

Diagnostic Sweat Testing: The Cystic Fibrosis Foundation Guidelines

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The Cystic Fibrosis Foundation (CFF) accredits cystic fibrosis (CF) centers, located in teaching and community hospitals nationwide, which provide comprehensive diagnosis and treatment for people with CF. The CF centers are evaluated by the CFF Center Committee according to specific criteria covering the areas of clinical care, teaching, and research. There are specific requirements for sweat testing, and adherence to them is required for accreditation. In 2006, the CFF Center Committee distributed a sweat testing guidelines memorandum to the CF center directors.¹ Although the guidelines are based on the Clinical Laboratory Standards Institute (CLSI), formerly National Committee for Clinical Laboratory Standards, sweat testing document C34-A2 and the College of American Pathologists (CAP) Laboratory Accreditation Program Inspection Checklist items for sweat testing, they are more prescriptive for uniformity and are focused on diagnostic rather than screening sweat tests.^{2,3} The guidelines are applicable to patients of all ages undergoing sweat chloride testing.

Adherence to the guidelines is mandatory for CFF centers; however, the requirements are appropriate and adaptable for any facility performing diagnostic testing for CF. Although it may be ideal for sweat testing to be centralized at CF centers, in practice this does not occur. According to enrollment in a national proficiency testing program for sweat analysis, more than 600 laboratories performed sweat testing in 2006.⁴ With widespread implementation of newborn screening programs for CF, the reliance on a well-performed and well-interpreted sweat test is critical to the success of accurately diagnosing CF. Sweat chloride testing should be performed on all infants with a positive newborn screen even in cases in which two CF-causing mutations have been identified.⁵

The following represent the 2006 CFF sweat testing guidelines, along with commentary discussing the specific guidelines.¹

GUIDELINES AND COMMENTARY

Guideline 1

The laboratory must perform quantitative pilocarpine iontophoresis sweat chloride testing according to the procedures outlined in CLSI document C34-A2 without modification.

COMMENTARY. A quantitative sweat test for diagnosis includes four steps described in detail in the CLSI C34-A2 document²:

- Stimulation of sweat using pilocarpine iontophoresis
- Collection of sweat into gauze, filter paper, or Macroduct coils (Wescor, Logan, UT)
- Evaluation of the amount collected either in weight (milligrams) or volume (microliters)
- Measurement of the sweat chloride concentration. This process is described in Guideline 12

Measurement of sweat conductivity, for example, Sweat Chek or Nanoduct (Wescor, Logan, UT) is not acceptable for diagnosis.

Guideline 2

The laboratory must have access to a copy of the above-referenced CLSI Guidelines document C34-A2, either paper copy or through electronic file (www.clsi.org).

COMMENTARY. Personnel performing the sweat collection and analysis should be knowledgeable about the contents of the CLSI document.

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CF	Cystic fibrosis	CQI	Continuous quality improvement
CFF	Cystic Fibrosis Foundation	QNS	Quantity not sufficient
CLSI	Clinical Laboratory Standards Institute		

Guideline 3

The iontophoresis equipment must be battery powered and regularly inspected.

COMMENTARY. For safety reasons, the iontophoretic current source needs to be battery powered. Inspection for current control and leakage must be periodically performed by biomedical engineering according to the manufacturer's recommendations.

Guideline 4

The minimum age for testing is 48 hours.

COMMENTARY. Sweat electrolytes may be transiently elevated during the first 24 hours of age.⁶ If after 48 hours of age, an adequate sweat sample can be obtained, sweat testing is appropriate.

Guideline 5

Only the arms or legs are to be used as collection sites. The iontophoresis current should not cross the heart.

COMMENTARY. Sweat is stimulated and collected from the patient's lower arm or upper leg, from a site that is free from inflammation, rash, or cuts to avoid contamination of the sample with serous fluid or blood.

Guideline 6

Sweat must be collected on gauze or filter paper or in a Macroduct coil (Wescor, Logan, UT) after iontophoresis.

- a. If gauze or filter paper collection is used, the stimulated area must be 2 × 2 inches (total area, 4 square inches). A slightly smaller electrode (eg, 1½ × 1½ inches) is used for iontophoresis. Other electrode sizes are permissible if they cover greater than 50% of the 2 × 2 inch area (ie, an area of greater than 2 square inches). The iontophoresis should be carried out using USP grade pilocarpine for 5 minutes. After stimulation, the sample must be collected from a single site, using 2 × 2 inch gauze or filter paper. The minimum sample weight using this method is 75 mg in 30 minutes.
- b. If a Macroduct coil is used for collection, then sweat must be stimulated with a disposable Pilogel electrode using the Webster Sweat Inducer (Wescor, Logan, UT) for 5 minutes. After a 30-minute collection, the minimum acceptable sample is 15 μL.

COMMENTARY. Adherence to a minimum sweat weight or volume from a single site is critical to obtain valid sweat testing results. The requirement for a minimum amount is to ensure an appropriate sweat rate and sweat electrolyte concentration. Sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat-electrolyte concentration decreases, and the opportunity for sample evaporation increases.

To ensure a valid result, the average sweat rate should exceed 1 g/m² per minute. The area of stimulation and collection must be of similar size to allow appropriate determination of sweat rate and to minimize evaporation or dilution of the chloride by nonstimulated sweat.

Guideline 7

Sweat must be collected for no more than 30 minutes.

COMMENTARY. If the collection time exceeds 30 minutes, the requirement for the amount of sweat needed to ensure adequate stimulation must increase. Extending the collection time can allow additional opportunity for sweat evaporation and practically does not increase the sweat yield significantly.

Guideline 8

The incidence of insufficient samples (ie, quantity not sufficient, or QNS samples) must be investigated and resolved if it exceeds 5% for patients older than 3 months of age.

COMMENTARY. Achieving a QNS rate below 5% for patients older than 3 months of age should not be a problem if the procedure in the CLSI document and the manufacturer's recommendations are followed. Factors influencing sweat collection include age, weight, race, skin condition, and collection system. For example, infants weighing less than 2000 grams, younger than 38 weeks age at testing, or of African-American race have an increased likelihood of producing an insufficient sample.⁷ Higher failure rates with the Macroduct coil compared with gauze collection have been reported.⁸ The calculation of a QNS rate is based on the percentage of tests where an adequate sweat sample is not obtained. If a bilateral (duplicate) sweat collection is performed, then the test is considered QNS only if an adequate sweat sample is not obtained from either site. For example, in an institution performing bilateral testing, a patient initially yields inadequate sweat samples on both sites (100% QNS). The same patient returns 1 week later and yields an adequate sample on one site and an inadequate sample on the other site (0% QNS). For this example, the overall QNS rate would be 50%.

Guideline 9

It is recommended that the collection and analysis be performed in duplicate.

COMMENTARY. Duplicate testing is recommended but not required as one mechanism for quality assurance. It should be noted that for diagnosis, duplicate testing done on the same day does not represent independent repeat testing.

Guideline 10

Insufficient samples should not be analyzed and must not be pooled for analysis.

COMMENTARY. Because the requirement for a minimum sample volume or weight is physiological, not analytical, each sweat sample must independently exceed a sweat rate of 1 g/m² per minute. Combining or analyzing insufficient samples may lead to false-positive and false-negative sweat tests, which have significant implications for patient care.

Guideline 11

Collection and analytical procedures must be designed to minimize evaporation and/or contamination. For specific techniques, refer to CLSI document C34-A2, Sections 8.1.3.1 and 8.1.4.

COMMENTARY. Sweat collected in gauze, once reweighed, can be stored with or without diluent in a tightly sealed container for up to 3 days at refrigerator temperature.⁹ Studies concerning the stability of sweat stored in Macrodut coils have not been published; therefore, laboratories should validate storage conditions.

Guideline 12

Sweat must be quantitatively analyzed for chloride by one of the following methods:

- a. Chloride by coulometric titration, using a chloridometer
- b. Chloride by a manual titration, using the Schales and Schales mercuric nitrate procedure
- c. Chloride by automated analyzers, using ion-selective electrodes that have been systematically validated against the methods described in a or b, above.

Analytical methods requiring the addition of extraneous chloride standard to patient samples to increase the analytical sensitivity should not be used.

COMMENTARY. Automated analyzers designed for quantifying serum chloride may lack the sensitivity needed for sweat analysis; therefore, validation studies using specimens with low chloride concentration (eg, 10 mmol/L) must be performed before use. It should be noted that automated analyzers using ion-selective electrodes for sweat chloride are different from the in situ or direct reading chloride electrode applied to the patient's skin.

Guideline 13

Perform and evaluate quality control with every sweat analysis run, using two levels of controls per the Clinical Laboratory Improvement Act of 1988 (CLIA, 1988).^{10,11}

COMMENTARY. A positive and negative control should be assayed with each patient run. If sweat is collected on gauze or filter paper, the control material should be applied directly to the collection surface and eluted for analysis.

Guideline 14

It is recommended that the sweat test be included in the laboratory's overall evaluation of CQI (continuous quality improvement).

COMMENTARY. The CQI evaluation should include the annual percentage of QNS samples, borderline and positive results, and adverse skin reactions. These variables should be reviewed by the CF Center Director and the laboratory director.

Guideline 15

Sweat samples must be appropriately labeled for patient identification throughout sweat collection and analysis. Reagents must be appropriately labeled.

Guideline 16

Appropriate reference values for sweat chloride must be used: <40 mmol/L = negative; 40 to 60 mmol/L = borderline/indeterminate; >60 mmol/L = consistent with the diagnosis of CF.

Note: Sweat chloride values <40 mmol/L have been documented in genetically proven CF patients. Clinical correlation is necessary.¹²

COMMENTARY. Results from sweat testing performed in infants suggest that sweat chloride values greater than 30 mmol/L should be considered abnormal, requiring further patient evaluation.^{5,13-17}

Guideline 17

The lower limit of detection should be determined by the laboratory and should be ≤10 mmol/L. The upper end of reportable results should be no more than 160 mmol/L.

COMMENTARY. The analytical method should be able to accurately measure sweat chloride at the mean normal concentration (around 10 mmol/L).¹⁸ Sweat concentrations below the lower limit of detection should be reported as "less than," for example, "<10 mmol/L." Although analytical instruments may have an upper measurement range of >160 mmol/L, concentrations above this are not physiologically possible and should not be reported.¹⁹ A patient with a sweat chloride of >160 mmol/L should be retested, as contamination or technical error is likely.

Guideline 18

All laboratories must document successful performance in the CAP proficiency testing survey for sweat test analysis.

COMMENTARY. In the sweat testing proficiency testing program, the CAP prepares three specimens that are mailed to laboratories twice a year. Laboratories are provided with feed-

back concerning their performance relative to others in their analytical group.²⁰

Guideline 19

We strongly suggest that the Center Director review all sweat test results by using procedures consistent with Health Insurance Portability and Accountability Act of 1996 (HIPAA) regulations.

COMMENTARY. Review of sweat tests by the Center Director provides an opportunity for quality improvement for CF diagnosis within the center. In addition, this review brings additional clinical expertise to the interpretation of sweat tests during the evaluation of “atypical” forms of CF. This is especially important as states embark on newborn screening and very young infants are being sweat-tested.

Guideline 20

All positive tests must be confirmed with a repeat sweat chloride test at a different time or another diagnostic test for CF.

COMMENTARY. For diagnosis, a positive sweat chloride test must be confirmed by repeating it at a different time or confirmed by identification of two cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutations known to cause CF, or abnormal electrophysiological studies of nasal epithelium.¹² All borderline sweat test results should be repeated. It is suggested that borderline sweat tests in patients identified by newborn screening be repeated within 1 to 2 months.⁵ If the repeated tests remain borderline, ancillary tests such as genotyping, assessment of pancreatic function, respiratory tract microbiology, and urogenital evaluation may be helpful.¹² Patients with borderline sweat tests should be monitored for respiratory problems and nutritional status.⁵ Sweat chloride tests should also be repeated in patients with confirmed CF who do not follow the expected clinical course.²

CFTR mutation analysis can be performed as a confirmation of an abnormal sweat test result. However, because many mutations are not detected by typical mutation panels used for CF screening, repeat sweat chloride testing is often required for diagnostic confirmation.

Guideline 21

Sweat testing must be available at least 2 days per week. Wait time for scheduling routine tests should be less than 2 weeks.

COMMENTARY. Adequate availability of sweat testing is critical to provide timely diagnosis and to lessen parental anxiety in anticipation of the testing.

Guideline 22

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. CLIA 1988 requires that new employees demonstrate competency every 6 months for the first year and annually thereafter.^{10,11}

COMMENTARY. Misdiagnosis of patients has been attributed to laboratories performing too few tests to maintain proficiency.^{21,22} However, the determination of what constitutes a “sufficient number” of sweat tests is subjective and not easily quantified. In not specifying the minimum number of sweat tests to be performed, the CFF has allowed each laboratory to determine the number of tests required for proficiency. The requirement that QNS rates be monitored and that the center director be involved in the review of sweat test results should ensure that laboratories are proficient at performing these tests.

Guideline 23

It is not appropriate to perform the sweat test using:

- a. Direct application of a chloride electrode to the patient’s skin.
- b. Chloride precipitation reaction by placing a patch directly on the patient’s skin.
- c. Measuring only potassium or sodium.
- d. Osmolality.
- e. Conductivity including Sweat Chek or Nanoduct (Wescor, Logan, UT).
- f. Any other screening (nonquantitative) tests.

COMMENTARY. The above methods are not appropriate for diagnosis at CF centers; however, the CFF has approved the Wescor Macroduct Sweat-Chek conductivity analyzer for screening at clinical sites, such as community hospitals, using the criteria that an individual having a sweat conductivity ≥ 50 mmol/L should be referred to an accredited CF care center for a quantitative sweat chloride test.²³

CONCLUSION

Despite the availability of genetic testing, a quantitative pilocarpine iontophoresis sweat chloride test remains the gold standard for the diagnosis of CF. Therefore, appropriate performance of sweat tests is vital to the function of the CF center. This is especially true as newborn screening for CF becomes more widespread.

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