NOTE: Sweat must not be stimulated or collected from the head or trunk. Sweat must not be stimulated or collected from an area of diffuse inflammation, such as a rash or eczematous lesion, because of the likelihood of contamination by serous fluid.

REFERENCES
1) Liebke C, *et al.* Sweat electrolyte concentrations in children with atopic dermatitis. *Lancet.* 1997;350:1678
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29400 Pilocarpine Grade **Phase II**

If the laboratory prepares the pilocarpine solution for iontophoresis, the source of the pilocarpine is USP grade or equivalent.

CHM.29500 Electrode Placement **Phase II**

Electrodes used for stimulation are placed such that iontophoretic current never crosses the patient's trunk.

NOTE: The protocol must specify that electrodes used for stimulation be placed so that current does not cross the patient's trunk. This is to avoid the possibility of current crossing the heart, which results in cardiac depolarization.

CHM.29600 Iontophoresis Conditions and Equipment Maintenance **Phase II**

Iontophoresis conditions and maintenance requirements are defined.

NOTE: For safety reasons, the iontophoretic current source must be battery-powered, to avoid the possibility of patient exposure to line voltage. For manually controlled devices, iontophoresis must be performed for no more than five minutes at a current less than 4 mA, to prevent burns. The iontophoresis unit must be tested by qualified personnel (such as engineering personnel) for current leakage and current control at defined frequencies and records retained.

CHM.29700 Iontophoresis Oxygen **Phase II**

Iontophoresis is not performed on patients receiving oxygen by an open delivery system.

NOTE: While the possibility of an explosion due to the generation of an electrical spark is remote, it cannot be ignored. Often, these patients can temporarily receive oxygen via a facemask or nasal cannula, in which case sweat testing can be done.

CHM.29800 Iontophoretic Stimulation **Phase II**

The area of iontophoretic stimulation is equivalent to the area of sweat collection.

NOTE: Sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat electrolyte concentration decreases. The average sweat rate should exceed 1 g/m^2/min.

To ensure adequate sweat stimulation and accurately reflect sweat electrolyte concentration, a minimum acceptable sweat volume or weight is required. This requirement is based on the size of the electrode and stimulation area, the type and size of collecting media, and the duration of sweat collection. To standardize the process, the stimulation and collection area should be equivalent, and the time of collection consistent. For example, for the positive electrode, use a 1.5 x 1.5 inch (3.8 x 3.8 cm) electrode over a 2 x 2 inch (5.1 x 5.1 cm) gauze pad saturated with pilocarpine for stimulation, then collect sweat onto a 2 x 2 inch pre-weighed gauze pad.

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29850 Appropriate Sweat Collection Device **Phase II**

The sweat collection device is appropriate for the iontophoresis system.

NOTE: The sweat collection device must be designed for use with the appropriate iontophoresis system so that the stimulation and collection area are equivalent and the appropriate minimum acceptable sweat volume or weight can be achieved. Examples of acceptable combinations include:

- *Stimulation with Pilogel iontophoresis and collection into Macroduct coils*
- *Stimulation with copper electrodes over gauze/filter paper pilocarpine pads and collection into gauze/filter paper*
- *Stimulation and collection into Nanoduct conductivity cell*

Examples of unacceptable combinations include:

- *Stimulation with Pilogel iontophoresis and collection into gauze/filter paper*
- *Stimulation with Polychrome iontophoresis and collection into Macroduct coils, gauze or filter paper*
- *Stimulation with copper electrodes over gauze/filter paper pilocarpine pads and collection into Macroduct coils*

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29900 Sweat Collection Time **Phase II**

For confirmatory sweat testing, collection time may not exceed 30 minutes.

NOTE: For quantitative testing, extending the collection time will not significantly increase the sweat yield and may lead to sample evaporation. Samples may be taken from less than maximally stimulated glands. This may lead to a false-negative result. In addition, altering the collection time will affect the minimum acceptable sweat weight or volume, because the time parameter of the rate equation has been changed. For screening kits, the manufacturer's instructions for collection times must be followed.

CHM.30000 Sweat Collection Parameters **Phase II**

The parameters of sweat collection are defined.

NOTE: These must include an established minimum acceptable sweat volume or weight based on the area of stimulation, area of collection and standardized time for collection. The average sweat rate should exceed 1 g/m^2/min, which in general corresponds to a minimum sample weight of about 75 mg of sweat collected on a 2 x 2 inch (5.1 x 5.1 cm) gauze or filter paper and about 15 µL of sweat collected in Macroduct coil in 30 minutes. Volume verification should be performed for any specimen that might be near the 15 µL threshold. Samples less than the required volume or weight must not be analyzed.

REFERENCES

1) Hjelm M, *et al.* Sweat sodium related to amount of sweat after sweat test in children with and without cystic fibrosis. *Acta Paediatr Scand.* 1986;75:652-656
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30100 Sweat Sample Rejection **Phase II**

Samples that do not meet the minimum sample size requirements are rejected and are not pooled for analysis.

NOTE: The average sweat rate of 1 g/m2/min is determined independently for each site. The requirement is a physiologic one, not an analytic one. Samples less than the required volume or

weight must not be pooled to achieve the weight/volume requirement. Measurement on samples from less than maximally stimulated sweat glands may lead to false-negative results.

Evidence of Compliance:
- ✓ Records of specimen rejection

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CHM.30150 Sweat Rejection Incidence Rate **Phase I**

The incidence of insufficient sweat samples is routinely monitored.

NOTE: For quality monitoring, laboratories must collect data on the number of patients from whom an insufficient sweat sample has been obtained (QNS - quantity not sufficient). For patients older than three months of age, the annual insufficient rate should not exceed 5%. For patients up to three months of age, the rate should not exceed 10%. If these rates are exceeded, the collection procedure should be reevaluated for consistency with the CLSI guideline C34 4th ed.

For bilateral sweat collections, a QNS patient is a patient with an insufficient sample collected from both sites (eg, right arm and left arm). Each patient encounter is counted to determine the total number of sweat collections; thus, the same patient may appear repeatedly in the total population as well as the QNS population.

Evidence of Compliance:
- ✓ Records of insufficient collection **AND**
- ✓ Records of corrective action if rate exceeds the norm

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30250 Sweat Sample Storage **Phase I**

Appropriate storage conditions for collected sweat samples are defined.

NOTE: If there is a significant delay between collection and analysis, appropriate storage conditions must be followed: a) Sweat collected on gauze is stable at refrigerator temperatures for up to 72 hours once reweighed and secured in a vial with a tightly fitting cap; and b) Sweat collected in Macroduct coils is stable at refrigeration or room temperature for up to 72 hours in a 0.2 mL microcentrifuge tube with a tight fitting cap. Storage of sweat in microbore tubing is not recommended.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30300 Sweat Collection Skin Reaction **Phase II**

Processes to recognize and treat patient skin reactions (allergic or burns) to pilocarpine and/or other reagents used in iontophoresis are defined.

NOTE: Rarely, some patients may develop an area of, urticaria (hives) or small localized burns. In such cases, the procedure must be discontinued immediately and appropriate medical attention obtained. Sweat must not be collected over areas of urticaria or burns.

Evidence of Compliance:
- ✓ Records of follow-up treatment