

## ***Sweat Testing Standards for CFF Accredited Care Centers*** *as approved by the CFF Center Committee, May 2006*

Sweat testing procedures should be performed in accordance with *Clinical and Laboratory Standards Institute* (CLSI, formerly NCCLS) guidelines as outlined in their document, **Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline – Second Edition, (C34-A2)**. Specific criteria required for CFF Center accreditation are listed below. **Failure to use the appropriate techniques outlined below will require immediate corrective action or the Center will not be accredited.**

1. The laboratory must perform quantitative pilocarpine iontophoresis sweat chloride testing<sup>a</sup> according to the procedures outlined in CLSI document C34-A2 without modification.
2. **The laboratory must have access to a copy of the above-referenced CLSI Guidelines document C34-A2, either via paper copy or electronic file ([www.clsi.org](http://www.clsi.org)).**
3. The iontophoresis equipment must be battery powered and regularly inspected.
4. The minimum age for testing is 48 hours.
5. Only the arms or legs are to be used as collection sites. The iontophoresis current should not cross the heart.
6. Sweat must be collected on gauze or filter paper or in a Macroduct<sup>®</sup> coil (Wescor, Logan, Utah) following iontophoresis.
  - a. If gauze or filter paper collection is used the stimulated area **MUST** be 2 x 2 inches (total area 4 square inches). A slightly smaller electrode (e.g. 1 ½ x 1 ½ inches) is used for iontophoresis. Other electrode sizes are permissible if they cover greater than 50% of the 2 x 2 inch area (i.e. 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 x 2 inch gauze or filter paper<sup>b</sup>. The minimal sample weight using this method is 75 mg in 30 minutes.
  - b. If a Macroduct<sup>®</sup> coil is used for collection then sweat must be stimulated with a disposable Pilogel<sup>®</sup> electrode<sup>b</sup> using the Webster Sweat Inducer (Wescor, Logan, Utah) for 5 minutes. After a 30 minute collection the minimum acceptable sample is 15 uL.
7. Sweat must be collected for **no more than 30 minutes**.
8. The incidence of insufficient samples (quantity not sufficient – **QNS**) must be investigated and resolved if it exceeds 5% for patients older than 3 months of age.
9. It is recommended that the collection and analysis be performed in duplicate.
10. Insufficient samples should not be analyzed and must not be pooled for analysis.
11. Collection and analytical procedures must be designed to minimize evaporation and/or contamination. For specific techniques, refer to CLSI document C34-A2, Section 8.1.3.1 and 8.1.4.
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 employing ion-selective electrodes which have been systematically validated against the methods described in a-b above.<sup>c</sup>Analytical methods requiring the addition of extraneous chloride standard to patient samples in order to increase the analytical sensitivity should not be used.
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 '88).

14. It is recommended that the sweat test be included in the laboratory's overall evaluation of CQI (Continuous Quality Improvement).
15. Sweat samples must be appropriately labeled for patient identification throughout sweat collection and analysis. Reagents must be appropriately labeled.
16. Appropriate reference values for sweat chloride must be used<sup>d</sup>:
  - <40 mmol/L = negative
  - 40-60 mmol/L = borderline/indeterminate
  - > 60 mmol/L = consistent with the diagnosis of CF
 NOTE: Sweat chloride values less than 40 mmol/L have been documented in genetically proven CF patients. Clinical correlation is necessary.
17. The lower limit of detection should be determined by the lab and should be equal to or less than 10 mmol/L. The upper end of reportable results should be no more than 160 mmol/L.
18. All laboratories must document successful performance in the College of American Pathologists (CAP) proficiency testing survey for sweat test analysis.
19. We strongly suggest that the Center Director review all sweat test results using procedures consistent with HIPAA regulations.
20. All positive tests must be repeated at a different time.
21. Sweat testing must be available at least 3 days/week. Wait time for scheduling routine tests should be <2 weeks.
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 '88 requires that new employees demonstrate competency every 6 months for the first year and annually thereafter.
- 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 employing a patch placed directly on the patient's skin.
  - c. Measuring only potassium or sodium.
  - d. Osmolality.
  - e. Conductivity including Sweat Chek<sup>®</sup> or Nanoduct<sup>®</sup> (Wescor, Logan, Utah).
  - f. Any other screening (non-quantitative) tests.

We hope this information will be helpful to you and your laboratory director. Thank you for your assistance in maintaining the CF Center network as a model of excellence.

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Footnotes:

- a. Chloride concentration in the sweat must be quantitatively determined. Measurement of sweat conductivity, e.g. Sweat Chek<sup>®</sup> or Nanoduct<sup>®</sup> (Wescor, Logan, Utah), is not acceptable.
- b. 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 non-stimulated sweat.
- c. There is a concern for the sensitivity of these analyzers in the lower electrolyte concentrations. (Automated analyzers using ion-selective electrodes for sweat chloride are different from the *in situ* or direct reading chloride electrode which is applied to the patient's skin.)
- d. 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.
  - Parad *et al*, *J Pediatr*. 2005;**147**(3 Suppl):S69-72
  - Rock *et al*, *J Pediatr*. 2005;**147**(3 Suppl):S73-7
  - Sontag *et al*. *J Pediatr*. 2005;**147**(3 Suppl):S83-8
  - Taccetti *et al*. *Arch. Dis. Child. Fetal Neonatal Ed* 2004;**89**: F463 - F464