Inconsistencies in sweat testing in UK laboratories

J M Kirk

Abstract

Background—Sweat testing procedures are perceived to vary widely.

Aim—To evaluate variability in sweat collection, analysis, and interpretation.

Methods—Questionnaire responses from 30 self selected centres: 15 paediatric centres, and 15 district general hospitals.

Results—Centres carried out 30–400 tests per year (median 100), with a diagnostic rate of 1:5–152 (median 1:30). Staff performed 5–268 tests per year. Minimum test age varied from 24 hours to four months. All stimulated sweating by pilocarpine iontophoresis using varying currents and times. Twenty six had observed urticaria or skin reddening, and nine blistering or burns. Sweat was collected for 10–60 minutes onto filter paper or into Macroduct coils. Between 2% and 25% of tests were considered insufficient. Twenty eight measured sodium, 24 chloride, and one osmolality and conductivity. Fifteen used literature and five in house reference ranges. Eleven would not test severely eczematous children.

Conclusions—Local audit is required to improve performance, as well as a national guideline to standardise collection, and external quality assessment to provide analytical feedback.

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Sweat testing has been the most widely used diagnostic test for cystic fibrosis for almost 50 years.¹ Diagnostic criteria have become less straightforward as referrals have widened to include neonates with abnormal screening results by immunoreactive trypsin and/or DNA mutation studies,² and less severely affected patients for investigation of atypical cystic fibrosis.³

In most laboratories sweat and routine plasma samples are analysed by separate methods. The United States has a well established external quality assessment (EQA) programme administered by the College of American Pathologists, and a guideline document on sweat test performance.⁴ Cystic fibrosis centres in the USA must comply with the guideline. Until a recent national pilot study of sweat analysis, no UK based EQA existed to assess analytical performance. Although laboratory regional audit groups have reviewed sweat collection and analysis procedures and produced consensus guidelines, such as the Welsh sweat standard,⁵ no national standard exists for the sweat test procedure.

This paper assesses sweat testing variability within a self selected group of UK centres, and considers how performance can be assessed and improved.

Methods

A questionnaire was circulated to Biochemical Investigations in Metabolic Disease Quality Assessment (BIMDG QA) committee members and participants enrolled for a sweat testing workshop at an Association of Clinical Biochemists national meeting. The responses represent large paediatric centres, and those with a particular interest or concern regarding sweat testing who were prompted to attend the workshop.

Results

Replies were received from all 15 BIMDG QA committee members and from 15 workshop participants representing eight children's hospitals, seven teaching hospitals, and 15 district general hospitals.

Centres carried out 30–400 sweat tests per year (median 100). The number of tests carried out for each diagnosis made varied from five to 152 (median 30). Tertiary referral centres had a higher ratio of abnormal tests. The highest ratio was reported by a centre which followed up infants in whom neonatal screening revealed increased immunoreactive trypsin. In most centres (n = 22) laboratory staff carried out sweat collection, varying from the most junior staff member to the head of department, with the majority (n = 12) using medical laboratory scientific officers (MLSOs). Some centres used nursing staff, physiotherapists, or respiratory measurement technicians. No centre reported the once common practice of using junior medical staff, medical students, or student nurses to carry out sweat tests as part of their training. Most centres restricted sweat collection to one or two trained staff. The extreme responses were a single operator carrying out 268 tests per year and 13 MLSOs sharing 64 tests per year. Centres using two staff either equally shared tests (n = 8) or had one operator carry out the majority, with the other as back up when necessary (n = 7). Five centres, all specialist hospitals, had a single trained operator.

The minimum test age quoted varied from 24 hours to four months. The commonest medical condition cited as a reason for deferring sweat testing was severe eczema (11 centres). Three centres were reluctant to test acutely ill children, and two were reluctant to test infectious children. Other conditions mentioned once each were: on mineralocorticoids, on flucloxacillin, on antibiotic, on oxygen, intravenous infusions in both arms, ichthyosis, and limbs too small for electrodes.

Department of
Paediatric
Biochemistry, Royal
Hospital for Sick
Children, Edinburgh,
Scotland, UK
J M Kirk

Correspondence to:
Dr Kirk
email:
Jean.M.Kirk@btinternet.com

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Figure 1 Sweat sodium and chloride reference ranges for normal individuals and for individuals with cystic fibrosis.

Seventeen centres used an Electro Medical Supplies power supply, and in house methodology.¹ Nine centres used a Wescor power supply, operated according to the manufacturer's instructions, and marketed with Macroduct collectors. Two used in house power supplies, one an Orion system, and the remainder did not specify. All centres stimulated sweating by pilocarpine iontophoresis. The nine Wescor users applied agarose discs containing 5 g/l pilocarpine at both electrodes. Others used anode pilocarpine concentrations varying from 2 to 5 g/l on gauze, lint, or filter paper and a variety of cathode electrolyte solutions. A current of 1.5 to 4 mA was applied for four to 10 minutes.

Twenty six centres had observed reddening/urticaria on the stimulated site, while blistering or burns was reported by nine centres, including two where operators carried out more than 100 sweat tests per year. Several centres commented that they had observed burns on a single occasion over many years testing.

Sweat was collected onto filter paper with a median area of 15.0 cm² (range 7–38.5) or into Macroduct collectors of area 7 cm². Collection time was usually 20–30 minutes, but varied from 10 to 60 minutes, and was not always accurately timed. Definition of an insufficient collection varied from 7 µl from a Macroduct user to 200 mg from a laboratory collecting over 38 cm² for 60 minutes. Because of the lack of information on sweat collection times, minimum sweat rates could not be compared across centres. Insufficient collections varied from 2% to 25% of the total and did not correlate with minimum sweat weight/area ($r = 0.18$, $p = 0.43$; two tailed test). In practice most laboratories analysed and

reported results on weights below their specified minimum. Patients especially likely to produce insufficient sweat included neonates/small infants (four centres), those with eczema or other skin conditions ($n = 4$), and Asian patients ($n = 2$). Laboratories also commented on particular "difficult" patients who repeatedly failed to sweat.

Twenty eight laboratories analysed sodium by flame photometry ($n = 23$), ion specific electrode ($n = 4$), or unspecified ($n = 1$). Twenty four laboratories measured chloride using six different methods. Twenty four centres measured sodium and chloride, four sodium alone, and two chloride alone. One laboratory measured sodium, chloride conductivity, and osmolality. Many in house and commercial standards and internal quality control (IQC) solutions were in use at different concentrations. IQC specimens should be handled in exactly the same way as patient samples. All 15 specialist centres, but only three workshop participants, added QC samples to weighed filter paper, and reweighed, diluted, and extracted them in parallel with patient samples.

Reference ranges (fig 1) were based on literature reports ($n = 15$), in house data ($n = 5$), manufacturer's quoted values ($n = 1$), not known ($n = 2$), or not specified ($n = 6$). Fifteen centres quoted an equivocal range, and two used a graph of sodium versus chloride to aid equivocal result interpretation. When a sweat test gave an abnormal result, 20 centres would repeat it, one by a different technique, and one by referral to their regional centre. One centre suggested fludrocortisone suppression. Eight centres would carry out DNA mutation testing, three pancreatic function assessment (by stool trypsin or chymotrypsin, plasma immunoreactive trypsin, or stool fat globules), and one nasal potential measurements.

Discussion

A recent NEQAS questionnaire identified more than 170 UK laboratories that measured sweat electrolytes. The 30 centres whose experience is reviewed here revealed wide variability in almost all aspects of sweat testing. Because they were a self selected group, with particular expertise and interest in sweat testing, even greater variability is likely if all testing centres were investigated. Some evidence for this is available from subsequent use of the questionnaire by regional audit groups, whose results suggest that more centres carry out very small numbers of tests, or use the Orion electrode, than would be extrapolated from the selected group reported here.

Sweat collection depends on care and skill in avoiding evaporation and contamination, both of which lead to artefactually raised electrolyte concentrations. The operator must assess when it would be inappropriate to test a child. This requires experience, and it is of concern that some individuals carry out fewer than 10 sweat tests annually, which occurs because of staff organisation in larger centres, as well as in centres with a small workload. Sporadic case reports document burns caused by iontophore-

sis, usually associated with testing by an inexperienced operator, and considered to be because of electrode-skin contact, or inadequate wetting of the support.⁶ This survey revealed that burns still occurred in centres with experienced operators, albeit rarely. Some conditions cited as reason to defer sweat testing are inappropriate. Reliable results have been obtained in infants less than 6 weeks old in many centres, and the false negative result reported in a patient taking flucloxacillin was not substantiated in a subsequent study.⁷

The 1959 methodological reference¹ described alternative sets of test conditions with variable iontophoresis time, and sweat stimulation and collection area. Local variations, introduced with or without audit and assessment of their effect, result in an almost total lack of standardisation and a lack of information as to which variables actually affect the result or the safety of the test. The group of laboratories using the commercial Macroduct system, which presumably follow the manufacturer's instructions, show less variability. However, even they differ in the iontophoresis current applied, depending on the model of their system.

Centres accepted a wide range of minimum sweat weights. Often these were literature derived, and inappropriate for the sweat collection area and time used. The minimum sweat weight quoted was often considerably greater than the sweat rate (1 g/m²/min) necessary for adequate results.⁴ Extending sweat collection time, particularly for the Macroduct coil where the yield is readily visible, cannot increase the sweat rate, and may not even increase sweat weight, while increasing the possibility of evaporation. The minimum volume required for analysis is a separate issue, and with modern technology should be achievable down to the minimum sweat rate. As the percentage of insufficient tests did not correlate with the minimum weight required, it appeared that patients who failed to sweat adequately usually failed completely, rather than representing the lower end of the normal distribution of sweat production.

The great majority of centres surveyed measured sodium and chloride. Increasingly laboratories use elderly flame photometers almost solely for sweat sodium samples, and therefore may have difficulty in funding their replacement. There is a greater range of chloride methods in use. The two most popular are a dedicated chloride meter with the same replacement problems as for a flame photometer, or a colorimetric method using standard laboratory equipment. For both sodium and chloride virtually all laboratories have entirely separate methods for the analysis of sweat and plasma, with only the plasma method monitored by EQA. Sweat conductivity and osmolality are not popular. The single centre measuring both also analysed sodium and chloride and had extensive in house data on all four parameters. There was wide variation in standardisation and internal quality control. Ideally both should be at a clinically important concentration, and should differ from each other. Large centres were more likely to make these solutions in house and to assess quality control in the equivocal range, while

smaller centres were more likely to use commercial sources.

More centres used literature reference ranges than in house data to interpret results. About half used a "grey" or equivocal range. Ongoing local audit will determine whether literature ranges are appropriate for the patient population. For example, the first Welsh sweat standard set a chloride cut off of 50 mmol/l. After introduction of two tier neonatal screening by immunoreactive trypsin (IRT) and a mutation analysis panel, the population tested was predominantly infants aged about 6 weeks with an abnormal IRT result and a single disease causing mutation identified. To achieve the maximum diagnostic rate in this population it proved necessary to lower the chloride cut off to 40 mmol/l. Local audit should also identify any change in the percentage of tests yielding insufficient sweat, or abnormal or equivocal results that are not confirmed when repeated. Such a change, without a change in the population referred, indicates that a significant variable has been inadvertently introduced into the sweat collection or analysis procedure.

Sweat testing organisation has changed over the last decade with recognition of the importance of restricting testing to experienced staff. Many more centres now measure chloride. However, these improvements have been matched by increasing complexity in result interpretation. DNA analysis, nasal potential measurements, and pancreatic function assessment provide additional diagnostic information. The sweat test still occupies an important place but until centres standardise their methodology, particularly in defining insufficient sweat rates, identifying analytical problems, and using reference ranges appropriate to their patient populations, it will fail to achieve its full diagnostic potential across the disease spectrum of cystic fibrosis.

For this to occur a threefold effort is recommended:

- Ongoing local audit of sweat test performance
- Development of a national EQA scheme to assess analytical variability and recommend optimal methods
- Dialogue between clinicians and laboratory specialists to produce UK guidelines on standardised procedures for sweat collection, analysis, and interpretation.

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