Sweat testing for the diagnosis of cystic fibrosis: Practical considerations

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Most pediatricians eventually encounter a patient with a clinical presentation that warrants the consideration of a sweat test to rule out or confirm the diagnosis of cystic fibrosis. This article discusses, in a series of questions and answers, the currently available sweat testing methods and describes the various methods' reliability, limitations, and frequency of use. In addition, sweat testing utilization and the interpretation and evaluation of test results are discussed so that the clinician can critically analyze the laboratory data. (J Pediatr 1996;129:892-7)

The understanding of the genetics and pathophysiology of cystic fibrosis has grown tremendously in the past decade. In an era of gene sequencing and gene transfer using viral vectors, it may seem like an anachronism to rely on the phenotypic diagnostic tool of the sweat test. However, because of the large number of mutations of the CFTR gene, confirmation of diagnosis by genetic testing is limited and the mainstay of diagnosis is the sweat test. There are several critical issues associated with sweat testing that can make the evaluation of a sweat test result challenging. Physicians need to be aware of the kind of sweat testing being performed on their patients because many of these methods have limitations. The following series of questions and answers can be helpful to the clinician faced with ordering and evaluating a sweat test result. These questions are based on those received from the practitioners referred to the author by the Cystic Fibrosis Foundation and are an outgrowth of a workshop presented at the 1995 North American Cystic Fibrosis Conference.

1. What does a sweat test encompass?

The term sweat testing is a general term referring to the quantitative or qualitative analysis of sweat to determine electrolyte concentration, conductivity, or osmolality for the confirmation of a CF diagnosis. Regardless of the method involved, a sweat test generally has three parts: sweat stimulation by pilocarpine iontophoresis; collection of the sweat onto gauze, filter paper, coil, patch, or capillary tube; and qualitative or quantitative analysis of the sweat for the chloride concentration, the sodium concentration, conductivity, or osmolality.

2. What is the difference between a qualitative and a quantitative sweat test?

A qualitative sweat test is a screening test for CF, and a patient with a positive or borderline result should have a quantitative sweat test. Screening tests may or may not measure the amount of sweat collected and may report a result as positive, negative, or borderline or give an actual concentration of sweat analytes. Examples of qualitative screening sweat tests are the Wescor Sweat Chek conductivity analyzer (Wescor, Logan, Utah), the Advanced Instruments conductivity analyzer (Advanced Instruments, Norwood, Mass.), the Orion skin electrode for chloride (Orion Research, Cambridge, Mass.), the Scandipharm CF Indicator System chloride patch (Scandipharm, Birmingham, Ala.), and sweat osmolality measurements. Some of these methods have documented problems, which will be described later.

In a quantitative sweat test, the amount of sweat collected is measured and then the chloride or sodium concentration in the sample is quantified.
3. According to the U.S. CF Foundation, what kind of sweat test is approved for diagnosis?

For diagnostic purposes, a sweat test consists of the quantitative analysis of sweat chloride, with or without sodium, using reliable methods. This procedure, often referred to as the quantitative pilocarpine iontophoresis test, involves the collection and quantitation of sweat after pilocarpine iontophoresis with the use of gauze, filter paper, or Macrodextrin coils and the quantitative analysis of sweat chloride with or without sodium. Chloride provides greater discrimination in diagnosis compared with sodium. The diagnosis of CF is based on at least two positive QPT results, together with an appropriate clinical presentation.

4. What clinical sites are appropriate locations for screening tests?

According to the U.S. CF Foundation, screening tests are appropriate for clinical sites such as community hospitals, but they are not appropriate for use at accredited CF care centers. These care centers should be performing QPT testing only.

5. What are the reference values for sweat chloride?

- Less than 40 mmol/L: negative
- A range of 40 to 60 mmol/L: borderline
- Greater than 60 mmol/L: consistent with CF

The interpretation of the chloride concentration is made with regard to the patient’s clinical presentation, family history, and age, and the knowledge that some rare mutations of the CFTR gene are associated with a borderline or negative sweat chloride concentration.

Sweat electrolyte concentration increases with age, and healthy adults can have sweat chloride concentrations greater than 60 mmol/L.

6. What is the distribution of CF patients within the chloride reference values?

Approximately 98% of patients with CF have sweat chloride concentrations greater than 60 mmol/L, and approximately 1% to 2% have sweat chloride concentrations less than 60 mmol/L. CF in patients whose sweat chloride concentrations are less than 60 mmol/L can be diagnosed on the basis of genotype, nasal potential difference studies, or clinical presentation.

7. What are the sweat conductivity reference values?

Sweat conductivity is a qualitative screening test. The U.S. CF Foundation has approved the use of the Wescor Macroduct Sweat Chek as a screening test for use at sites other than CF care centers. According to the Foundation, a patient having a sweat conductivity greater than or equal to 50 mmol/L should be referred to an accredited CF care center for a QPT. The Sweat Chek manufacturer’s decision levels are higher than those of the CF Foundation, which can lead to confusion between the laboratory and the physician as to the appropriate decision level. The Foundation’s medical advisory committee, in deciding to set the decision limit at 50 mmol/L, did so cautiously, realizing that the goal of screening tests is to tolerate some false-positive results and minimize or avoid false-negative results. False-positive test results will be resolved on further testing, but it would be unacceptable to miss CF in a patient. When evaluating sweat conductivity results, physicians should be aware that sweat conductivity is approximately 15 mmol/L higher than the sweat chloride concentration because of the presence of unmeasured anions such as lactate and bicarbonate.

8. What about the analysis of both sodium and chloride concentrations in the same sample?

Some laboratories analyze both chloride and sodium in the patient’s sweat samples. The determination of both electrolytes can be a useful technique to monitor quality control but may have limited usefulness in diagnosis. For quality control purposes, a significant discordance between the two electrolyte concentrations can indicate technical error in collection, analysis, or both. Diagnostically, several studies have evaluated the use of sweat Cl/Na ratios in discriminating patients with and those without CF. Because of analytic variation in the procedures, individual chloride and sodium concentrations can overlap, thereby limiting the diagnostic usefulness of a Cl/Na ratio.

9. What factors affect sweat test accuracy?

Unreliable methods, technical errors, and errors in interpretation can all lead to false sweat test results. There are problems reported with some of the methods used for sweat testing. The Orion electrode does not quantitate the amount of sweat collected and is subject to sample evaporation, and the electrode may be pressure sensitive, whereas older conductivity analyzers using unheated collection cups do not quantitate the amount of sweat collected and are subject to sample evaporation, condensation, or both. Methods that do not quantitate sweat collected or do not have an established minimum sample volume or weight are subject to false-negative results because an adequate sweat rate cannot be ensured.

Other problems with sweat testing include technical errors of evaporation and contamination, and errors in dilution, instrument calibration, and result reporting. These errors occur more often in institutions doing relatively few tests. Interpretation errors in sweat testing include lack of knowledge of the laboratory method, failure to repeat tests with borderline or positive results, failure to repeat tests with negative results when inconsistent with the clinical picture, and failure to repeat testing in patients with a diagnosis of CF that does not follow the expected clinical course.

10. What sweat test methods are laboratories currently using?

The results of a recent survey designed to assess sweat test methodology of laboratories in North America appear in Table I. Chloride is the most popular analyte for sweat test-
Table I. Method of analysis

<table>
<thead>
<tr>
<th>Methods</th>
<th>Frequency No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride methods (n = 574)</td>
<td></td>
</tr>
<tr>
<td>Chloridometer</td>
<td>199 (34.7)</td>
</tr>
<tr>
<td>Direct skin electrode</td>
<td>197 (34.3)</td>
</tr>
<tr>
<td>ISEs</td>
<td>59 (10.3)</td>
</tr>
<tr>
<td>Manual titration</td>
<td>46 (8.0)</td>
</tr>
<tr>
<td>Test patch</td>
<td>17 (3.0)</td>
</tr>
<tr>
<td>Other</td>
<td>36 (6.3)</td>
</tr>
<tr>
<td>Conductivity (n = 325)</td>
<td></td>
</tr>
<tr>
<td>Wescor Sweat-Chek test</td>
<td>206 (63.4)</td>
</tr>
<tr>
<td>Advanced instruments</td>
<td>60 (18.5)</td>
</tr>
<tr>
<td>Other</td>
<td>59 (18.1)</td>
</tr>
<tr>
<td>Sodium methods (n = 77)</td>
<td></td>
</tr>
<tr>
<td>Flame photometer</td>
<td>41 (53.2)</td>
</tr>
<tr>
<td>ISEs</td>
<td>29 (37.7)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (9.1)</td>
</tr>
<tr>
<td>Osmolality (n = 64)</td>
<td></td>
</tr>
<tr>
<td>Vapor pressure osmometer</td>
<td>47 (73.4)</td>
</tr>
<tr>
<td>Freezing point depression osmometer</td>
<td>17 (26.6)</td>
</tr>
</tbody>
</table>


ISEs, Ion-selective electrodes.

The appropriate methods of sweat collection and analysis, quality control, and the evaluation and reporting of the test results.

11. Why is it important to establish a minimum acceptable sweat volume or weight?

Sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat electrolyte concentration decreases. The average sweat rate should exceed 1 gam/my per minute. The minimum acceptable sample for analysis from a single site, with a 2 x 2-inch gauze or filter paper used for stimulation and collection, is 75 mg collected in 30 minutes. With the use of the Wescor Macroduct coil system, the minimum acceptable sample is 15 ml collected in 30 minutes. The minimum acceptable volume or weight will depend on the size of the electrode and stimulation area, the type and size of collecting media, and the duration of the sweat collection period.

12. Are there standard procedures established for sweat testing?

The National Committee for Clinical Laboratory Standards, a voluntary organization that develops and promotes the use of national and international standards for performing clinical laboratory tests, has published document C34-A, entitled Sweat Testing: Sample Collection and Quantitative Analysis—Approved Guideline. The document describes the appropriate methods of sweat collection and analysis, quality control, and the evaluation and reporting of the test results.

13. Are there national programs available for assessing accuracy in sweat testing?

The College of American Pathologists, a professional association involved in promoting accuracy in clinical laboratory testing, established proficiency testing for sweat analysis in 1994. The goal of the program is to provide participants with an external evaluation of sweat analysis accuracy, along with education concerning collection, interpretation, and testing limitations.

14. What other disorders besides CF can cause sweat electrolytes to be elevated?

There are a variety of disorders in addition to CF that can cause elevations in sweat electrolyte concentrations. Most of these disorders are distinguishable from CF on the basis of clinical presentation and are listed in Table II.

15. How common are false-positive and false-negative sweat test results?

The answer depends on the method used. False-positive results have been reported to be as high as 15% and false-negative results as high as 12%. There are a variety of disorders in addition to CF that can cause elevations in sweat electrolyte concentrations. Most of these disorders are distinguishable from CF on the basis of clinical presentation and are listed in Table II.

16. What are the causes of false-positive and false-negative sweat test results?

False-positive results can arise because of disorders caus-
ing elevations of sweat electrolyte concentrations (see Table II), eczema, methods that allow sample evaporation to occur, and other methodologic and technical errors.\textsuperscript{17}

False-negative results can occur if the patient is edematous, if an inadequate quantity of sweat is collected and analyzed, and because of other methodologic and technical errors.\textsuperscript{17}

17. What patient conditions can affect sweat test results?
The patient’s nutritional status, hydration status, use of mineralcorticoids, skin condition (eczema, rash), and age can all affect the sweat electrolyte concentration. Malnutrition, dehydration, eczema, and rash can increase sweat electrolyte concentrations, whereas edema and the administration of mineralcorticoids can decrease sweat electrolyte concentrations.

18. How should a sweat test result of 213 be interpreted?
Physiologically, the maximum sweat chloride concentration is around 160 mmol/L.\textsuperscript{20} When evaluating a result significantly higher than 160 mmol/L, one should consider the following:

a. The units associated with the number. Perhaps 213 is the weight of the sweat sample in milligrams.

b. The analyte being measured. Perhaps the laboratory is analyzing the sweat for osmolality. The reference ranges for sweat osmolality are 50 to 150 mmol/kg (normal), 150 to 200 mmol/kg (borderline), and greater than 200 mmol/kg (consistent with CF).\textsuperscript{3}

c. Laboratory error.

d. Munchausen syndrome by proxy.\textsuperscript{21} Order a second collection and analysis, asking the laboratory to observe the patient during the entire collection process.

19. Why do some patients fail to produce an adequate sweat volume/weight?
Insufficient sweat samples, also referred to as “quantity not sufficient” samples, can be due to several factors such as age, race, skin condition, and collection system.\textsuperscript{22} Though it may seem to be more difficult to obtain an adequate sweat sample in very young infants, studies have shown no statistically significant difference in the proportion of QNS results in patients younger than 6 weeks of age in comparison with patients older than 6 weeks of age.\textsuperscript{22,33} Differences in skin resistance because of ethnicity or individual patient variability may lead to QNS samples. For example, in the United States a higher prevalence of QNS samples have been reported with black patients than with white patients.\textsuperscript{24} Collection systems vary with regard to insufficient samples; for example, there is a 0.7% failure rate associated with collection onto gauze or filter paper compared with a 6.1% failure rate associated with the use of the Wescor Macroduct coils.\textsuperscript{10}

20. Can sweat collection be extended if the patient does not provide sufficient amounts of sweat in 30 minutes?
No, the amount of sweat required is derived from a rate equation using 30 minutes as a variable. Extending the collection beyond 30 minutes will increase the amount of sweat required. In addition, extending the collection time can allow additional opportunity for sample evaporation and, in practice, does not significantly add to the sample quantity.

21. Why cannot two insufficient sweat samples be combined for analysis?
The average sweat rate of 1 gm/m\textsuperscript{2} per minute is determined independently for each site. The requirement is a physiologic one, not an analytic one, because sweat electrolyte concentration increases with increased sweat rate.\textsuperscript{18} Samples below the average sweat rate can cause false-negative sweat electrolyte results.

22. How old does a patient have to be to undergo a sweat test?
Because of a report of transient elevations in sweat electrolyte concentrations in the first 24 hours after birth, it is recommended that patients be at least 48 hours old before undergoing a sweat test.\textsuperscript{3,25}

23. When can a sweat test be repeated?
Anytime, preferably when the patient is physiologically and nutritionally stable.

24. What body sites are appropriate for sweat collection?
Arms or legs. The skin should be free of rash or inflammation. The stimulation site should be selected so that the iontophoresis current will not cross the heart.

25. Is there any difference in sweat electrolyte concentration collected from different body sites?
No.

26. Should laboratories be performing the sweat test in duplicate on each patient (i.e., collection and analysis from two different sites)? In this situation, what is the acceptable variation between the results?
Because of the potential for significant preanalytic variation with the sweat test, it is suggested that the test be performed in duplicate from two sites for quality assurance.\textsuperscript{3} This should not be misconstrued as representing multiple, independent sweat tests for diagnosis. For diagnostic purposes, patients with a positive sweat test result should be retested on a separate occasion for confirmation.

Most patients’ sweat chloride concentrations on duplicate testing agree within 1 to 5 mmol/L. The laboratory can set an appropriate range based on the standard deviation in their laboratory. One suggestion is that for patients with chloride concentrations less than 60 mmol/L the duplicate results must agree within 10 mmol/L, and for patients with chloride concentrations greater than 60 mmol/L agreement must be within 15 mmol/L.\textsuperscript{3}
27. Is there any risk to the patient undergoing a sweat test?
There is a very slight (probably <1%) risk of a burn or urticaria.

28. Why would a patient get burned, and how should the burn be treated?
A skin burn can occur if the iontophoresis current is greater than 4 mA, if the bare metal of the electrode touches the skin, if the reagent interface is not sufficiently moist, or if the electrode surface is damaged or oxidized. In addition, patients vary with regard to their skin resistance and their sensitivity to the iontophoresis procedure, which may account for the occurrence of burns despite appropriate techniques. Depending on the severity of the burn, either no treatment or an antibiotic ointment is sufficient. The sweat should not be collected over the burn site.

29. Why does localized urticaria occur, and how frequent is it? How should the urticaria be treated?
The occurrence of localized urticaria is rare. The patient may react to the pilocarpine or to the phenomenon of electrical stimulation. If it is the latter, urticaria will appear under both electrodes. The test should be discontinued, and sweat should not be collected over the site of urticaria. Depending on the severity of the reaction, antihistamines can be administered.

30. Can a sweat test be performed on a patient receiving oxygen?
Yes, if the oxygen is delivered by nasal cannula or face mask. Because of the remote risk of electrical sparking, patients should not undergo sweat testing while receiving oxygen by an open delivery system.

31. Can a sweat test be performed on a patient receiving fluid intravenously?
Yes, as long as the infusion does not interfere with good skin-pad-electrode contact and the iontophoresis does not interfere with venous flow. In addition, care should be taken to ensure that there is no contamination of the collection site by the fluid.

32. Can the sweat test be performed on either the left or right limb?
Yes, particularly if the iontophoresis unit is battery powered. The original caution concerning sweat testing on the right arm was in reference to iontophoresis units operating from house current.

33. What kind of quality control should the laboratory be performing?
Laboratories should document competency testing of all personnel performing sweat test collection or analysis. Analytically, laboratories should be running a positive and a negative control with every patient sample, using procedures described in the National Committee for Clinical Laboratory Standards document. The controls can be electrolyte standards, appropriately diluted urine control material, or commercially prepared sweat control material. If electrolyte standards are used as controls, they should not be the standards used to calibrate the instrument.

34. What kind of information should be on the laboratory report for a sweat test?
Specific information concerning what analyte of sweat is being measured. For example, does the test measure chloride, sodium, conductivity, or osmolality? The interpretation of the results depends on the physician’s being knowledgeable about the analytic method, because decision levels vary considerably with each analyte. In addition, the sweat weight or volume should also be reported to ensure adequate sample collection.

35. What kind of questions should the physician ask the laboratory concerning sweat testing?
- a. Is the sweat test a screening or a definitive (quantitative) test?
- b. What does the test measure?
- c. What is the minimum sample volume/weight?
- d. How many tests are performed annually?
- e. Who performs the test? How is competency evaluated?
- f. What kind of quality control and quality assurance is ongoing?

SUMMARY
A reliable sweat test result depends on many factors, including the use of appropriate methods, performance of a sufficient number of tests to maintain proficiency, performance of the test by competent technologists, active quality control and quality assurance programs that include the use of two controls, consistently acceptable performance on College of American Pathologists proficiency testing, and knowledgeable interpretation of the test results in light of the patient’s clinical presentation.

REFERENCES