sweat analysis [Grade D]

3.2.4 What safety issues need to be considered during iontophoresis?

1. The definitive paper by Schwarz ⁽²²⁾ examined hazards in detail. The paper describes a series of experiments that assessed factors that predisposed to blisters and burns. Schwarz showed that atropine injection produced an oedematous swollen area and reduced skin resistance by a factor of 40-100. Iontophoresis at 4 mA for 5 min to introduce atropine into the skin resulted in pain, pitting and oedematous swelling. 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.
 - ◆ The lowest pH and worst blisters were found with unbuffered hydrochloric acid at concentrations down to 0.01 mmol/L. Using 4.5 g/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 4 mA for 5 min.

- ◆ Fewer filter papers led to more blistering
- ◆ Dryer filter papers led to more blistering

(Evidence level 2+)

The original iontophoresis paper ⁽³²⁾ refers to the rare occurrence of burns, attributed to skin-electrode contact. A large-scale survey ⁽²⁹⁾ of 7,200 tests carried out by an experienced technician reported superficial burns at the cathode at a rate of <1:200 (Evidence level 3)

2. Reports of adverse effects are all small number studies

a. One anecdotal report ⁽⁴⁸⁾ details a burn suffered during a sweat test carried out by an inexperienced SHO without monitoring the patient during iontophoresis (Evidence level 3)

b. Rattenbury⁽⁴⁹⁾ 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. A questionnaire returned by 6/10 paediatric labs indicated that all had seen burns at some time. Skin reddening was seen frequently (Evidence level 3)

c. Two incidents ⁽⁵⁰⁾ 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⁽⁵¹⁾.(Evidence level 3)

3. The UK surveys ⁽³⁶⁾ 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 ^(52, 53).(Evidence level 3)

4. There is a theoretical risk of atrial fibrillation but this has never been documented (Evidence level 4)

Burns or blisters are sporadically reported resulting from electrode-skin contact or inadequate reservoir of electrolyte solution between skin and electrode. 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.

Due to the reddening of the skin that occurs during the sweat collection procedure and the rare reports of burns in patients (outlined above) patients should be advised regarding the small risks involved prior to giving consent for the sweat test to be undertaken. Such information may be included in a patient information sheet (See Appendix 1 – The Sweat Test: What it is and how is it performed?)

Recommendations

- Suitably thick pads must be used for the electrolyte solutions to minimise the risk of acid burns [Grade C]
- Hybrid systems, e.g. Wescor® power supplies or electrodes with aqueous electrolyte solutions, or Wescor® gel discs used with non-Wescor electrodes, should not be used as this may lead to injury [Grade D]

3.3 Which collection medium should be used and how long should sweat be collected for?

Sweat must be collected in sufficient quantity for accurate and precise analysis. During collection it must be protected from contamination and evaporation (see example SOPs in Appendices 2a and 2b)(Evidence level 4).

3.3.1 Which collection medium should be used?

1. Gibson and Cooke ⁽³²⁾ described collection of sweat onto filter paper circles 2.5 cm in diameter or gauze 3x3 inch squares. Concern about possible sodium chloride contamination led some authors to wash and dry filter papers or gauze before use ⁽⁴¹⁾(Evidence level 2-)

2. 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 4)

Recommendations

- Sweat should be collected onto pre-weighed chloride free filter paper or gauze of approximately equal size to the stimulated area (i.e. the lint pads used in iontophoresis) or into Wescor Macroduct® disposable collectors [Grade D]
- Filter paper or gauze 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 [Grade D]
- During collection by any method, sweat must be protected from contamination, as well as condensation and evaporation (see example SOPs in Appendices 2a and 2b) [Grade D]

3.3.2 How long should sweat be collected for?

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

1. Differential sweat collections have been carried out by collecting for 5 min periods onto different filter papers ⁽²⁹⁾, collecting onto Macroduct® ⁽⁵⁴⁾ or other tubing ⁽⁵⁵⁾ and sectioning the tubing, or measuring conductivity continuously ⁽³⁰⁾. 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 2+).
2. Osmolality or conductivity measurements have shown that sweat concentration decreases in tandem with sweat secretion rate ^(28, 30, 34, 54, 55, 56) (Evidence level 2+)
3. Decreasing sweat collection time from 60 to 30 minutes leads to a statistically insignificant decrease in the mean weight of sweat collected from 520 to 490 mg ⁽¹⁶⁾. Extending the sweat collection time beyond 30 minutes will produce little, if any, additional weight/volume (Evidence level 3)

4. Measurements in situ (Orion electrode) should not be used ⁽⁵⁷⁾ (Evidence level 2+)

Recommendations

- Sweat should be collected for not less than 20 minutes (unless Macroduct tubing is full earlier) and not more than 30 minutes [Grade C]

3.3.3 How should the Macroduct sample be removed?

A common cause of failed collection by Macroduct® system is incorrect technique in removing the Macroduct from the patients arm. The manufacturer advises collections with Macroduct should have the free end of the tubing stopped with a syringe or clamp and then cutting the attachment to the Macroduct. Attempts to remove the Macroduct from the patient's arm with the tubing still coiled on the collector usually lead to loss of the sample (Evidence level 4).

Recommendations

- Collections with Macroduct should be removed by stopping the free end of the tubing with a syringe or clamp and cutting the attachment to the Macroduct [Grade D]

Addendum: Availability of replacement equipment in UK:

The formerly widely used Electro Medical Supplies power supply is no longer manufactured. Suppliers of a battery powered, current limited 'Gibson-Cooke' power supply are C&S Electronics Inc, 2565 16th Ave, Columbus, NE 68601⁽⁵⁸⁾ - this is widely used in the USA, and Sherwood Scientific Ltd, 1 The Paddocks, Cherry Hinton Road, Cambridge, CB1 8DH UK

Wescor® systems are supplied in the UK by Chemlab Scientific Products Ltd., Laindon, Essex, SS15 6TQ.

4. How should sweat be analysed?

4.1 Which pre analytical aspects need to be considered?

4.1.1. How should samples be stored before analysis?

Evaporation of sweat during collection, transfer and transport is a potential source of error in the sweat test⁽⁹⁾.

- Sweat samples collected on filter paper, reweighed and secured in a vial with a tightly fitting lid, are stable for up to 72 hours at 4°C, with or without diluent⁽⁵⁹⁾ (Evidence level 2+)
- Storage times and conditions should be validated individually by each laboratory⁽⁶⁰⁾ (Evidence level 4)
- Liquid sweat is also stable for up to 72 hours at 4°C, if stored in heat sealed Macroduct® tubing⁽⁶¹⁾, but not at room temperature where evaporation will occur within 48 hours (Evidence level 3)
- Liquid sweat is stable in 200µl PCR tubes with dome caps for up to 72 hours refrigerated (3-7°C) or heated (35-39°C)⁽¹⁴⁸⁾ (Evidence level 2-)
- Liquid sweat can also be stored in 100µl capillary tubes sealed with plasticine, provided an air gap is left between the sweat and plasticine, for up to 6 hours⁽⁶²⁾ (Evidence level 4)

Recommendations

- Throughout sweat collection, transport and analysis, every effort should be made to minimise evaporation of the sample [Grade D]
- 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 [Grade C]
- Liquid sweat from Macroduct® collections can be stored in either sealed Macroduct® tubing or capped PCR tubes 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. [Grade D]
- Storage of liquid sweat for up to 72 hours at higher temperatures should be in capped PCR tubes [Grade C]
- Sweat may be collected at remote sites and transported to the laboratory for analysis provided there is attention to storage details [Grade D]

4.1.2. How should sweat weight/volume be determined?

In order to accurately weigh sweat to the nearest milligram, a balance sensitive to 0.0001g is required as recommended by CLSI⁽⁹⁾. 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. Always use powder free gloves (Evidence level 4)

Recommendations

- The same balance must be used throughout [Grade D]
- A balance sensitive to 0.0001g must be used to weigh sweat [Grade D]
- Sweat collections onto paper pads should be weighed and analysed as soon as practicable [Grade D]

4.1.3 What is an adequate sample?

A minimum sweat secretion rate of $1\text{g}/\text{m}^2/\text{min}$ averaged over the collection period is considered adequate⁽⁹⁾, where m^2 = area of collection site. 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 sweat has been lost by leakage or evaporation⁽¹⁵⁾. Goldberg et al⁽¹⁶¹⁾ identified discordant sweat conductivity between paired sweat collections where one sample was $<15\mu\text{l}$ and the other $>15\mu\text{l}$ (a volume that equated to the minimum recommended sweat secretion rate) such that mean conductivity was greater in the samples $<15\mu\text{l}$. The study also showed that although 97% of sweat chloride concentrations were comparable (within 1.96 SD of mean), irrespective of volume, sweat chloride concentration was significantly higher in the low volume samples (28.4 ± 15.7 vs $25.1\pm 15.2\text{mmol/L}$). See Appendix 3 for calculation of average sweat rate over the collection period (Evidence level 2-)

Recommendations

- The sweat secretion rate measured as an average rate over the collection period should not be less than 1g/ m²/min. Collections below this rate should not be analysed. Insufficient sweat collections must not be pooled. The full sweat test should be repeated [Grade D]

4.2 How should the sweat sample be prepared for analysis?

4.2.1 How should sweat be eluted from filter paper?

The most common time period allowed for elution is 30 minutes ⁽⁵²⁾, but more recent data ⁽⁶³⁾ shows elution for any length of time, from 1 minute up to 180 minutes, to have no effect on chloride concentration (Evidence level 2+). Before any analysis takes place, the sample must be homogenised and mixed thoroughly (Evidence level 4)

4.2.2 How should sweat be prepared when collected by the Macroduct® system?

Sweat collected using the WescorMacroduct® system should be carefully expelled and mixed prior to analysis to ensure consistent measurements (Evidence level 4)

Recommendations

- **When sweat is collected onto filter paper (section 3.2.1) elution time should be greater than 1min and less than 3 hours [Grade C]**
- **Samples from filter paper should be homogenised and mixed thoroughly before analysis [Grade D]**
- **Sweat collected using the WescorMacroduct® system should be carefully expelled and mixed prior to analysis [Grade D]**

4.3 Which analytes should be measured in sweat for diagnosis of CF?

4.3.1 Should chloride concentration be measured?

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 ^(64, 65, 66), and is recommended as the analyte of choice. ^(9, 60)(Evidence level 2++)

4.3.2. Should sodium, potassium and/or osmolality be measured?

As these analytes do not discriminate as well as chloride between control and CF populations ^(67, 68, 69, 70, 71, 72, 115), their use is no longer recommended (Evidence level 2-).

4.3.3. Should conductivity be measured?

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 ⁽⁷³⁾ provides evidence of better-between-laboratory agreement for conductivity measurements compared to chloride (section 5.4) (Evidence level 3)

The US Cystic Fibrosis Foundation advises that it is not appropriate to perform the sweat test using conductivity⁽⁷⁴⁾. The CLSI guidelines ⁽⁹⁾only accept conductivity measurement as a 'screening test' as does the Welsh Standard ⁽⁷⁵⁾and this view is supported by a number of studies^(76, 77).(Evidence level 2+)

Although it has been suggested that conductivity could be used for diagnosis ⁽⁴⁴⁾, and there is evidence that conductivity, when combined with genotype analysis, may be as reliable as chloride for diagnosis ⁽⁷⁸⁾

Conductivity measurement, when associated with chloride concentration has been found to be useful in cases that are not absolutely clear cut ⁽⁷⁹⁾(Evidence level 3)

Recommendations

- Chloride is the analyte of choice [Grade B]
- Sweat conductivity measurement alone is not an adequate diagnostic test for the investigation of CF. Conductivity may be used as a screening test [Grade D]
- In infants under the age of 6 months, sweat chloride concentration must be measured even if conductivity levels are normal (due to the limited data available in this cohort) [Grade D]
- In infants and children over the age of 6 months, chloride concentration must be measured if borderline or positive conductivity levels are obtained [Grade D]
- Sweat sodium/potassium/osmolality measurement are not recommended [Grade D]

4.4 What methodology should be used for measurement?

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

4.4.1 What methods are available for Chloride measurement?

The following methods are available for sweat chloride.

- a. Coulometry⁽⁸⁰⁾
- b. Indirect Ion Selective Electrode^(81, 82)
- c. Direct Ion Selective Electrode (ISE). A small number of laboratories report in the UK NEQAS ⁽⁷³⁾ using a variety of methods which they classify as 'Direct ISE'. There is insufficient data to assess the performance of these methods.
- d. Colorimetry^(83, 71)
- e. Mercurimetric determination ⁽⁸⁴⁾

Other methods have been described in literature⁽¹⁴²⁾ but have not progressed to routine use.

All methods must be fully validated before use. Where sufficient sample is available, duplicate analytical measurements of chloride should be made. A recent study reported on a comparison of sweat chloride analysed by ISE and coulometry and showed them to have comparable results ⁽⁸⁵⁾. Whichever method

is used, it should be able to detect sweat chloride at the lower end of the reference interval (10 mmol/L, CV <20%)⁽⁶⁰⁾ (Evidence level 4).

Recommendations

- Colorimetry, coulometry and ISEs are satisfactory methods for analysis of sweat chloride. Where possible, duplicate analytical measurements of chloride should be undertaken [Grade D]

4.4.2 What methods are available for Conductivity measurement?

Measurement of sweat conductivity in the UK is primarily users of WescorMacroduct® equipment, who achieve CV <3% at all concentrations of electrolyte circulated in the UK NEQAS Sweat Testing Scheme⁽⁷³⁾

Sweat collected using the WescorMacroduct® system should be expelled and mixed prior to analysis using the Wescor Sweat Cheker™ to ensure consistent measurements (Evidence level 4).

The Nanoduct® system is not currently recommended for diagnosis as there is insufficient evidence to support its use^(60, 46) (Evidence level 3). Once an adequate sweat rate has been achieved, as little as 3 µL of sweat are required for analysis, the reportable result being derived from a 5 minute averaging sequence following an initial stabilisation period.

Barben⁽⁴⁴⁾ measured sweat conductivity using the Nanoduct® in 94 subjects, comparing it with the Macroduct® collection system and chloride measurement. Using the Nanoduct®, they correctly identified 20 patients known to have classical CF, differentiating them from healthy controls (n = 73). They had fewer insufficient collections than with the Macroduct® system. However, the mean difference between conductivity and chloride concentrations in the patients with classical CF was lower than expected (i.e. ~7 mmol/L rather than the ~15 mmol/L that would be anticipated, since conductivity is a measure of all ions present, not just chloride). This finding was alluded to in the discussion but no explanation

was given (Evidence level 2-). A further paper ⁽⁴⁷⁾ extended their study to 1041 patients across three different sites, using the Nanoduct® as the primary testing device, with conductivity results of > 50 mmol/L being confirmed with a sweat chloride analysis (using the Macroduct® system for collection). The authors noted a high failure rate for the Nanoduct® in newborns (~50%)(Evidence level 2-)

A more recent study on 487 infants ⁽¹⁶²⁾ compared sweat testing by Gibson and Cook method and Nanoduct® as part of a New Born Screening programme. The study found that Nanoduct® conductivity discriminated equally well between CF and CF-patients as sweat chloride concentration by Gibson and Cook method.

Losty⁽⁴⁶⁾ obtained an unacceptably high false negative rate when using the Nanoduct® to measure conductivity in sweat samples from 100 subjects (36 with classical CF, 6 with non-classical CF, 58 without CF). They observed a negative bias between conductivity measured with the Nanoduct® and conductivity measured following Macroduct® collection, and also between Nanoduct® conductivity and sweat chloride results. The authors criticised the lack of an external mechanism for verifying the accuracy of the Nanoduct® sensors; the high false negative rate and unexpected negative bias of conductivity compared with chloride results in sweat samples from patients with CF was found to be owing to a fault with a batch of sensors (Evidence level 2+)

In conclusion, there are limited data available comparing Nanoduct® conductivity directly with Macroduct® conductivity; data that are available indicate that conductivities obtained using the Nanoduct® system can be negatively biased compared with Macroduct® conductivity values and chloride values. Until further data are available or these findings are explained satisfactorily, it is not possible to recommend the use of the Nanoduct® as part of the diagnosis of CF.

Recommendations

- Conductivity measurement using the Wescor Sweat-Chek™ equipment is a satisfactory method of analysis [Grade D]
- The WescorNanoduct® is not currently recommended for routine sweat collection and analysis [Grade D]

4.5 What format should the report take?

A report format is detailed in the CPA standards ⁽²⁷⁾, with some additional comments specific to sweat test reporting in the Welsh Consensus Guidelines ^(75, 27) which form the basis of the recommendations for reporting (Evidence level 4).

Recommendations

The report format should include:-

- i. Full patient identification
Requester and delivery address
- ii. Date and time of test and date and time of report
- iii. Analytical results (mmol/L)
It should be explicit on the report form which analyte(s) have been measured.
i.e. chloride
conductivity (sodium chloride equivalent)
Reason if no result is available/performed
- iv. Reference interval (see section 6)
- v. Interpretation of results (see section 6)

[Grade D]

5. What are the quality requirements for a sweat testing service?

There are published reports ^(86, 87, 88, 89, 90) 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 ⁽⁹¹⁾. 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, reporting ⁽¹⁰¹⁾ 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 and conductivity measurements.

5.1 Should sweat be analysed if contamination or evaporation are suspected?

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 4)

Recommendations

- Sweat which has been subject to evaporation and/or contamination must not be measured. [Grade D]

5.2 What considerations should be given when selecting analytical methods?

Suitable methods (see section 4.4) should be used which enable measurement of analytes in the concentration ranges likely to be encountered for both normals and subjects with cystic fibrosis (i.e. for chloride 0-150 mmol/L). The lower limit of detection for chloride 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) ⁽²⁷⁾. The SOP should include the analytical method(s), quality procedures, reporting, interpretation and all safety aspects (Evidence level 4).

Recommendations

- The analytical range of the methods used must cover the concentration ranges found in normals and subjects with cystic fibrosis [Grade D]
- The analytical methods must be fully documented as standard operating procedures (SOPs) to comply with Clinical Pathology Accreditation (or equivalent standard).

5.3 What Internal Quality Control (IQC) should be used?

When analysing sweat collected onto paper or gauze, QC material should be treated in the same way. If undiluted sweat (i.e. Wescor System®) is used, then it is acceptable to directly analyse the QC solutions. (Evidence level 4)

CPA Standards and Guidelines state that quantitative analyses require multiple levels of internal quality control ⁽²⁷⁾. Quality control materials should be at clinically important concentrations and should differ from standards.

Acceptable limits for each analyte should be established for each QC material (evidence level 4).

An audit of 73 UK labs against the 2003 guidelines carried out in 2006 ⁽¹²⁾ showed that only 5 labs did not comply with the recommendation for having 2 levels of IQC and only 5 labs did not achieve between batch CVs of <5% (Evidence level 3).

Recommendations

- 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 [Grade D]

5.3.1 What imprecision should be achieved for chloride measurement?

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 Macroduct® collections respectively ⁽⁷¹⁾. Coefficients of variation of 11%, 8% and 4% at chloride concentrations of 30, 60 and 120 respectively, were reported by using an ion selective electrode ⁽⁹²⁾. Heeley et al ⁽⁷⁰⁾ using a colorimetric method for chloride quoted CV% of 2.5% at a concentrations of 50 mmol/L. Using a modified mercurimetric titration method (20ul sample volume) for chloride on sweat samples collected by the Wescor Macroduct® system, between batch CVs of 2.1%, 4.7% and 9.4% were achieved at chloride levels of 75, 30 and 15 mmol/L ⁽⁸⁴⁾ (Evidence level 2+).

Recommendations

- The chloride method used should have a between batch CV of 5% (or less) at a concentration of 40-50 mmol/L [Grade C]

5.3.2 What imprecision should be achieved for conductivity measurement?

A between batch coefficient of variation of 1.0% is reported by Hammond ⁽⁹³⁾ at a concentration of 67 mmol/L NaCl equivalents.

Between-run analytical variation was calculated as 1.32% at 40 mmol/L and as 1.15% at 123 mmol/L ⁽¹⁰²⁾ (Evidence level 3).

Recommendations

- The conductivity method used should have a between batch CV of 2% (or less) at a concentration of 50 mmol/L [Grade D]

5.4 What considerations need to be given to External Quality Assessment (EQA)?

External quality assessment is essential in order to:-

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

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

The UK NEQAS for Sweat Testing commenced on a pilot basis in June 1999 ⁽⁹⁴⁾ and has developed into a full CPA Accredited monthly Scheme. Over fifty distributions of three aqueous-based specimens, covering the range of concentrations seen in the clinical setting, have been carried out to date. In addition to numerical results for Chloride, Sodium and Conductivity, the participating laboratory is also asked to make a clinical interpretation.

In February 2009 177 UK laboratories (215 in total) were performing sweat test analyses ⁽¹⁵⁷⁾. The number of UK laboratories performing each test was as follows:

159	Cl
97	Na
67	conductivity

The breakdown of test repertoires for UK laboratories was as follows:

76	-	Na and Cl
33	-	Cl only
30	-	Cl and conductivity
20	-	Na, Cl and conductivity
17	-	Conductivity only
1	-	Na only

Approximately 80% of laboratories collect sweat using the WescorMacroduct® system, with a dwindling proportion, currently at 20%, using filter paper.

Data from the Scheme shows that the measured analyte concentrations for sodium and chloride agree with the weighed in values. As expected, conductivity does not equate to chloride concentration equivalents in a simple relationship when anything other than pure sodium chloride is used to construct the specimens. Conductivity has the best between laboratory agreement with coefficients of variation (CVs) of 3% compared to chloride, 5%, at concentrations of 50 mmol/L. At lower concentrations of 25 mmol/L, the between-laboratory agreement worsens for chloride (CV 8%), while the between-laboratory agreement remains constant for Conductivity (CV of 3%).

From the outset, data from the Scheme has consistently demonstrated the far superior *analytical* performance of conductivity compared with chloride (Evidence level 3).

CPA Standards and Guidelines ⁽²⁷⁾ state that the department must participate in approved External Quality Assessment schemes corresponding to its repertoire, and evidence of satisfactory performance will be sought. The UK NEQAS has agreed limits of acceptable performance and at any one time, for Chloride, around 3% of laboratories may exhibit rolling time-window performance outside these limits. Reassuringly, only a small proportion of laboratories will go on to become “persistent poor performers”. Very rarely does any laboratory ever exceed the acceptable limits for conductivity.

Since its inception, the Scheme has observed improvement in the overall performance for all analytes (Evidence level 3).

Recommendations

- The laboratory must participate in a suitable external quality assessment scheme [Grade D]

5.5 What are the expected upper limits for chloride and conductivity concentrations?

Chloride concentrations in sweat gland fluid do not exceed 160 mmol/L⁽⁹⁵⁾ (Evidence level 3). Causes of concentrations above this include laboratory error, fabricated or illness induced by carer⁽⁹⁶⁾, and pseudohypoaldosteronism⁽⁹⁷⁾ (Evidence level 3). A working upper limit for conductivity 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.⁽⁹³⁾

Recommendations

- Results which are not physiological should be questioned, i.e. chloride > 150 mmol/L [Grade D]
- For conductivity a provisional upper limit of 170 mmol/L may be used pending further evidence [Grade D]

5.6 What are acceptable 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 percentage 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 ⁽⁹⁸⁾ an insufficient rate of 4.7% in the neonatal period, and 1.6% across all age groups. Collection systems may vary in performance. Hammond et al ⁽⁹³⁾ quoted a 0.7% failure rate for collection on to gauze or filter paper compared with 6.1% using the WescorMacoduct®, although Heeley et al ⁽⁷⁰⁾ reported only 1.4% with the latter. Denning reported a failure rate of 1.9% using iontophoresis and collection on gauze ⁽⁵⁷⁾(Evidence level 3)

Eng et al ⁽³⁾ identified predictors of successful sweat testing in pre-term and full-term infants under 6 weeks of age. African-American race, infant weight <2000g, preterm birth and gestational age<36 weeks were all associated with increased odds of failure, although with a multivariate logistic model, the only significant predictors were African-American race and post gestational age. Parad et al ⁽⁴⁾ reported a failure rate of 17% at the second week compared with a rate of between 3% and 11% between the third and eighth week of life. Beauchamp et al ⁽⁹⁹⁾ in reviewing sweat testing performance in Canadian labs, identified that insufficient sweat volume results in children <3 months occurred in a median of 18.3% of tests, whereas in the remaining population it was 4.5%. According to Le Grys⁽¹⁰⁰⁾, 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 2-).

Failure rate is reported to be reduced by sequential stimulation and simultaneous collection from both forearms ^(19, 149)with one study quoting a failure rate of 10.8% for unilateral testing but 4.1% with bilateral testing⁽¹⁴⁹⁾ (see section 3.1.2).

Audit of failure rate will identify diversion from recommendations/standards. Subsequent implementation of quality improvement processes have been successfully used to can be used to achieve significant reduction in failure rate at some centres ⁽¹⁶⁴⁾.

No evidence has been identified to suggest the number of times a failed sweat test should be repeated on an individual child, nor the time interval between repeat tests.

With regards to the WescorNanoduct®, Desax et al ⁽⁴⁷⁾ found in term infants the WescorNanoduct® was unsuccessful 45% of times during the first 4 weeks of life. In premature infants (n=14, 32-37weeks gestation) collection was unsuccessful in 69%. However, in infants >1 month failure rate was only 9.7% (n=237)(Evidence level 3).

Recommendations

- Centres should monitor repeat rates and investigate any significant increase in failure rate [Grade D]
- 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 less than 5% in children over 6 months of age [Grade D]
- In children under 6 months of age failed sweat collections should not exceed 20% of the tested population [Grade D]

5.7 How should service performance be assessed?

Quality of sweat testing can be assessed by collecting data on population means and range, percentage 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 4)

It is good clinical practice to follow up/audit all positive and intermediate results.
(Evidence level 4)

Recommendations

- Performance of sweat testing should be reviewed on a regular basis.
This should include:-
 - insufficient collections - as % of total tests
 - per operator
 - analytical failure rate (i.e. % outside accepted QC range)
 - external quality assessment performance [Grade D]
- 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 [Grade D]

6. What reference values and interpretive comments should be used?

A sweat chloride concentration of more than 60 mmol/L was considered to be consistent with a diagnosis of cystic fibrosis^(103, 104). This should however, be interpreted in the context of the patient's age and phenotype. The diagnosis was made when there was elevation of sweat chloride 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⁽¹⁰⁴⁾. 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, however the UK Newborn Screening Programme does currently require a sweat test after a positive newborn screening test, even in the presence of two CFTR mutations.

6.1 What reference values and interpretive comments should be used for sweat chloride concentrations?

A number of studies have published ranges for sweat electrolytes for individuals with and without CF. The following major studies report sweat chloride concentrations in patients with CF and in various groups of healthy controls^(105, 106, 69, 107, 68, 108, 32, 15). Many of these earlier studies were carried out prior to genotyping being available and so some control individuals could have had CF or CF related disorders. The advent of newborn screening programmes during the last decade has led to a number of publications with both sweat chloride concentration and genotype being available. The population studied is also significantly younger than previously described^(85, 109, 110, 13, 111, 112, 4, 113). Other work⁽¹¹⁴⁾ has reviewed genotype in those patients previously falling within the intermediate sweat chloride range. Data suggest a need to use age related reference intervals for the interpretation of sweat chloride concentration.

A recent survey by Mishra et al⁽¹¹⁵⁾ further examined reference intervals from age 5 years into adulthood (non –CF healthy subjects). There was an increasing median sweat chloride concentration that plateaued at approximately 20 years, but data suggested that the upper limit of the reference interval for sweat chloride

in the non-CF population at 5-9 years (n=40) was 40mmol/L, at 10-14years (n=40) was 49mmol/L, at 15-19years (n=40) was 53mmol/L, and 20years+ (n=162) was 60mmol/L. The data suggests further evaluation of age-related reference intervals in older children may be necessary. Senthilarasu et al⁽¹¹⁶⁾ have also reported preliminary data with higher sweat chloride concentrations in the adult population and supported by recent data from Traeger et al⁽¹⁶³⁾ (Evidence level 2+).

A study⁽⁷¹⁾ 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.

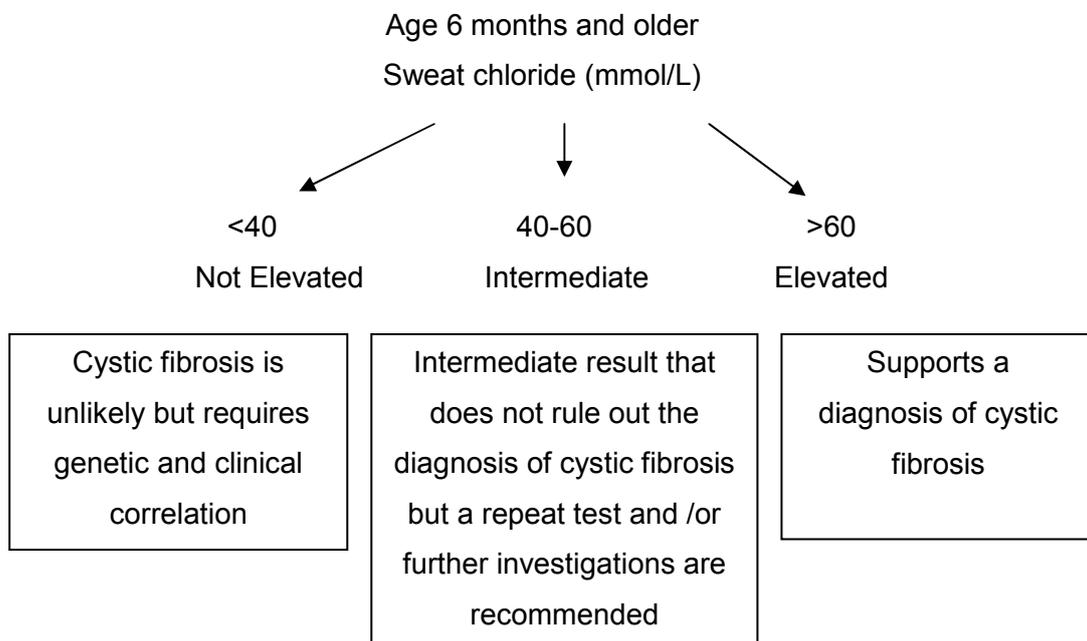
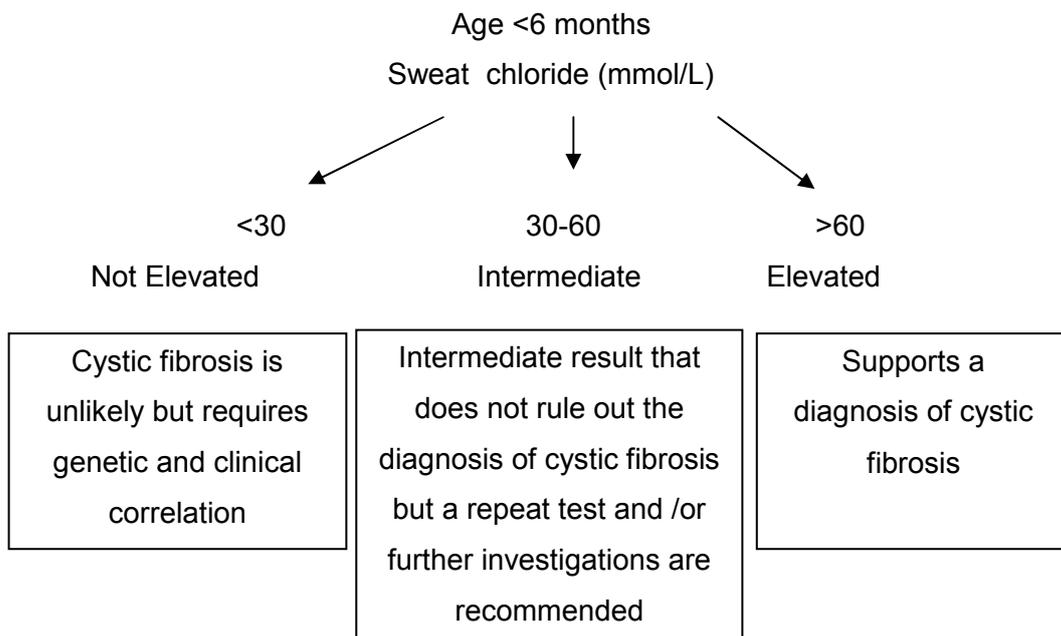
However, a newborn screening study⁽⁴⁾ showed a decrease in measured sweat chloride with age in infants with elevated IRT, 1 mutation and sweat chloride <30mmol/L (between 1 and 8 weeks post gestational age). Review of false negatives in a screening programme by Padoan et al⁽¹¹⁰⁾ identified a slight change in measured chloride concentration such that equivocal concentrations became positive – this study however did have very small numbers (Evidence level 3)

Review of data suggest the following ranges be applied for interpretation of results (see table)

Age	Sweat chloride concentration (mmol/L)	Interpretation	References
<6months	<30	Cystic fibrosis is unlikely but requires genetic and clinical correlation	13, 60, 85,112
6 months and older	<40	Cystic fibrosis is unlikely but requires genetic and clinical correlation	60, 110
<6months	30-60	Intermediate result which requires further cystic fibrosis assessment	13, 60, 85, 111, 112
6 months and older	40-60	Intermediate result which requires further cystic fibrosis assessment	60, 110
All ages	>60	Supports a diagnosis of cystic fibrosis	13

Figure 1
Schematic of sweat test chloride interpretation

Guidelines for the Performance of the Sweat Test
for the Investigation of Cystic Fibrosis in the UK v.2



Recommendations

- A sweat chloride concentration of > 60 mmol/L supports the diagnosis of CF [Grade C]
- A chloride concentration of 40 - 60 mmol/L or if less than 6 months of age 30-60 mmol/L is an intermediate result which requires further cystic fibrosis assessment such as a repeat test and /or further investigations [Grade C]
- A sweat chloride of less than 40 mmol/L (or less than 30 mmol/l in patients less than 6 months of age) makes CF unlikely but requires genetic and clinical correlation [Grade C]

6.2 What reference values and interpretive comments should be used for sweat conductivity?

Hammond ⁽⁹³⁾ carried out a large scale study comparing sodium, chloride and conductivity. 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 CLSI Guidelines ⁽⁹⁾ and the Cystic Fibrosis Foundation would equate to a chloride of 33 mmol/L and include a significant number of the normal population. The study by Heeley ⁽⁷⁰⁾ 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. 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/L⁽⁵⁸⁾. 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 ⁽¹⁰²⁾. A recent study ⁽⁷⁶⁾ comparing sweat chloride concentration and sweat conductivity in 1164 patients (age range 3days -85 years)found 62 discrepant results, these were positive for conductivity (>50mmol/L) but negative for sweat chloride (<30mmol/L or <40mmol/L dependent on age). Sensitivity for sweat conductivity was 100% at >50mmol/L,

but 5% of patients tested by conductivity alone would require additional testing. It was concluded that conductivity was acceptable as a screening test for CF but not for diagnosis. All patients positive for conductivity should undergo further testing (Evidence level 2+)

The manufacturer's manual dated 1997⁽¹³⁹⁾ 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 4)

A cut off >90mmol/L as diagnostic of CF is supported by data from Katherisan et al⁽⁷⁸⁾, Cinel et al⁽⁷⁷⁾, Lezana et al⁽¹¹⁷⁾, all quoting 100% specificity at this point (Evidence level 2+)

Recommendations

- A value below 50 mmol/L (NaCl equivalents) is unlikely to be associated with cystic fibrosis. Values above 90 mmol/L support a diagnosis of cystic fibrosis [Grade C]
- Cystic fibrosis should not be diagnosed based on conductivity measurement alone. Confirmation should be sought using sweat chloride and/or genotyping (See also Section 4.3.3) in all patients with conductivity equal or greater than 50mmol/L. [Grade C]

6.3 What is the intra-individual biological variation of sweat analytes?

6.3.1 What is the intra-individual biological variation of sweat chloride?

Few data are available regarding intra-individual variation of sweat chloride concentration. Koerbin et al⁽¹¹⁸⁾ undertook repeated sweat tests in 4 healthy adults using the WescorMacroduct® system over a period of 2 years to determine intra-individual biological variation for sweat chloride concentration. This was calculated to be >25% in 3 subjects, with two patients spanning the classification of intermediate and normal. Analytical imprecision (CV) was less than 2% (Evidence level 3). Mackay et al⁽¹⁶⁰⁾ reported a study in which sweat was collected from 2 sites

simultaneously (using Gibson Cook method, n=295), for sweat chloride concentration the overall total CV was 20.2% (between pair standard deviation 4.3mmol/L). In the vast majority of patients chloride variability did not change interpretation, in the intermediate group classification would have changed in 48% (13 patients). Analytical imprecision was reported to be 4.1% at 8.9mmol/L chloride (Evidence level 2+)

6.3.2 What is the intra-individual biological variation of sweat conductivity?

Van de Merwe et al⁽¹⁰²⁾ collected sweat by WescorMacroduct® system from 55 subjects once a week for 5 weeks. Conductivity was measured using the WescorSweatChek™ analyser. The intra individual CV(%) in healthy infants, adults and CF patients was 18%, 12% and 7.3%, respectively (Evidence level 3)

Recommendations

- Consideration should be given to intra individual biological variation during the interpretation of the sweat test, particularly around cut-off points. Biological variation is considerably greater than analytical imprecision [Grade D]

6.4 How does intermediate sweat chloride relate to genotype?

There have been large numbers of case reports and studies examining the relationship of intermediate sweat tests to the CF genotype (^{119, 120, 71, 121, 122, 123, 124, 110, 125, 79}) many of which are associated with non-classical CF. An extensive detailed review of this data is beyond the scope of the guideline.

Recommendations

- Patients falling into this area of intermediate sweat chloride require further clinical evaluation by cystic fibrosis physicians prior to diagnostic labelling [Grade D]

6.5 What other disorders are 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 ^(126, 127, 128, 129, 130). 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 ⁽¹³¹⁾.

6.6 What other diseases and conditions are associated with increased sweat electrolyte concentrations?

A large number of conditions have been associated with abnormalities of sweat concentrations of sodium and chloride ^(143,144, 145, 146). In most of these studies only one or two patients are reported and in many the sweat electrolytes returned 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 ^(103, 132) and is not considered in the scope of these guidelines and there is no recommendation.

6.7 What are the indications for a repeating a sweat test?

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 is not necessary.

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. Data from Calvin et al ⁽¹²⁵⁾ reported 6 babies identified as part of a screening programme with raised IRT but having normal/borderline sweat tests. Extended DNA testing revealed all had 2 mutations consistent with CF. Although repeat sweat testing in the immediate period confirmed previous results, in one child, sweat electrolytes became elevated into the CF range at 2 years of age.

All borderline sweat chloride concentrations not confirmed by genotype should be repeated ^(133,110, 134) (Evidence level 3)

Recommendations

- A repeat sweat test is recommended when the sweat test result is not in keeping with the clinical phenotype and/or genotype[Grade D]

6.8 What further investigations can be undertaken to diagnose cystic fibrosis?

(a) Genotype Analysis

This is beyond the scope of the guideline and there is no recommendation.

(b) Nasal Potential Difference Measurement

This is beyond the scope of the guideline, but consensus is that nasal pd is not recommended in isolation ⁽¹³⁵⁾(Evidence level 4)

7. Who has responsibility for testing and training?

7.1 Who should perform the sweat test - What skills are required and what are the 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 ^(86,88).

Shwachman and Mahmoodian⁽⁸⁸⁾ 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 4)

An audit of UK labs in 2006 showed that there were 20 labs among the 73 respondents that collected /or analysed less than 50 sweat tests in the previous 12 months. 2 labs performed less than 10 per annum.

Consensus Guidelines

The Cystic Fibrosis Foundation guidelines suggest that sweat testing must be performed on a sufficient number of patients by a limited number of experienced, well-trained personnel who pass periodic documented competency testing. CLSI ⁽⁹⁾ requires that sweat testing be performed on sufficient number of patients by experienced well-trained personnel in order that proficiency is maintained. Competency assessment should be undertaken periodically and documented. (Evidence level 4).

UKNEQAS

There is no direct correlation between workload and analytical performance. However, by its very nature the EQA data is looking only at the analytical aspects and has to make the assumption that the more technically demanding stimulation and collection aspects of the sweat testing protocol have all been carried out without error (Evidence level 3)

Recommendations

- 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 and collection of a sufficient number per annum is required to maintain competency and quality [Grade D]
- Sweat collection must be performed by fully trained and experienced personnel:-
 - training schedules should be fully documented
 - the procedure should be documented as a standard operating procedure
 - appropriate revalidation procedures should be in place [Grade C]
- Sweat collection can be undertaken by a variety of healthcare professionals [Grade C]
- Sweat analysis should be performed by qualified and experienced biomedical scientists or clinical scientists who are fully trained with regular validation:-
 - training and validation schedules should be fully documented [Grade C]

7.2 Who has responsibility for analysis and training?

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 ⁽²⁷⁾ require that staff must be appropriately qualified for the work they are performing (Evidence level 4).

Recommendations

- A consultant clinical scientist or consultant chemical pathologist should have responsibility for training, assessment of competence and revalidation for all staff undertaking sweat tests [Grade C]
- The responsibilities 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 [Grade C].

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PROCESS OF GUIDELINE REVIEW

METHODOLOGY

Guidelines were first published in 2003⁽¹⁴¹⁾. A review of the guidelines was undertaken in during the period 2008-2014. Methodology involved in the review process is outlined below. The process for guideline review is summarised as Figure 2.

Guideline Review Group

A multidisciplinary Guideline Review Group was established. The group included health professionals of scientific, clinical and nursing disciplines from a range of stakeholder organisations. Details of all the members and stakeholder organisations are listed at the beginning of the document. Each member of the group was assigned subsection/s of the guideline to review. Assignment was based on expertise in that area.

Development of the Scope

The Guideline Review Group agreed a scope for the guideline at an initial meeting.

Identifying Evidence

A number of mechanisms were used to identify evidence including computerised literature search, hand searching, specific searching, review of existing Consensus Based Guidelines and National UK Laboratory Sweat Test Subgroup This identified evidence since initial guideline, as well as expanding evidence identified during the first 2003 guideline by use of a wider search base.

Review of Evidence

The evidence and recommendations for sweat testing were reviewed as seven separate subsections (see scope). Each subsection had at least two members of the Guideline Review Group review and grade evidence. Subsections were presented at a guideline review group meeting for discussion and informal agreement of summary evidence statements and recommendations in order that a first draft guideline is produced.

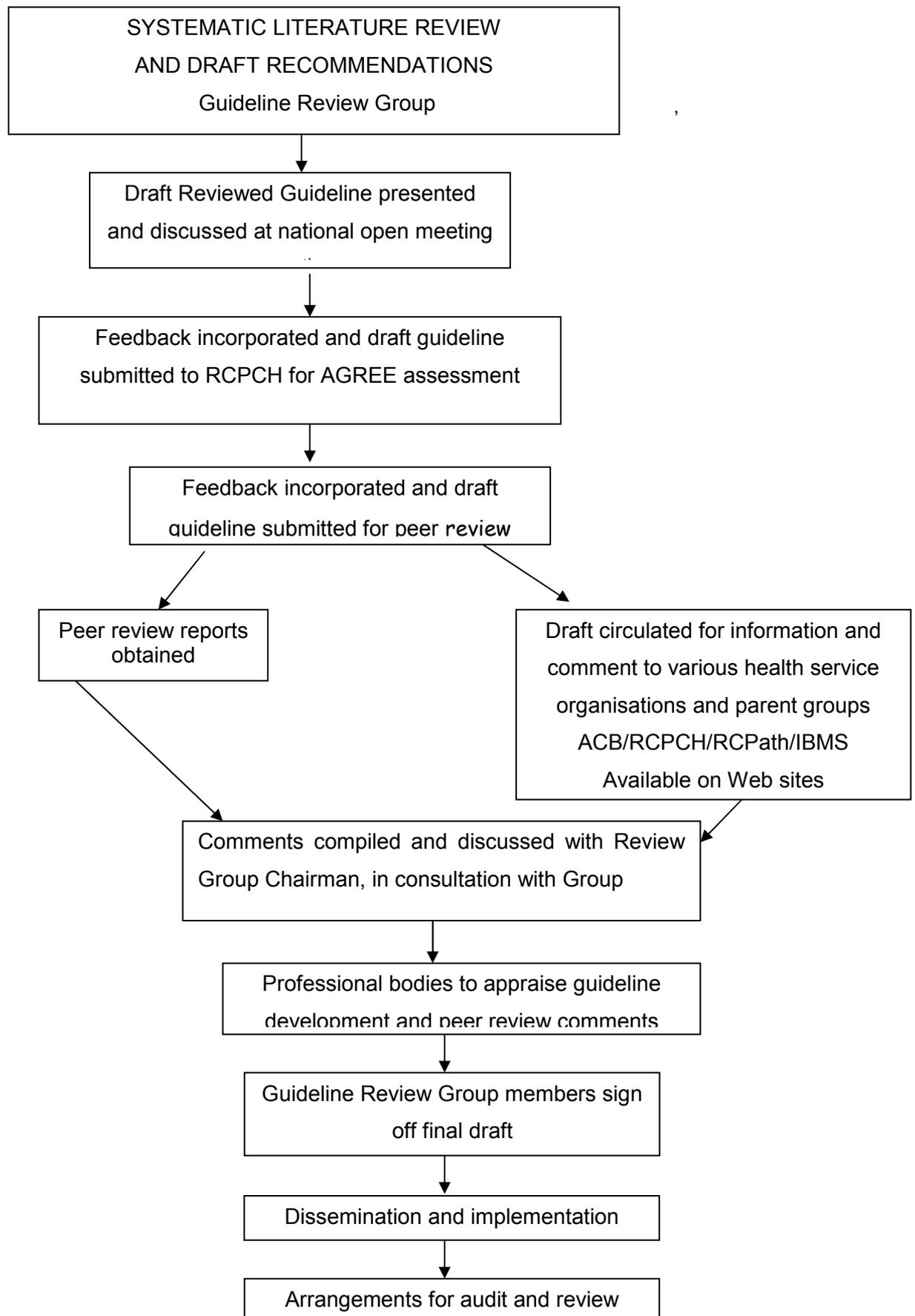
Consultation

The first draft of the reviewed guideline was presented at an open meeting for comment and discussion and responses were incorporated where appropriate. The guideline was then passed for AGREE assessment by RCPCH. A second draft guideline was circulated to organisations and individuals listed at the beginning of the document. Again responses were incorporated where appropriate.

Dissemination

The reviewed guideline will be distributed to professional bodies and be made available on websites.

Figure 2. Process for Guideline Review



GRADING OF EVIDENCE AND RECOMMENDATIONS

The Guideline Development Group has undertaken a systematic review of evidence in accordance with SIGN methodology and the evidence and recommendations have been graded according to this ^(i,ii,iii,iv). The group note that the SIGN grading system has been revised, hence the grading of some evidence/recommendations are changed since the original guideline in 2003.

Additional points of note are:-

- publication is not essential to be considered as good evidence.
- 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 informal consensus agreement of the working group members.
- grading of all evidence was reviewed by the same two members of the working group to ensure consistency throughout the guideline.

Grading Scheme for Recommendations

The criteria for the grading of recommendations in this document are based upon those used in 'Standards for Development of Clinical Guidelines and Implementation in Paediatrics and Child Health' produced by the Royal College of Paediatrics and Child Health ⁽¹³⁶⁾, and are based on the Scottish Intercollegiate Guidelines Network (SIGN) ⁽¹³⁷⁾ where level of evidence is used to determine grade of recommendation.

Levels of evidence:-

Level	Classification of evidence (based on SIGN 2000 ¹³⁷)
1++	Evidence from high quality meta analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+	Evidence from well conducted meta analyses, systematic reviews of RCTs, or RCTs with a low risk of bias
1-	Evidence from meta analyses, systematic reviews of RCTs, or RCTs with a high risk of bias
2++	Evidence from high quality systematic reviews of case-control or cohort studies or high quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a moderate probability that the relationship is causal
2+	Evidence from well conducted case control or cohort studies with a low risk of confounding, bias, or chance and a moderate probability that the relationship is causal
2-	Evidence from case control or cohort studies with a high risk of confounding, bias, or chance and a significant risk that the relationship is not causal
3	Evidence from non-analytical studies e.g. case reports, case series
4	Evidence from expert opinion

Grading of Recommendations

Grade	Type of recommendation (based on SIGN 2000 ¹³⁷)
A (level 1++)	Requires at least one meta analysis or randomised controlled trial rated as 1++ , and directly applicable to the target population, and demonstrating overall consistency of results
B (levels 1++, 1+ and 2++)	Requires a body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results, or Extrapolated evidence from studies rated as 1++ or 1+
C (level 2+ or 2++)	Requires a body of evidence including studies rated as 2+, directly applicable in the target population and demonstrating overall consistency of results, or Extrapolated evidence from studies rated as 2++
D (level 2+, 3 or 4)	Evidence level 3 or 4 , or Extrapolated evidence from studies rated as 2+

The process of developing the recommendations has been according to guidance from the RCPCH ⁽¹³⁶⁾; further details are provided in the process of guidelines development section.

References

- i. SIGN Guidelines: Standards for development of Clinical Guidelines in Paediatrics and Child Health 3rd Edition (2006) Royal College Paediatrics and Child Health ISBN:1 900954 97 4.
- ii. The guideline development process: an overview for stakeholders, the public and the NHS 3rd edition (April 2007) National Institute for Health and Clinical Excellence
- iii. Royal College of Paediatrics and Child Health. Standards for Development of Clinical Guidelines and Implementation in Paediatrics and Child Health. 3rd Edition 2006
- iv. Scottish Intercollegiate Guideline Network (2000) A guideline developers' handbook. *SIGN* 50

SYSTEMATIC REVIEW

The search process comprised the different electronic search strategies for version 1 and 2 of the guideline:-

Electronic search strategy for guideline version 1 (2003).

- **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.

During review of the guidelines four further searches were undertaken – these are shown below. In all cases non-English publications were not included on the basis that translation of the full text article would not be available. All articles were for humans only in order that literature would be directly comparable to the population to be tested and not require extrapolation to another species or be assumed from *in vitro* data.

Electronic search strategy for guideline version 2 (Finalised March 2014, update of Version 1 2003)

1. May 2008 :(January 2000 to May 2008), Medline, The Cochrane library, Cinahl, Embase (1996 - May 2008), NLH Specialist Libraries, PubMed using the following PICO formula search components: Patient – All children, Intervention - sweat test, comparison – none, outcome – cystic fibrosis, diagnostic test.

Keywords and search terms used were:

Patient - Newborn(s), Baby, Babies, Infant(s), Child(s), Children, Childhood, Adolescent(s), Adolescence

Intervention – Sweat, Sweat test(s), Sweat testing, Sweat chloride test(s), Sweat chloride testing, Sodium chloride, Chloride(s), Sodium

Comparison – None

Outcome - Cystic fibrosis, CF, Diagnosis laboratory, Diagnostic test routine, Diagnostic accuracy, Diagnostic procedure, Laboratory test, Clinical laboratories, Neonatal assessment, Neonatal screening

English Language only, Humans only

2. June 2009 : (from Jan 2008 – June 2009) Medline, The Cochrane Library, Cinahl, Embase, PubMed, Google Scholar, Zetoc

3. October 2010:(from 1970 to 2000)Cinahl, 1965-2000 Embase, The Cochrane Library pre-2000. These earlier searches were undertaken to address inadequacies in the previous guideline.

Searches 2 and 3 used the following PICO formula search components: Patient – as previous

Intervention – Sweat, Sweating, Sweat gland(s), Sweat test(s), Sweat testing, Sweat chloride test(s), Sweat chloride testing, Sweat chloride, Sweat sodium, Sweat electrolyte(s), Chloride(s), Sodium, Pilocarpine iontophoresis

Comparison – None

Outcome - Cystic fibrosis, CF

4. November 2013 : (January 2009 to November 2013). Medline, The Cochrane Library, Cinahl, Embase, PubMed and Google Scholar. These further searches were undertaken to capture any final publications prior to finalisation of the guideline. As with previous searches (see 3 above) the same PICO headings were used.

Other searches undertaken:

▪ **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) and audit data collected from 73 laboratories in 2006 on behalf of the Sweat Test Guidelines Group in partnership with UKNEQAS (supported by the ACB)⁽¹²⁾.
 3. Wescor® instruction manuals (Webster sweat collection system model 3500. 1979; Macroduct® sweat collection system model 3700, Sweat Chek™ 3120, Nanoduct®, Patient Simulator) and Website (<http://www.wescor.com>)
 4. Data collected by Internet enquiry (Association for Clinical Biochemistry Mailbase 2009) 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, Landon, 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 text word searching
2. UK National External Quality Assurance Schemes Sweat Test External Quality Assurance Surveys

Quality

1. UK Audits on Sweat Testing (unpublished data ^(46,47,57,122)).
 2. UK National External Quality Assessment Schemes Data from Sweat External Quality Assessment Surveys⁽¹²⁾
- **Review of existing Consensus Based Guidelines**
 - CLSI 2009 ⁽⁹⁾
 - Welsh Sweat Standard 1999 ⁽²⁸⁾
 - **National UK Laboratory Sweat Test Subgroup ⁽¹⁵⁾**
 - this comprises evidence from a 'Consensus of experts' collected from the National UK Laboratory Subgroup⁽¹⁴¹⁾

Assessment of Quality of Evidence

Data identified from computerised and other searches was circulated to 4 members of the review group (search 1) for evaluation for relevance to the sweat test process. Members scored literature independently for need to obtain a complete article. A full text article was not thought to be required if, for example, the literature was:

- not relevant to the sweat test process
- had already been reviewed as part of the first guideline
- a review article without original evidence
- primarily related to genotype studies

Brief reasons for none inclusion were recorded. Responses were tabulated and full text articles were obtained for literature identified as relevant, as well as articles where it was impossible to assess relevance. In cases where there was a split in scoring the full text article was obtained when 2:2 or 3:1 (inclusion:exclusion, respectively) split occurred. Due to the smaller amount of literature identified during search 2 this was scored by 2 members only. All full text articles were provided to all members of the review group. During review of literature data were assessed and scored using SIGN 2000¹³⁷ to assess the quality of the evidence.

EXPECTED IMPLICATIONS OF THE NEW GUIDELINES

Changes introduced in this guideline are not expected to have significant resource implications for Departments and Trusts offering a sweat test service. On the contrary, the move to competence in assuring a quality service has reduced the requirement for undertaking a minimum number of tests such that service provision across the UK may be maintained and reduce the requirement for patients to travel significant distances for testing. Documentation of competence in performing the sweat test mirrors requirements for laboratory accreditation and thus should have little additional training requirement. Review of reference intervals will better reflect the population being served and support those centres providing newborn screening tests for cystic fibrosis. The expansion of storage of sweat before analysis (4.1.1) clarifies the position for those centres in whom provision of a full analytical service would not be cost effective in full. It supports collection on site (a benefit to the patient via reduced travel time) and referral of the sweat samples for analysis to centres of excellence. The importance of audit of results/outcome and failure rates is re-iterated and this should be used to facilitate review of service provision. Providers should review workload and whilst working within the guidelines determine whether referral of patients and/or sweat samples for analysis may provide a more efficient service.

Potential facilitators

Governance is increasingly important within the NHS and the guidelines will provide support to those leading sweat testing services in ensuring a consistent approach to testing and analysis.

It is believed that the restructuring and redesign of NHS services that is currently underway will support review of sweat services and may increase uptake of referral of sweat samples.

Potential barriers

There is the possibility that centres having high failure rates for sweat collection or those with very low workload to adequately demonstrate competence will continue

offering services rather than seek alternate providers or work with centres of excellence to improve performance.

Sweat chloride alone cannot be used to diagnose cystic fibrosis and this may impact upon Trusts utilising the WescorNanoduct® system.

Appendix 1

The sweat test

Introduction

The sweat test is a test used to diagnose cystic fibrosis (CF). This factsheet explains how the test works, why it is used and what the results mean.

Written by Professor Anne Green, Consultant Clinical Biochemist (Honorary), and Dr Peter Weller, Consultant Paediatrician (Respiratory Medicine), Birmingham Children's Hospital NHS Trust.
Last updated 2010 (currently under review).

What is the sweat test?

The sweat test measures the amount of salt (usually measured as chloride) in sweat. This is done by collecting a small amount of sweat from the arm, or sometimes the upper part of the leg, in a small baby.

Why is it used?

In people with cystic fibrosis there is a problem in the transport of chloride across cell membranes. This results in higher concentrations of chloride (as salt) in sweat compared to those who do not have cystic fibrosis. So, if there is a family history or a possibility of CF, the sweat test is part of the special tests to help make, or exclude, a diagnosis of cystic fibrosis. Screening for cystic fibrosis is part of the national newborn blood spot screening programme. The sweat test is done in those babies suspected of having cystic fibrosis, as part of the follow up of the screening process. As part of the investigations to look for possible causes of illness, the sweat test may be done in children with no family history of cystic fibrosis but are having lots of chest infections, unexplained diarrhoea, or who are not putting on weight or growing normally. In these circumstances the test is often used to exclude a diagnosis of cystic fibrosis. It is also helpful in investigating adults with problems like bronchiectasis, infertility and pancreatitis.

How is the test performed?

A small area of skin on the arm or leg is cleaned with water, and two gels or special pads are attached. These gels/pads contain a substance called Pilocarpine, which will make the skin sweat. In order to get the Pilocarpine into the skin, the area is stimulated by a small current from a battery for about five minutes. This may produce a tingling sensation but does no harm and does not hurt.

The gels/pads are removed, the skin is cleaned and a small coil device or a piece of special paper is placed onto the arm/leg. The sweat is collected into the coil or on the paper for about 20–30 minutes. The sweat in the coil/on the paper is then taken to the laboratory for analysis. The whole test usually takes about 30 minutes.

The area of the arm or leg which was stimulated may stay red for a few hours after the test, but this is normal and nothing to worry about. The test is very safe and the risk of any problems is extremely small.

Occasionally it is necessary to repeat the test if insufficient sweat has been collected or there has been some contamination. This does not necessarily mean that your baby or child is more likely to have Cystic Fibrosis. However, sometimes a borderline chloride result is obtained, and a repeat test will be necessary.

The result of the sweat test

The result of the test will usually be available to you within a few days from the doctor who requested the test. The test can help your doctor to decide what is wrong but he/she will also rely on the symptoms and the results of other tests.

If your baby is being tested because of a newborn screening test result, arrangements will be made for the sweat test result to be explained to you by a doctor in your CF Clinic, as part of the follow up from the screening results – this will usually be within 24 hours.

If you have any questions about why this test is being performed, you should ask your doctor. You should not telephone the laboratory for results. Laboratory staff are not allowed to give out results on the telephone, as they may not know the background for a specific patient.

Further information

The Cystic Fibrosis Trust provides information about cystic fibrosis through our factsheets, leaflets and other publications.

Most of our publications can be downloaded from our website ordered using our online publications order form.

Visit www.cysticfibrosis.org.uk/publications.

Alternatively, to order hard copies of our publications you can telephone the CF Trust on 020 8464 7211.

If you would like further information about cystic fibrosis please contact:

Cystic Fibrosis Trust
11 London Road
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Kent BR1 1BY
T 020 8464 7211

cysticfibrosis.org.uk

enquiries@cysticfibrosis.org.uk

Helpline 0300 373 1000

We would welcome your feedback on this or any other of our publications.

Please email publications@cysticfibrosis.org.uk.

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The information included in this publication is not intended to replace any advice you may receive from your doctor or CF multidisciplinary team and it is important that you seek medical advice whenever considering a change of treatment.

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Appendix 2a

EXAMPLE STANDARD OPERATING PROCEDURE FOR SWEAT TESTING USING 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	TOXIC
Sweat	BIOHAZARD
Electrode contact	BURNS

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 <2 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 3700).
Check the following components.
 - (a) Sweat inducer (Power supply box)
iontophoresis electrode sets (red and black) with Velcro straps.
 - (c) Velcro straps in different sizes, to fit Macroduct®.
 - (d) Sweat extractor tool or syringe with blunt needle and scissors or nippers.
2. Sealable tubes or cups for sweat transport and storage. Eg. Autoanalyser cups and caps, or 200ul PCR cups and caps, 100 µL Haematocrit capillary tubes and PlasticineMiniseal block.
3. Mediswabs
4. Kleenex medical wipes or cotton wool balls.
5. Adhesive labels for sample identification.

REAGENTS:

1. *Macroduct® Test Kit*. WescorCat no SS-032 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 Sweat Inducer (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®, i.e. 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. Follow manufacturer's instructions for performing the iontophoresis. The instructions below apply to the current Model 3700.

Push the control switch to the RUN (I) position and hold momentarily until you hear a short "beep". A steady tone indicates excessive external circuit resistance, a break in the line, or weak batteries. If this occurs, move the control switch to STOP (0) and correct the fault condition before proceeding.

If everything is normal, the CURRENT FLOW indicator reaches full brightness in approximately 20 seconds, and diminishes in brightness during the last 5 seconds of iontophoresis as the current is reduced to zero.

After iontophoresis is complete "chirrup" will sound briefly and the green CURRENT FLOW indicator LED 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 (NB. 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

15 uL) has collected after 20 min but should never be left longer than 30 minutes. Although suggested in the manufacturer's instruction manuals , extending the collection time beyond 30 minutes does not increase the weight of sweat collected by a significant amount.

15. Label PCR cup with patient name and laboratory number.
16. Leaving Macroduct® in position on arm remove clear plastic 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 the coil as close to the Macroduct® as possible. Put cut end into PCR 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 1g/m²/min is 12 uL or mg for a 20 minute collection, or 18 uL 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 PCR cup and reweighing immediately. Assess sweat samples for adequacy immediately after collection. Collections of less than 12 uL or mg in 20 minutes or 18 uL 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 conductivity may also be measured). Sweat chloride may be analysed by a coulometric, ion selective electrode or colorimetric 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 72 hours before analysis.

INSTRUMENTATION AND APPARATUS:

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

REAGENTS:

Appropriate to local methodology. See separate SOPs.

STANDARDS:

The method should be standardised using commercial or in-house materials at concentrations appropriate for sweat samples, i.e. 0-150 mmol/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 10 mmol/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 preparations or in-house solutions.

PROCEDURE:

1. Sample directly from analyser cup or score the ends of the glass capillary and carefully break open. After sampling, transfer any remaining sweat to PCR cup or 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% for chloride (at a concentration of 40-50 mmol/L) and <2% for conductivity (at a concentration of 50 mmol/L). 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. Analytical results (chloride, conductivity) in mmol/L. It should be explicit on the report form which analyte(s) have been measured.
 - Reference intervals (see Section 6 of these guidelines)
- vi. Interpretation of results, based on the reference intervals outlined in Section 6 of these guidelines, and any further information supplied about the patient (e.g. 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, i.e. chloride >150 mmol/L, conductivity > 170 mmol/L.

REFERENCES

Instruction Manuals - Macroduct® Sweat Collection System appropriate to model used.

Medical Devices Agency. Safety Notice: Prevention of burns during iontophoresis. MDA SN1999 (05).

Guidelines for the performance of the Sweat Test for the investigation of Cystic Fibrosis in the UK (Version 2).

¹Appendix 2b

**EXAMPLE STANDARD OPERATING PROCEDURE FOR
SWEAT TESTING USING
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 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	TOXIC
Cathode electrolyte	POTENTIALLY TOXIC
Sweat	BIOHAZARD
Electrode contact	BURNS

¹

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, and who satisfy regular competency assessments should perform this test.

The sweat test should be deferred in babies <14 days old and/or <2 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 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. Batches of filter paper should be checked for chloride levels prior to use.
2. Airtight container for weighing and eluting filter paper e.g. Universal container
3. Laboratory balance sensitive to 0.0001g
4. Iontophoresis power supply e.g. C & IS Electronics Gibson – Cooke Power Supply
5. 2 electrodes
6. Rubber or Velcro electrode straps of adjustable size.
7. Electrolyte support pads e.g. 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 e.g. Traysin.
12. 2 Containers suitable for soaking lint pads in electrolyte solutions e.g. 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. Slek or Setonplast

REAGENTS:

- (1) *Pilocarpine nitrate solution (0.2 – 0.5%). Pharmaceutical Grade*
- (2) *Cathode electrolyte, if different e.g. 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 i.e. 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 cut-out 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 cut-out 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 $1\text{g/m}^2/\text{min}$ as follows: (See guidelines 4.1.3), where m^2 = area of collection site.

Calculate the area of the filter paper collector in cm^2 as πr^2 where r is the radius.

Then sweat rate ($\text{g/m}^2/\text{min}$) =

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

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

e.g., for a 30 minute collection on 5.5cm diameter filter paper $1\text{g/m}^2/\text{min} = 71\text{ mg}$

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

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 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:

Immediately proceed to analysis or store weighed sweat collections on filter paper pads at 4°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 i.e. 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 period of between 1 minute and 3 hours. 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, where possible, 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. Analytical results (chloride) in mmol/l. It should be explicit on the report form which analyte has been measured.
- iv. Reference intervals as described in the table in section 6.1
- vi. Interpretation of results, based on the above reference intervals and any further information supplied about the patient (eg pancreatic sufficient, unusual CF mutation etc)

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

Calculation of average sweat rate over the collection period

The average sweat production rate over the collection area is calculated in $\text{g/m}^2/\text{min}$ 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 cm^2 as πr^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 ($\text{g/m}^2/\text{min}$) =

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

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

For a 20 minute Macroduct® collection $1\text{g/m}^2/\text{min} = 12\text{uL}$. For a 30 minute Macroduct® collection $1\text{g/m}^2/\text{min} = 18\text{uL}$. The manufacturer's recommendation of 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.