A

Welsh Standard

for

Sweat Testing

Version 2 (provisional)

1999

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INTRODUCTION

The analysis of sweat electrolytes is the acknowledged gold standard for the diagnosis of cystic fibrosis[1]. However, deficiencies of technique, particularly with the collection of sweat, have been responsible for misdiagnosis[2].

In order to minimise misdiagnosis of cystic fibrosis in Wales, a workshop was convened in April 1993 with representation from all Welsh hospitals performing sweat tests. A consensus standard was prepared[3].

This standard was reviewed in 1996 under the auspices of the Welsh Standing Specialist Advisory Group in Clinical Chemistry of the Welsh Scientific Advisory Committee, to incorporate the National Committee for Clinical Laboratory Standards recommendations for sweat testing[4] and in preparation for the introduction of Neonatal Screening for Cystic Fibrosis in Wales.

Further revision was undertaken in 1999 to incorporate the experience of three years of neonatal screening and recent published developments in sweat testing. This (provisional) version will be further reviewed once the new UK multidisciplinary guidelines for performance of the sweat test are finalised (expected to be in 2003).

PRINCIPLE OF SWEAT TESTING

The sweat glands on a localised area of skin are activated by the iontophoretic introduction of pilocarpine. In the process of iontophoresis, an electric potential is established so that pharmacologically active ions carry a current and are thereby introduced into the skin. In this case a positive electrode moistened with pilocarpine nitrate is used.

The positively charged pilocarpine ions move away from the electrode and into the skin. A negative electrode is applied to the same extremity. After the sweat glands have been stimulated, the electrodes are removed, the skin cleaned, and sweat collected over the exact region where the pilocarpine was iontophoresed. Sweat electrolytes are then measured.

GENERAL CONSIDERATIONS

1. The hospital biochemistry department will determine the Standard Operating Procedure (SOP) for the sweat test including sweat collection, analyses, reporting, interpretation and all safety aspects of the test.

2. In conjunction with the Paediatric Department, patient/parent written information concerning the test should be prepared for distribution on request (Appendix 1). Patient/parent(s) must have access to paediatric staff concerning the test if required. Individual departments may wish to obtain written parental/patient consent before proceeding with the test.

3. Sweat tests should NOT be performed before seven days of age or if the infant is acutely unwell.

COLLECTION AND ANALYSIS OF SWEAT ELECTROLYTES

1. Sweat Collection

1.1 Personnel

The staff performing the collection of sweat may be laboratory or nursing staff, but must be trained by the laboratory and adhere precisely to the technique described within the SOP.

Annual workload will dictate the frequency of sweat testing, but a minimum of 10 sweat stimulations/annum/staff member should be performed to maintain expertise.

1.2 Methodology

Sweat stimulation should be by pilocarpine iontophoresis.

Personnel should be familiar with the guidance of the Medical Devices Agency Safety Notice 1999 (5) *Prevention of Burns During Iontophoresis*[5].

1.3 Equipment

The equipment used for iontophoresis should be EMS (Electro Medical Supplies, Wantage, Oxon) or Wescor (Chem Lab Scientific Products Ltd, Hornchurch, Essex).

The equipment should be maintained as per manufacturer's recommendations with particular reference to safety aspects. Records of electrode inspection and maintenance must be kept for independent inspection if required.

1.4 Iontophoresis

1.4.1 Site of Iontophoresis

Sweat should only be collected from the arms or legs. The area for stimulation must be free of any skin lesion. The skin should be cleaned by distilled water wash followed by drying with paper tissues.

1.4.2 Electrodes and Electrolyte

i) EMS - Uniformly saturate hospital lint (BPC Plain 500 gram 4 - 8 thicknesses) or filter paper (Whatman 42/44 10 - 15 papers) with electrolyte.

Anode: 0.2% pilocarpine nitrate (in distilled water).
Cathode: As for anode or 0.1M magnesium sulphate.

ii) Wescor - As per manufacturer's instructions using supplied electrode gels.

The electrodes should be spaced as follows:

EMS > 10 cm but < 20 cm apart.
Wescor > 5 cm but < 10 cm apart.

Ensure the electrodes do NOT directly touch bare skin[6] and that the skin between them is dry.

1.4.3 Iontophoresis

i) EMS - the current is increased slowly over about 20 seconds to a maximum of 4mA and is manually controlled at this level by adjustment of the output control. The duration of iontophoresis is 5 minutes[2]. Iontophoresis is terminated by reducing the current slowly over 30 seconds.

ii) Wescor - this is controlled automatically with a programmed increase in current to 1.5mA for 5 minutes, and then a programmed decrease in current to completion.

During the iontophoresis the patient should be monitored at all times.

1.5 Sweat Collection

1.5.1 Post iontophoresis the skin should be further washed with distilled water followed by drying with paper tissue.

1.5.2 Sweat is collected either:

i) EMS - Whatman No. 42/44 pre-weighed filter paper. Evaporation controlled by overlying a water proof membrane, e.g. polythene sheet completely sealed at the edges by water proof tape ("Sleek", Smith and Nephew Ltd).

ii) Wescor - into customised capillary tubing (Macroduct) as per manufacturer's instructions.

1.5.3 The sweat should be collected at ambient temperature for 30 minutes[1].

2. Sweat Analysis

2.1 Personnel

The analysis should be performed by fully trained laboratory staff. A minimum of 10 procedures/annum/staff member should be performed to maintain expertise.

2.2 Minimum Sweat Weight or Volume

For a sweat test to be valid adherence to minimum amounts is critical. Sweat electrolyte concentration is related to sweat rate which should exceed $1g/m^2/min$[4]. The minimum amounts standardised for collection area and time are:

i) EMS - 100mg
ii) Wescor - 15 µl

A minimum of 75mg is recommended in North America[4].

If the incidence of insufficient sweat collections exceeds 5% the iontophoresis and collection procedures should be reviewed.

2.3 Qualitative v Quantitative Analysis

Quantitative measurement of both chloride and sodium is recommended.

Qualitative measurement of sweat conductivity and/or osmolality are regarded as screening tests and should be confirmed by quantitative analysis.

2.4 Analytical Range

The analytical equipment should be validated for measurement in the range 1 - 165 mmol/l.

2.5 Reference Ranges

Chloride concentrations should take priority over sodium for interpretation. The reference range for chloride (age independent) is:

Unequivocally normal	< 40 mmol/l
Equivocal	40 - 60 mmol/l
Unequivocally abnormal	> 60 mmol/l

(This data has also been validated for the neonatal period[7]).

2.6 Quality Control

An internal quality control material (sodium chloride 60 - 70 mmol/l) should be prepared for the analysis step. Acceptance criteria ±2 mmol/l.

2.7 External Quality Assessment

Laboratories should participate in either the UKNEQAS Sweat Testing Scheme or the College of American Pathologists (CAP) Sweat Analysis Scheme.

2.8 Criteria for Repeat Testing

The sweat test should be repeated in full:

i) If there is insufficient sweat weight/volume

ii) If the interpretation of the result is equivocal.

iii) If the first test results are unequivocally abnormal.

2.9 Report Format

The report format should include:

i) Full patient identification.

ii) Date of test, and date and time of report.

iii) Sweat weight/volume collected.

iv) Analytical results.

v) Interpretation of results.

vi) Recommendation for repeat testing if appropriate.

NEONATAL SCREENING

Neonatal screening for Cystic Fibrosis commenced in Wales (2/12/96) and employs a two-stage protocol (Appendix 2). Immunoreactive trypsin is initially (Stage 1) measured followed by genotyping (Stage 2) for the 5 most common CF mutations in Wales.

i) Sweat Testing is undertaken to confirm a Cystic Fibrosis diagnosed by screening.

ii) Sweat Testing (two successive determinations) is required in stage 2 to distinguish between carriers for cystic fibrosis with 1 mutant allele (normal sweat electrolytes) and compound heterozygotes in whom the second CF mutation is unknown.

Experience with genotyping has demonstrated that some mutations are associated with mild expression of the disease and normal sweat electrolytes[8]. Therefore an equivocal or normal sweat test performed at Stage 2 may **NOT** exclude the diagnosis of Cystic Fibrosis and further investigation (more extensive genotyping, evaluation of pancreatic exocrine function testing or nasal transepithelial voltage testing) may be required.

Because an estimated 10% of cases (2 out of 16 cases per annum will be missed on screening (either from opting-out or testing normal for IRT) clinical vigilance and proficiency in laboratory sweat testing should be maintained.

FUTURE DEVELOPMENTS

Any diagnostic test for Cystic Fibrosis which is proposed to replace the Sweat Test should be fully evaluated for its effectiveness before being introduced.

ACKNOWLEDGEMENTS

I should like to thank Dr Mary Goodchild (previously Associate Specialist, Cystic Fibrosis Unit, UHW) who pioneered this initiative.

I recognise the significant contribution of Dr M Penney (Department of Medical Biochemistry, Royal Gwent Hospital, Newport) in the preparation of the original version of this standard.

Finally, I should like to acknowledge the fruitful dialogue with Professor Vicky LeGrys (School of Medicine, University of North Carolina, Chapel Hill, USA) and Dr Iolo Doull (Cystic Fibrosis Unit, UHW) in revising this standard.

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SWEAT TEST

PATIENT INFORMATION

Introduction

The sweat test is performed so that the concentration of salt in sweat can be measured. The collection of sweat is usually made from the arm but occasionally the leg is used. The test is usually performed on children who have had recurrent chest infections, unexplained episodes of loose or pale stools, or in children who are not gaining weight or growing normally.

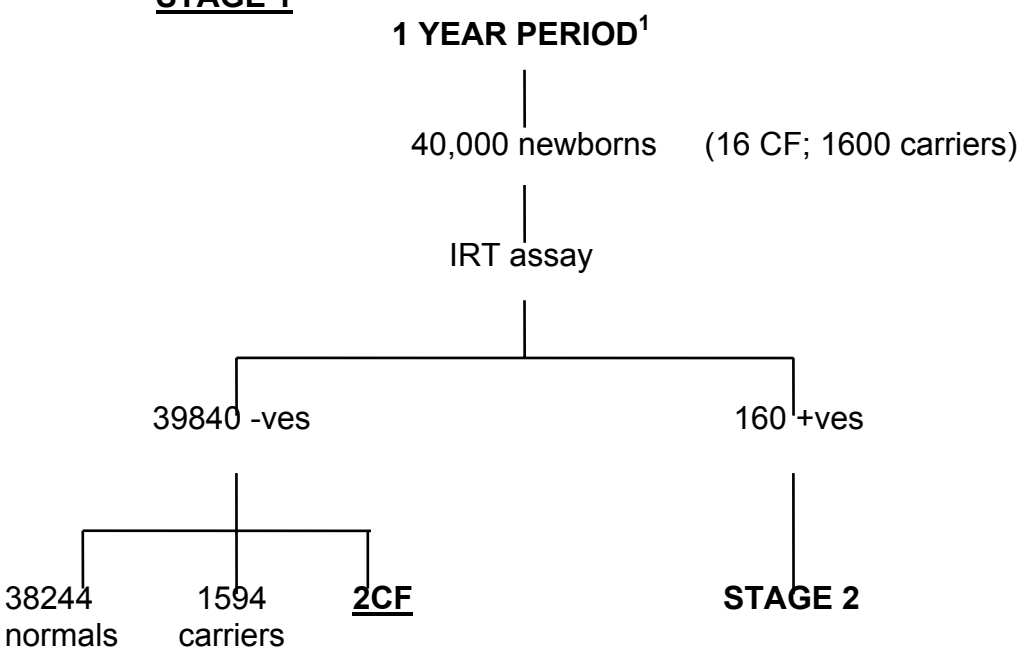
A positive result may mean that your child has the chest disease called cystic fibrosis; however this diagnosis would not depend on one sweat test result alone, but would be made in discussion with your doctor, taking into account symptoms, clinical findings and other tests.

Procedure

A small area of skin is cleaned with water and dried and a paper disc is applied which has been previously soaked in a solution containing a drug to stimulate sweating. A small electrical current from a torch battery is applied to the disc and a circuit formed by a second disc applied to nearby skin. The small electric current causes the drug to be drawn into the sweat glands in the skin to cause local sweating, producing a tingling sensation. After 5 minutes the discs are removed and the skin cleaned. A dry paper disc is now applied to the stimulated area of skin and covered. The sweat is absorbed into the paper disc over the following 30 minutes and at the end of the test the absorbed sweat is sent to the laboratory for detailed analysis.

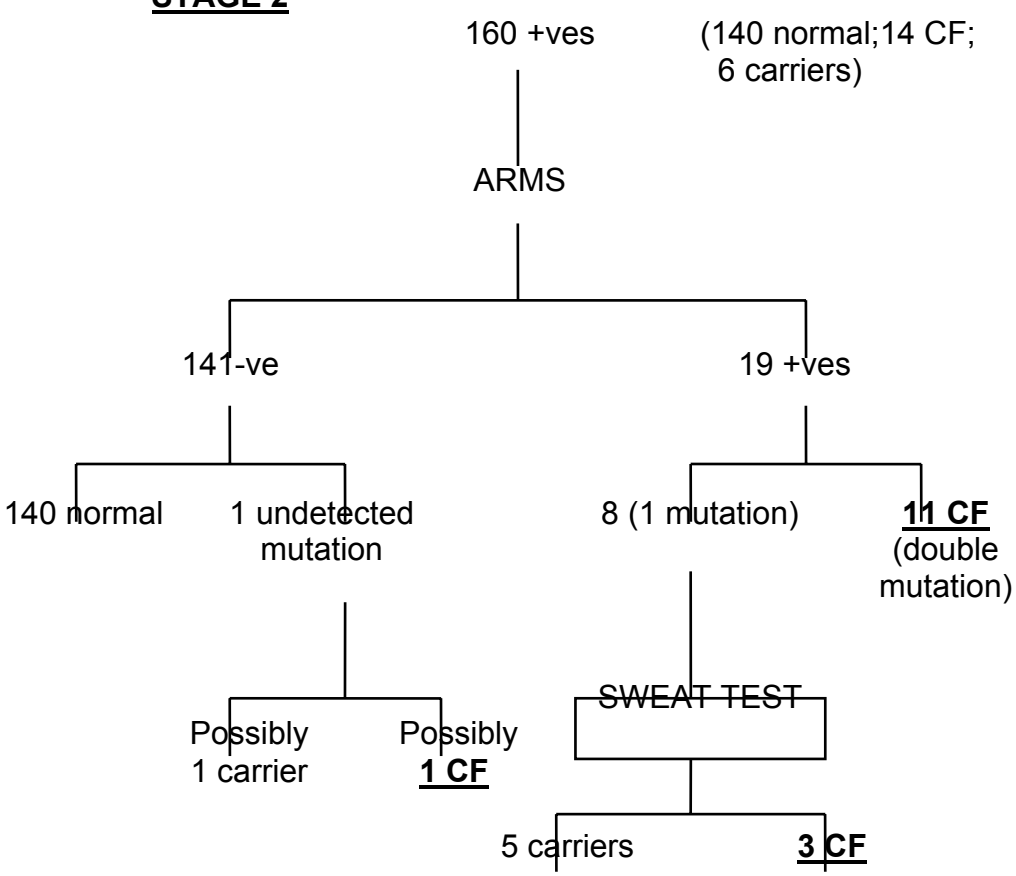
TWO STAGE NEONATAL SCREENING FOR CYSTIC FIBROSIS

Appendix 2



ASSUMPTIONS:

1. Annual birth rate in Wales of 40,000
2. CF affected frequency 1 : 2,500
   Carrier frequency 1 : 25
3. False negatives (possible over-estimate) 10% for IRT assay
4. Positive rate of 0.4% for IRT assay



ASSUMPTIONS: Detection of 88% of CF mutations by ARMS

SUMMARY: Neonatal screening detects 14/16 CF patients.

[1] Predicted figures for 1 year

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